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Analysis of paternity index of 164 paternity trios DNA-typed by either 10 STR or 4 RFLP loci

E. Raimondi *, U. Toscanini, E. Haas

PRICAI-Favaloro Foundation, Av. Belgrano 1782-1er Subsuelo, 1093, Buenos Aires, Argentina

Abstract

In the past few years, short tandem repeat (STR) typing has become the method of choice for many, if not most, laboratories to perform paternity testing. The aim of this study was to show that a set of carefully chosen and well-known STR loci may provide as reliable results as Restriction Fragment Length Polymorphism (RFLP) typing does. We analyzed the Paternity Index (PI) and Residual Paternity Index (RPI) obtained in 67 non-exclusion cases and 22 exclusion cases typed by 10 STR loci, and in 61 non-exclusions and 14 exclusions typed by four RFLP loci. PI was calculated for the trios and also for child and alleged father in motherless cases using local frequency tables and it was assigned to one out of six categories. The 10 STR locus analysis for paternity testing led to conclusive results for all trio cases. In paternity tests lacking a mother, more than 10 STRs should be analyzed to get similar results.

Keywords: STR; RFLP; Paternity Index; DNA typing

1. Introduction

In the past few years, short tandem repeat (STR) typing has become the method of choice for many, if not most, laboratories to perform paternity testing. We began with STR typing in 1996 (6 loci). Up to that time, Restriction Fragment Length Polymorphism (RFLP) and HLA typing had been the methodologies applied in our laboratory for paternity testing. In the past 4 years, there has been a gradual, though not complete, transition from RFLP to STR typing. Currently, we analyze most paternity cases by STR typing and we perform RFLP and HLA typing mainly in incomplete cases in which our 18-locus STR battery is not enough to reach conclusive results.

* Corresponding author. Tel.: +54-11-4384-5400; fax: +54-11-4383-1197.
E-mail address: eraimondi@ffavaloro.org (E. Raimondi).

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2. Materials and methods

We analyzed the Paternity Index (PI) and Residual Paternity Index (RPI) obtained in 67 non-exclusion and 22 exclusion cases typed by 10 STR loci (CSF1PO, TPOX, THO1, D12S1090, D3S1744, D18S849, FGA, D7S820, D1S533, D9S304), and in 61 non-exclusions and 14 exclusions cases typed by four RFLP loci (D12S11, D17S79, D4S163, D7S467). Cases were all true trios from the Buenos Aires metropolitan area. Ten cases in which only one locus showed a difference between child and alleged father (probably mutations) were excluded from this study.

DNA was extracted by a non-organic procedure from EDTA collected blood [1].

STR loci were amplified in multiplex polymerase chain reactions (PCR): one triplex reaction for CTT loci (CSF1PO, TPOX, THO1) [2]; one triplex reaction for Multiplex-I loci (D12S1090, D3S1744, D18S849) [3]; and one tetraplex reaction for Multiplex-II loci (FGA, D7S820, D1S533, D9S304) [3]. Amplified products were electrophoresed in 4% polyacrylamide gels and detection was carried out by silver staining. Alleles were assigned by directly comparing with the allelic ladders provided with each typing kit [2,3].

DNA for RFLP typing was *Pst*I-digested, run in 0.9% agarose gels and transferred to neutral nylon membranes. Membranes were hybridized with alkaline phosphatase-labeled probes and LumiPhos 480™ was used as enzyme substrate. Autoradiograph images were analyzed using a digitizing tablet and the LIFEPRINT™ Sizing Software (Lifecodes).

PI was calculated for trios (mother, child, and alleged father) and duos (child and alleged father) using an Excel-based program for STR typed trios, and the LIFEPRINT™ Analysis Software (Lifecodes) for RFLP typed cases. PI was also calculated for STR-typed trios considering either six loci (CTT plus Multiplex-I) or seven loci (CTT plus Multiplex-II). RPI was calculated for all exclusion cases. Calculated PI were assigned one out of six categories (Table 1).

3. Results

Results are detailed in Tables 1 and 2. The highest value for PI was 53,655,218 for STRs and 578,015 for RFLPs. The highest PIs for duos were 341,876 and 36,125 for STRs and RFLPs, respectively.

Table 1
Number of trios and duos in each PI category for either 10 STR and 4 RFLP loci

PI category	Trio cases		Duo cases	
	10 STR loci typed	4 RFLP loci typed	10 STR loci typed	4 RFLP loci typed
<1000	0 (0%)	1 (1.7%)	15 (22.4%)	34 (58.6%)
1000–10,000	1 (1.5%)	26 (44.8%)	31 (46.3%)	22 (37.9%)
10,000–100,000	21 (31.3%)	26 (44.8%)	17 (25.4%)	2 (3.4%)
100,000–1 million	29 (43.3%)	5 (8.6%)	4 (6%)	0 (0%)
1 million–10 million	12 (17.9%)	0 (0%)	0 (0%)	0 (0%)
>10 million	4 (6%)	0 (0%)	0 (0%)	0 (0%)

Table 2
Number of trios in each PI category using either six or seven STR loci

PI category	6 STRs used for PI calculation	7 STRs used for PI calculation
< 1000	36 (53.7%)	17 (25.4%)
1000–10,000	21 (31.3%)	30 (44.8%)
10,000–100,000	10 (14.9%)	18 (26.9%)
100,000–1 million	0 (0%)	2 (3.0%)
1 million–10 million	0 (0%)	0 (0%)
>10 million	0 (0%)	0 (0%)

Exclusion cases always had RPIs < 1000 for both trios or duos using either STR or RFLP typing except for one RFLP case which yielded a RPI of 3676. The number of loci that excluded the alleged father was between 3 and 8 out of the 10 STR loci and between 1 and 4 for RFLP loci (cases with only one exclusion were confirmed with more probes).

4. Conclusion

Analyzing these 10 STR loci for paternity testing may lead to conclusive results for trio cases, usually yielding higher PIs than those expected with four RFLP probes and a high probability of excluding non-biological fathers. In cases where the mother cannot be typed, more than 10 STRs should be analyzed to get similar results.

References

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