International Congress Series 1239 (2003) 943–946

Use of STRs in paternity testing in the Flemish population

G. Mertens [a,b,*], N. Mommers [a], H. Heylen [a], L. Boutrand [c], A. Vandenberghe [a,c], Z.N. Berneman [b]

[a] *Antwerp Blood Transfusion Centre, Edegem, Belgium*
[b] *Faculty of Medicine, Antwerp University, Antwerp, Belgium*
[c] *Faculty of Pharmacy, University Claude Bernard Lyon I, Lyon, France*

Abstract

A review is presented of 6 years of paternity testing using almost only short tandem repeats (STRs). In 1995, we replaced RFLP hybridisation analysis by PCR of STRs and silver staining. Initially, nine STRs amplified in three home-made triplex reactions were used, yielding a combined power of exclusion (PEX) of 99.66%. This has evolved to a first line of nine STRs, including two commercial and one home-made triplex, supplemented by five more STRs as well as HLA A-B-DR, resulting in a PEX of 99.9998%. The requirement to exclude paternity was eventually sharpened from two to three Mendelian inconsistencies. Among the 951 cases examined, we observed 11 mutations in seven STRs, the overall mutation rate across all tested loci being 8.3 per 1000 meioses. In the fall of 2001, we will acquire an ABI 310 analyzer and start typing of 16 STRs in one multiplex. The silver staining system will be kept in reserve but HLA typing will be abolished, so that we will truly rely on ''STRs only''.

Keywords: Paternity testing; Short tandem repeat (STR)

1. Introduction

Paternity testing exclusively based on DNA polymorphisms has been practised in Antwerp since 1987. At that time, RFLP hybridisation analysis using one multi-locus and three single locus probes was performed. In 1995, paternity testing moved from the University to the Blood Transfusion Centre. On that occasion, we decided to drop

* Corresponding author.

0531-5131/03
PII: S0531-5131(02)00560-5

detection with radiolabeled probes and switch to PCR amplification of STRs and silver staining after electrophoresis in a denaturing polyacrylamide gel. Here, we describe our experience of the subsequent 6 years.

2. Evolution

Table 1 summarises the different combinations of STRs that were applied in paternity casework and their statistics. The first set of STRs we used were the three triplexes validated for paternity testing by Alford et al. [1]. In the Flemish population, the combined power of exclusion (PEX) of that system is 99.66%. Furthermore, the X-located HPRTB can be informative only when the child is a girl. We thus replaced the Alford triplex with HPRTB by the Multiplex I (D12S1090–D3S1744–D18S849) from Lifecodes. From 1997 onwards, each individual was typed for nine "first line" STRs (see Table 1). If two or more Mendelian inconsistencies were observed, paternity was excluded and no more testing was carried out. Otherwise, the Alford triplex with HPRTB was added. Due to the D12S1090 locus in particular (30 alleles, heterozygosity 93%, PEX 78%), the combined PEX could be raised to 99.987%. The condition to exclude paternity was eventually sharpened to require three loci. The observed median number of excluding loci per case of excluded paternity rose from four out of nine first line STRs with the Alford system, to five from 1997 onwards. In the middle of 2000, Lifecodes discontinued the distribution of Multiplex I, which we exchanged for another commercial STR-triplex for silver staining

Table 1
Statistical review

Period	Markers	PEX %	# Paternity cases	% Exclusions	Median # excluding markers in case of non-paternity
05/95–10/97	**CSF1PO–TH01–PLA2A1** (H), **F13A01–CYAR04–LIPOL** (H), **HPRTB–FABP–CD4** (H)	99.66	329	62.0	4
10/97–07/00	**D12S1090–D3S1744–D18S849** (L), **CSF1PO–TH01–PLA2A1** (H), **F13A01–CYAR04–LIPOL** (H), *HPRTB–FABP–CD4* (H)	99.97	421	62.2	5
07/00–12/00	**D16S539–D7S820–D13S317** (P), **CSF1PO–TH01–PLA2A1** (H), F13A01–CYAR04–LIPOL (H), *HPRTB–FABP–CD4* (H) + HLA DR (I)	99.996	73	52.1	5
12/00–07/01	**D16S539–D7S820–D13S317** (P), **F13A01–FESFPS–vWA** (P), **CSF1PO–TH01–PLA2A1** (H), *F13A01–CYAR04–LIPOL* (H), HPRTB–FABP–CD4 (H) +HLA A-B-DR (I)	99.9998	128	57.8	5

PEX = combined power of exclusion; (H) = home made; (L) = Lifecodes; (P) = Promega; (I) = Innogenetics BOLD = first line STRs; italics = second line STRs; underscored = third line STRs; + = supplementary markers.

(D16S539–D7S820–D13S317). To compensate for the loss of the extremely "powerful" D12S1090 locus, we introduced as a "third line" HLA DR typing by PCR and a line probe assay (Innogenetics). This yielded an overall PEX of 99.996%. In 2001, another triplex from Promega was inserted (F13A01–FESFPS–vWA) in the first line, driving the F13A01–CYAR04–LIPOL triplex to the second, and the triplex with HPRTB to the third line. Purchasing a ready-to-use kit from a reliable producer offers several advantages, including the availability of a comprehensive, sequenced allelic ladder. The overlapping of two triplexes, a commercial one and a home-made one, for F13A01, is not necessarily redundant or wasteful. On the contrary, it can be considered an internal control. As a final supplement, full HLA A-B-DR typing was added. For inclusion calculation purposes, HLA A-B-DR haplotype frequencies can be consulted of each country having a registry of candidate bone marrow donors [2]. The whole database actually includes more than 7 million typed individuals! We now have arrived at a combined PEX of 99.9998%.

The overall exclusion rate amounts to 61%. This relatively elevated figure can be explained by Belgian legislation. Indeed, if a child is born less than 200 days after a divorce has been officially decreed, the ex-husband remains the "legal" father, even when both divorcing parties completely agree that not the ex-husband but the ex-wife's new partner is the biological father. Only a "DNA test" is accepted as legal proof of the ex-husband's non-paternity.

Over a period of 6 years, the number of requests rose by 38%. An increasing demand for paternity tests is clearly observed in other European countries and the USA. The area our laboratory serves has a population of about 3 million. For the year 2000, the relative number of test cases amounted to 60 per million inhabitants. A similar case rate is seen in the neighbouring country of the Netherlands (40 per million), while the testing volume is definitely larger in the USA (1000 per million).

3. Unexpected observations

Doing paternity casework, we observed 11 mutations, the key data of which can be found in Table 2. This information is regularly forwarded to the ISFG by means of the questionnaire of the annual Paternity Testing Interlaboratory Comparison. Taking into account all 951 paternity tests performed, the cumulative mutation rate across all loci examined becomes 8.3 per 1000 meioses. All mutations were characterised at the molecular level by cloning and sequencing. The overall paternal:maternal mutation ratio

Table 2
Mutations

STR	#/1000 meioses	Paternal:Maternal	Structure
FESFPS	5.5	1 P	1 extension
TH01	0.75	1 P	1 extension
D13S317	3.5	1 P	1 extension
D3S1744	3.5	1 P:1 M	2 extension
CSF1PO	1.5	2 P	1 extension:1 retraction
D12S1090	5.2	1 P:2 M	1 extension:2 retraction

was 8:3, while the ratio of repeat gain:repeat loss was also 8:3. In all of the 11 mutations, structural analysis revealed a change of one full repeat. As expected, the most heterozygous and complex locus (D12S1090) showed the highest mutation rate, most mutations occurring in the longer stretch of repeats. An apparent mutation of CD4 was caused by drop-out of a paternal allele with a rare sequence variant in the penultimate position of the reverse primer.

While sequencing primers and alleles of the Multiplex I kit from Lifecodes, we discovered that for locus D18S849 the actual primer sequence completely differed from the description accessible on the National Center for Biotechnology Information [3]. Shortly thereafter, Multiplex I was no longer available.

4. Conclusion and future perspectives

Our 6 years of experience illustrates the evolution of paternity testing by typing of STRs. While a first line of nine STRs is still sufficient for exclusion in the majority of cases, the total number of STR markers we use has grown to 14; the power of exclusion from 99.96% to 99.9998%. In parallel, three instead of previously two excluding loci are required to prove non-paternity. In the first line, two commercial triplexes have replaced home-made ones. The Lifecodes story however shows the need for in-house validation, also of commercial kits.

In the third quarter of 2001, we will acquire an ABI 310 Genetic Analyzer replacing the traditional system of manual pouring of gels and visual—by two independent persons—reading of the silver stained pattern. While we have decided to use one megaplex PCR, coamplifying 16 STRs, the choice between both systems on the market still has to be made. We will keep the STRs of the traditional system described above as supplementary markers. This will permit HLA typing to be abolished, leading to a truly ‘‘STR only’’ strategy for paternity testing.

References

[1] R.L. Alford, H.A. Hammond, I. Coto, C.T. Caskey, Rapid and efficient resolution of parentage by amplification of short tandem repeats, Am. J. Hum. Genet. 55 (1994) 190–195.
[2] Available at: http://www.bmdw.org/.
[3] Available at: http://www.ncbi.nlm.nih.gov/.