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De novo mutations at D3S1358, D8S1179 and D18S51 loci emerged during paternity testing: confirmation of biological paternal lineage by using a panel of Y-chromosome STRs

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Abstract

In three paternity tests performed in our laboratories with a battery of autosomal STRs, we discovered three separate incompatibilities for the loci D3S1358, D8S1179 and D18S51. Since the other employed markers confirmed the paternity of the alleged father, the observed incompatibility for these markers was probably due to mutation events. However, taking into account the mutation value for the markers, the paternity index (PI) and posterior probability (*W*) results were very low. In these cases, we normally analyse an extra panel of polymorphic markers to increase the PI value. However, in these three cases, since the disputed children were males and the Y-chromosome is transmitted exclusively to the male lineage, we studied a battery of 10 Y-STR in the children and in the alleged fathers. Evaluation of Y-STR haplotype frequencies was deduced from a European available database.

Keywords: STR polymorphism; Paternity test; STR mutation

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1. Introduction

The analysis of Y-chromosome polymorphisms acquires for various purposes a growing value in the forensic genetics practice. Here, we report the role of the Y-chromosome DNA markers for the confirmation of biological paternity in three cases of mutations of autosomal polymorphic loci in the paternal meiosis. Since most Y-STR loci do not recombine, the haplotype frequencies cannot be estimated simply as a product of allele frequencies. Estimates involving our database in a Tuscany population [1] could be just employed like the inverse of the database size, with a too conservative value. For this reason, we applied the "surveying" method suggested by Roewer et al. [2] to estimate the frequencies for the observed haplotypes.

In the first part of the study, the two different laboratories performed the analyses of a variable battery of autosomal STRs by using different protocols and instruments. In alternative to the analysis of an extra panel of autosomal markers, given that the children were all males in the three families, we decided to use a battery of 10 Y-STR (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS385I/II, DXYS156Y and YCAII) to investigate the biological paternity.

2. Material and methods

2.1. DNA extraction

DNA was extracted with the Puregene system (Gentra) and with the phenol–chloroform method and was measured with the spectrophotometer at 260 nm.

2.2. Amplification and detection

To perform the paternity testing, we used two different strategies. One method is based on an ABI Prism® 310 Genetic Analyser (Applied Biosystems) with the AmpFlSTR Profiler Plus PCR Amplification kit and SGM Amplification kit, according to the manufacturer's recommendation. The second approach consists in the use of an Automated DNA Sequencer IR-based (LI-COR) with a previously described protocol [3].

We performed the Y-STR analysis by using a PCR monoplex amplification protocol. The characterisation of the alleles was achieved with the IR-based Automated DNA Sequencer (LI-COR 4200). For the exact detection of the alleles, we used specific allelic ladders and positive controls, partly provided by the Italian Collaborative Project of the Ge.F.I Group (Fiuggi 2), and partly by the European Y-Users Group [2].

2.3. Data analysis

The paternity index (PI) and a posteriori probability (W) were calculated as suggested by Evett and Weir [4]. The mutation value for paternal meioses was deduced from the report of American Association of Blood Bank (D18S51 = 0.3%; D8S1179 = 0.29%;

D3S1358 = 0.11%) [5]. The observed and estimated haplotype frequency for Y-chromosome was derived from the Y STR database, using directly a computer program available on the site http://ystr.charite.de/index_mkl.html [2]. The mean value of the posterior frequency distribution of minimal haplotypes was used as the most conservative estimate. These Y-STR haplotype frequencies were used to evaluate the evidence of compatibility using the likelihood ratio method.

3. Results and discussion

In each of the three paternity tests, we identified a genetic incompatibility between the alleged father and the child for one of the panel of the analysed STRs (Fig. 1). In particular, the loci in which we observed a single exclusion were D3S1358, D8S1179 and D18S51. The sequence analysis at these loci will clarify the exact conformation of each allele in the patterns of the alleged fathers and in the children. However, the evaluation of the results suggested that for D8S1179, the mutation is represented by the contraction of a single repeat in the child in comparison to the alleged father's sequence. Probably also in the mutation observed for D3S1358, a contraction event occurred. For D18S51, it was impossible to understand the mechanism of the mutation very well because of the haplotype of the mother and of the alleged father. These data were in agreement with previously reported data [6].

Table 1 reports the results obtained for the Y-STR analysis and the evaluation of the data. Taking into account the mutation rate for these STRs [5], the PI value for 12–14 analysed markers resulted in very low numbers, between 3.9 and 127, with *W* (50%) between 79.8% and 99.2%. In family 1, we also reported the results for a brother (child 2), to show the efficiency of the panel of autosomal STRs employed.

The Y-chromosome analysis of 10 loci showed a complete compatibility between the child and the alleged father, in each of the three families we studied. The mean value for the haplotype frequency, derived from the posterior frequency distribution, was calculated for the "minimal haplotype" (excluding DXY156Y and YCAII). The most probable value for the haplotype frequency resulting was between 1.3×10^{-5} and for 3.2×10^{-5}.

The occurrence of the extremely rare haplotype in the alleged fathers and in the children strongly supported the relationship of biological paternity. The PI cumulative value increases drastically to between 1,746,875 and 9,769,230, with *W* (50%) between 99.99994% and 99.999989%.

	FAMILY 1				FAMILY 2			FAMILY 3		
Locus	**Father**	**Mother**	**Child 1**	**Child 2**	**Father**	**Mother**	**Child**	**Father**	**Mother**	**Child**
D3S1358	14, 17	14, 16	14, 16	16, 17	14, 15	14, 17	14, 17	14, 19	16, 18	**16, 18**
D8S1179	10, 14	12, 16	14, 16	14, 16	14, 16	14, 15	**13, 14**	12, 13	12, 14	13, 14
D18S51	16, 18	17, 19	**17, 19**	16, 17	12, 20	16, 17	12, 16	15, 17	13, 17	15, 17

Fig. 1. Genotypes observed in the families at the mutated loci.

Table 1
Evaluation of the results obtained before and after Y-STR analysis

	Family 1			Family 2		Family 3	
Locus	Father	Child 1	Child 2	Father	Child	Father	Child
DYS385	11, 14	11, 14	11, 14	13, 15	13, 15	11, 14	11, 14
DYS389I	13	13	13	10	10	13	13
DYS389II	29	29	29	26	26	29	29
DYS390	24	24	24	22	22	25	25
DYS391	10	10	10	10	10	11	11
DYS392	11	11	11	12	12	13	13
DYS393	14	14	14	12	12	14	14
DYS19	16	16	16	15	15	16	16
YCA-II	3, 7	3, 7	3, 7	4, 5	4, 5	3, 7	3, 7
DXYS156Y	12	12	12	11	11	12	12
Mutation (frequency)		D18S51 (0.30%)	–		D8S1179 (0.26%)		D3S1358 (0.11%)
PI value		55.9	28844467		127		3.96
W (50%)		98.2	99.9999996		99.2		79.8
Mean f_{ext} for Y-STR		3.2×10^{-5}	3.2×10^{-5}		1.3×10^{-5}		2.8×10^{-5}
LR for Y-STR		31,250	31,250		76,923		35,714
PI value cumulative		1,746,875	9×10^{11}		9,769,230		141,427
W (50%) cumulative		99.99994	$\rightarrow 100$		99.99998		99.9992

We are able to confirm the utility of the Y-chromosome polymorphic markers in paternity testing with a male child and to underline the role of the Y-chromosome markers to indirectly establish the mutational event in the paternal meiosis.

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