Disease Markers 20 (2004) 313–315
IOS Press

Mutation rate at commonly used forensic STR loci: Paternity testing experience

Faruk Aşıcıoğlu[a], Fatma Oguz-Savran[b] and Ugur Ozbek[a,c,*]
[a]*The Council of Forensic Medicine, Cerrahpaşa, 34300, Istanbul, Turkey*
[b]*Istanbul Medical Faculty, Medical Biology Department, Istanbul University, Turkey*
[c]*Genetics Department, Institute for Experimental Medicine* (*DETAE*)*, Istanbul University, 34280, Istanbul, Turkey*

Abstract. Paternity tests are carried out by the analysis of hypervariable short tandem repeat DNA loci. These microsatellite sequences mutate at a higher rate than that of bulk DNA. The occurrence of germline mutations at STR loci posses problems in interpretation of resulting genetic profiles. We recently analyzed 59–159 parent/child allele transfers at 13 microsatellite loci. We identified 12 mutations in 7 microsatellite loci. No mutations were occurred in other 6 loci. The highest mutation rate was observed with 5 mutations at D8S1179 locus at different alleles. The event was always single repeat related. The mutation rate was between 0 and 1.5×10^{-2} per locus per gamete per generation. The mutation event is very crucial for forensic DNA testing and accumulation of STR mutation data is extremely important for genetic profile interpretation.

Keywords: STR, mutation, paternity testing, short tandem repeat

1. Introduction

Microsatellites are tandem repeats of short units of DNA that occur with high frequency throughout the genomes of many organisms [7]. Microsatellites are genotyped by PCR using primers flanking the repeat sequence. Paternity tests are carried out by the analysis of family groups at these hypervariable loci. Microsatellite loci have a high degree of variability that is caused by a high rate of mutations that alter microsatellite length [5]. A mutation event is typically recognized as a shift in allelic mobility in the comparison of parent and off-spring [9].

The primary mutational mechanism leading to changes in microsatellite length is polymerase template slippage [4,6]. During replication of a repetitive region, DNA strands may dissociate and then reassociate incorrectly. Renewed replication in this misaligned state leads to insertion or deletion of repeat units, thus altering allele length. At the triplet repeat loci associated with various genetic disorders, there is also some possibility of very rapid growth in allele size [1,2,8]. General estimates of STR mutational rates have been shown to depend on the consensus repeat length. It is important for paternity tests with a seeming paternal exclusion, to account for the possibility of mutation in gene transmission. The mutation rate of microsatellites is often quoted in range of 10^{-3} to 10^{-4} per locus per generation. Mutation rates in microsatellites influence the structure and length of tandem repeat [1]. Microsatellite mutations constitute a significant part of all the mutations within a genome transferred from one generation to another.

In this report we present data on mutational events from 59–159 parent/child allele transfer at 13 microsatellite loci commonly used in forensics and paternity testing.

2. Materials and methods

The material comprised paternity testing samples analyzed during 2001–2002 at the Institute of Forensic Medicine, Ministry of Justice, Turkey. DNA was

*Corresponding author. Tel.:/Fax: +90 212 631 1351; E-mail: uozbek@istanbul.edu.tr.

ISSN 0278-0240/04/$17.00

Table 1
Mother, father, child allele segregation of 10 families mutations observed at 7 loci. (mutated alleles underlined)

	STR loci	Mother	Father	Child
1	D21S11	32, 32.2	31, 32.2	31, 31
	D8S1179	11, 12	14, 15	12, 13
	D7S820	9, 11	12, 12	11, 11
2	D8S1179	13, 15	11, 15	14, 15
3	D8S1179	13, 13	13, 17	13, 18
4	D8S1179	12, 13	13, 14	12, 12
5	D8S1179	13, 15	13, 13	14, 15
6	D21S11	28, 30.2	29, 33.2	28, 32.2
7	D18S51	16, 18	17, 20	16, 18
8	D13S317	8, 8	8, 10	8, 11
9	HumvWA	17, 17	16, 18	17, 19
10	HumFGA	21, 25	22, 23	21, 25

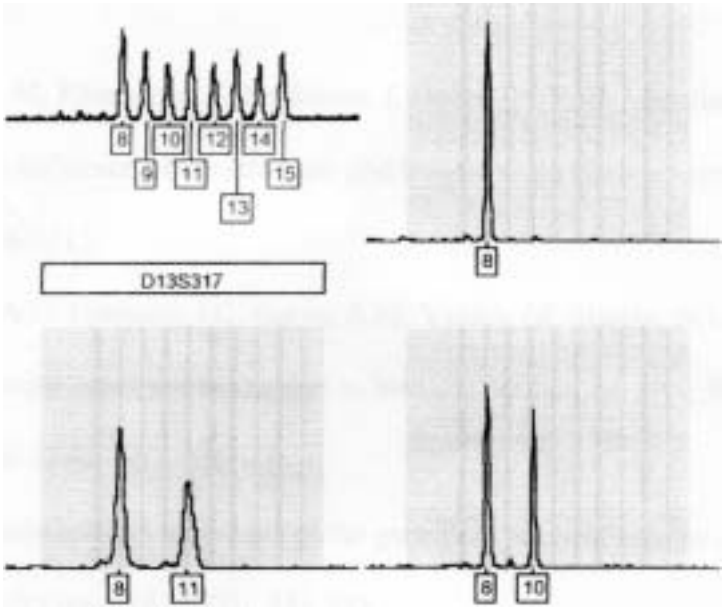

Fig. 1. Electropherogram of D13S317 locus analysis of a mother, father, child allele segregation (family # 8). (Mother, father, child genotyped as 8,8;8,11,;8,10 respectively.)

extracted from EDTA blood by Chelex-resin method. PCR amplification was performed by strictly following the manufacturer's recommended protocol in a GeneAmp PCR System 2700 (PE Applied Biosystems, USA). Typing was done by using the commercially available AmpFeSTR Profiler Plus Kit (PE Applied Biosystems, USA) in ABI Prism 3100 Genetic Analyzer equipped with 16 capillaries and Performance Optimized Polymer 4 (POP-4) with 28 min run times. The Hum CSF1PO, HumTHO1, HumTPOX, HumVWA, HumFGA, D3S1358, D7S820, D16S539, D8S1179, D21S11, D5S818, D13S317, D18S51 loci amplified by commercially available kit (Applied Biosystems, USA). The Gene Scan 2.1 and Genotyper 2.0 (Applied Biosystems, USA) software used for sizing and typing. The samples were re-extracted, re-amplified and run twice to confirm the genotypes.

3. Results and discussion

The number of analyzed allele transfer between child and parents were 118 to 318. We identified 12 mutations in 7 microsatellite loci in a total of 3248 paternal alleles and in 1 locus in a total of 3240 maternal allele transfers (Table 1). In each family with a mu-

tation, we calculated the power of evidence for parenthood by using the other genotyped loci data, this was from 99.99% to over 99.999%. No mutations occurred in 6 loci (HumCSF1PO, HumTHO1, HumTPOX, D3S1358, D16S539, D5S818). Five mutations were observed in D8S1179 locus at different alleles. The event was always single repeat related. The mutation rates were between 0 and 1.5×10^{-2} per locus per gamete per generation. The mutation event is very crucial for forensic DNA testing and accumulation of STR mutation data is extremely important for genetic profile interpretation. Mutation rate differ between the species and within the species between the loci [3,5, 11]. The mutation rates of individual alleles at a given locus vary according to allele length [1]. Longer alleles exhibit higher mutation rates than shorter ones. It is plausible that the positive correlation mutation rate and allele size can be explained by the fact that replication slippage can take place at more locations in a repetitive sequence with many repeat units [3]. In our samples, we observed that D8S1179 locus mutated 5 times out of 8 total loci mutated. This result does not confirm Brinkmann's observation [1]. This might be explained by the small population size. Most of the mutation at microsatellite loci originated in males [1,10]. This is in concordance with observations of other species as well [3,11]. In our study we observed similar incidence for male mutation predisposition.

To our knowledge, this is the first report on the mutation rate of minisatellite loci in Turkish population. As more data become available, more certainty will be gained for paternity probability calculation.

References

[1] B. Brinkmann, M. Klintschar, F. Neuhuber, J. Huhne and B. Rolf, Mutation rate in human microsatellites: influence of the structure and length of the tandem repeat, *Am J Hum Genet* **62** (1998), 1408–1415.

[2] A. Di Rienzo, A.C. Peterson, J.C. Garza, A.M. Valdes, M. Slatkin and N.B. Freimer, Mutational processes of simple-sequence repeat loci in human populations, *Proc Natl Acad Sci USA* **91** (1994), 3166–3170.

[3] H. Ellegren, Microsatellite mutations in the germline: implications for evolutionary inference, *Trends Genet* **16** (2000), 551–558.

[4] M. Farrall and D.E. Weeks, Mutational mechanisms for generating microsatellite allele-frequency distributions: an analysis of 4,558 markers, *Am J Hum Genet* **62** (1998), 1260–1262.

[5] J. Henke and L. Henke, Mutation rate in human microsatellites, *Am J Hum Genet* **64** (1999), 1473–1474.

[6] A.J. Jeffreys, D.L. Neil and R. Neumann, Repeat instability at human minisatellites arising from meiotic recombination, *Embo J* **17** (1998), 4147–4157.

[7] A.J. Jeffreys, V. Wilson and S.L. Thein, Hypervariable minisatellite regions in human DNA, *Nature* **314** (1985), 67–73.

[8] L. Jin, C. Macaubas, J. Hallmayer, A. Kimura and E. Mignot, Mutation rate varies among alleles at a microsatellite locus: phylogenetic evidence, *Proc Natl Acad Sci USA* **93** (1996), 15285–15288.

[9] S. Kruglyak, R.T. Durrett, M.D. Schug and C.F. Aquadro, Equilibrium distributions of microsatellite repeat length resulting from a balance between slippage events and point mutations, *Proc Natl Acad Sci USA* **95** (1998), 10774–10778.

[10] B. Rolf, P. Wiegand and B. Brinkmann, Somatic mutations at STR loci-a reason for three-allele pattern and mosaicism, *Forensic Sci Int* **126** (2002), 200–202.

[11] D.C. Rubinsztein, W. Amos, J. Leggo, S. Goodburn, S. Jain, S.H. Li, R.L. Margolis, C.A. Ross and M.A. Ferguson-Smith, Microsatellite evolution–evidence for directionality and variation in rate between species, *Nat Genet* **10** (1995), 337–343.