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ORIGINAL ARTICLE

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The evidential value of STRs

An analysis of exclusion cases

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Abstract In this study, a total of 191 cases with STR exclusions out of 591 paternity cases were analysed using 2 STR sets, i.e. (set a) 5 STRs in 462 cases with 150 exclusions and (set b) 9 STRs in 129 cases with 41 exclusions. Set (a) was associated with four exclusions on average while set (b) showed five exclusionary loci on average. Double exclusions were observed in 18 cases and further elaborated. Of these, 2 ended up with probabilities of paternity of 0.1% and 0.4%, respectively and with a random occurrence of the hypothesis "mutation" of 1:20,000 and 1:50,000, respectively, while all other cases were associated with much lower frequencies. The conclusion is that the evidential value of a set of highly polymorphic STRs applied in paternity cases is usually extremely high.

Key words Short tandem repeats · STR · Paternity testing · Exclusion cases

Introduction

Short tandem repeat (STR) systems are highly polymorphic, the allele distribution is discrete and the length measurement is achieved with standard deviations below ± 0.5 bp (Tagliabracci et al. 1999). The precision of the allele definition does not seem to know a comparable level under previous genetic marker systems. Deletions or silent alleles seem to be rare (Gusmão et al. 1996; Watanabe et al. 1998) and deviations from the Hardy-Weinberg equilibrium have not been observed in an abundance of related studies (e.g. Hammond et al. 1994; Lareu et al. 1996; Möller et al. 1994; Wiegand et al. 1999). When applied in parentage testing, the efficiency is usually expressed by the general exclusion chance (GEC). If a series of such systems is applied in combination, this value usually reaches or exceeds 99.99%, meaning that on average only 1 out of 10,000 non-fathers would remain unexcluded. The only disadvantage of this system category lies in the occurrence of genetic mutations which appear to be more frequent than in classical marker systems. The risk of finding a pseudo-exclusion therefore needs to be properly addressed. In this study we have further examined routine paternity cases where the alleged father was excluded by STRs.

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Materials and methods

Out of a total of 591 paternity cases, 191 were examined where the alleged father had been excluded by STR analyses. According to the systems tested, the following subdivision was performed:

– A set of 7 classical markers plus 5 STRs: 150 out of 462 cases
– A set of 6 classical markers plus 9 STRs: 41 out of 129 cases

Analytical procedure

The testing for paternity was carried out using the following system categories:

1. Classical systems: AB0, MNSs, Gc subtypes, Pi subtypes, EAP, PGM (Evett et al. 1996)
2. Set (a): 5 STRs FGA (Barber et al. 1996; Rolf et al. 1998), VWA (Möller et al. 1994), TH01 (Edwards et al. 1992), D12S391 (Lareu et al. 1996), ACTBP2 (Rolf et al. 1997) with a combined general exclusion chance of 99.86%.
3. Set (b): 9 STRs (AmpF*l* STR Profiler PCR Amplification Kit, PE Applied Biosystems) with a combined general exclusion chance of 99.95%.

The calculated probability of observing only a single exclusion in a case of non-paternity is 2.11% for set (a) and 0.65% for set (b). The first value is comparable to the value that Thomson et al. (1999) reported for the SGM (6 STR loci; 2.08%), while the Profiler loci were even more efficient than the PowerPlex 1 systems (8 loci; 1.87%).

Results and discussion

Set (a) with the application of 5 STRs was associated with 3.7 exclusionary loci on average. In 10% (15 cases) there

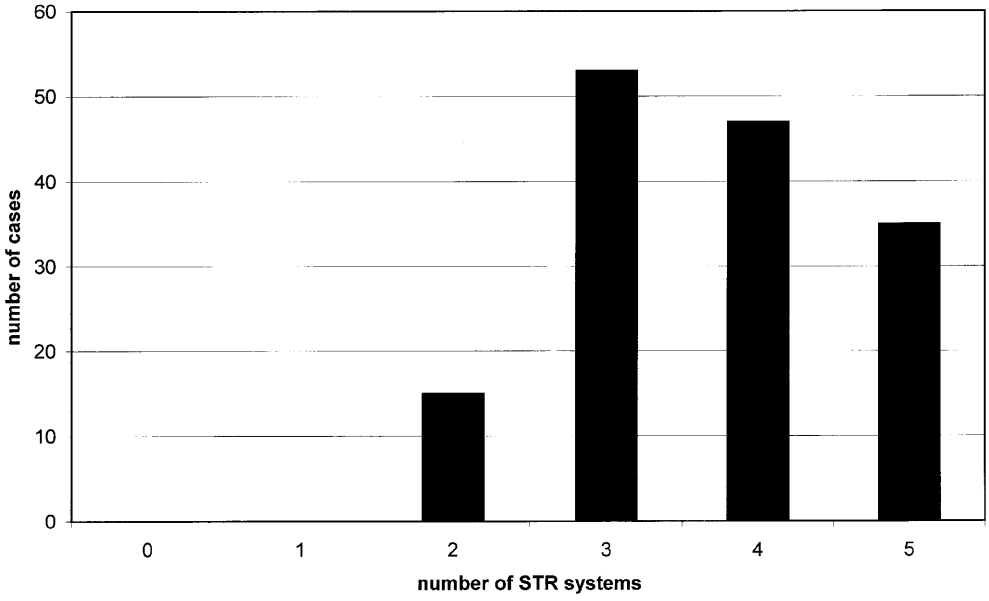

Fig. 1 The distribution of the number of exclusionary DNA loci after including five STR systems in routine paternity testing (n = 150)

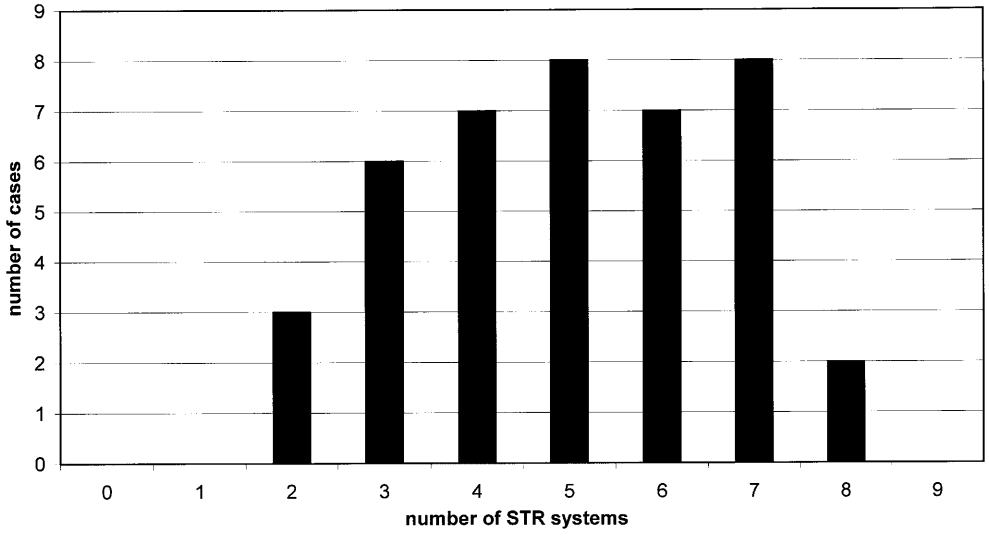

Fig. 2 The distribution of the number of exclusionary DNA loci after including nine STR systems in routine paternity testing (n = 41)

occurred double exclusions only (Fig. 1). Set b was associated with 5.2 exclusionary STR loci on average and in 3 cases (7%) there existed double exclusions only (Fig. 2).

Cases with double exclusions were further elaborated (Table 1), most of them were associated with ≥ 2-step differences and 3 double exclusions remained one-step differences at both loci involved (Table 1).

Assuming a mutational event, the rate for each individual step was estimated from published data (e.g. Brinkmann et al. 1998). Systems not included in this publication were given estimates as derived from analogies in structure and repeat numbers. The male mutation rate was elaborated from a sex ratio of 5:1 (m:f), 2-step mutation rates were estimated from 1-step rates using a frequency ratio of 20:1 (1-step:2-step; Brinkmann et al. 1998). Although 3-step mutations have not yet been observed, one contribution made by Sajantila et al. (1999) is under critical consideration since TH01 has an extremely low rate even for 1-step mutations (no mutation in more than 6,000 meioses investigated as yet, B. Brinkmann, unpublished observation), they were estimated from two-step mutation rates using a further ratio of 20:1.

During a complete genome scan of 337 nuclear families with a total of 287,786 parent-offspring allele transfers, Xu et al. (2000) identified 236 mutations at 122 tetranucleotide STR loci and found that the rate of contraction mutations increased exponentially with allele size, whereas the rate of expansion mutations was constant across the entire allele distribution. One-step mutations were reported to occur 10 times more frequently than 2-step mutations and the paternal mutation rate was more than 3 times higher than the maternal mutation rate. Since the identity of the loci investigated was not given in that publication we used the frequencies that were estimated for forensically relevant loci for the following calculations:

Table 1 Statistical evaluation of double exclusions (n = 18) (Minimum allele differences between alleged father and child are indicated by an arrow, N_{all} number of all systems applied, N_{STR} number of STR systems applied, *W-value in % under assumption of mutation)

	N_{all}/ N_{STR}	Exclusions in classical systems	FGA	VWA	TH01	D12S391	ACTBP2	TPOX	CSF1PO	D3S1358	D5S818	D13S317	W-value (%)*	Combined frequency of double mutation
1	12/5	3	22→21	–	–	17→18	–	–	–	–	–	–	3.93×10^{-1}	5×10^{-4}
2	12/5	2	22→26	–	–	–	18→16	–	–	–	–	–	2.99×10^{-7}	1×10^{-10}
3	12/5	2	24→25	–	–	–	27.2→24.2	–	–	–	–	–	9.42×10^{-4}	5×10^{-6}
4	12/5	3	–	–	9.3→9	–	27.2→20	–	–	–	–	–	3.88×10^{-4}	1×10^{-7}
5	12/5	1	22→27	–	–	–	32.2→29.2	–	–	–	–	–	6.35×10^{-10}	1×10^{-10}
6	12/5	2	–	–	7→8	–	27.2→12	–	–	–	–	–	3.90×10^{-21}	–
7	12/5	1	–	17→16	–	18→20	–	–	–	–	–	–	1.26×10^{-3}	–
8	12/5	3	–	15→17	–	–	24.2→26.2	–	–	–	–	–	1.52×10^{-4}	–
9	12/5	3	23→24	–	7→9.3	–	–	–	–	–	–	–	3.87×10^{-5}	–
10	12/5	4	–	15→16	–	–	22.2→16	–	–	–	–	–	3.52×10^{-8}	–
11	12/5	3	–	17→18	–	–	29.2→30.2	–	–	–	–	–	1.26×10^{-1}	2×10^{-4}
12	12/5	2	–	–	–	18→19	28.2→30.2	–	–	–	–	–	4.35×10^{-3}	5×10^{-5}
13	12/5	2	19→20	–	–	21→16	–	–	–	–	–	–	3.65×10^{-7}	–
14	12/5	1	–	–	–	21→17	20.2→24.2	–	–	–	–	–	1.33×10^{-6}	–
15	12/5	2	–	–	8→6	19.3→22	–	–	–	–	–	–	1.98×10^{-6}	–
16	15/9	2	–	–	–	–	–	–	12→11	15→14	–	–	5.96×10^{-5}	–
17	15/9	0	–	16→19	–	–	–	–	–	–	–	11→12	1.26×10^{-6}	–
18	15/9	0	–	–	–	–	–	8→10	–	–	12→13	–	7.91×10^{-6}	–

1. Calculation of the probability of paternity assuming mutation (Fimmers et al. 1992; see Table 1).
2. Calculation of the frequency of two such occurrences (mutations) in one germ cell (Table 1). For both calculations it was assumed that the mutations had occurred independently.

The highest probability values obtained in our material were 0.1 and 0.4%, respectively (Table 1). These two double mutations would occur with frequencies of 2×10^{-4} and 5×10^{-4}, respectively. The figures obtained in the remaining cases were several orders of magnitude lower (Table 1).

The evidential value of the STR "exclusions" in our cases is further emphasised by the fact that further exclusions were observed in classical systems (Table 1). Biostatistically, classical systems were not considered in this study because the study aimed to simulate a situation with the exclusive usage of STRs.

In more than 100 cases with a single mismatch (B. Brinkmann, unpublished observations), the final probability value (after inclusion of the mutation) was always higher than 99.99% and from this data we concluded on mutation. The mutations observed were always 1-step mutations and, very occasionally, 2-step mutations.

Conclusion

1. Once double mismatches have been reached after applying a reasonable set of STRs, they must be further elaborated. Only if they are associated with hitherto unpublished fragment lengths and structures and if two such occurrences exist in combination, then further calculation does not seem to make sense.
2. It must be critically emphasised that calculations are based on rather small numbers of observations (Brinkmann et al. 1998). In a given system there often exist expressed differences in the mutation rates between small alleles and long alleles (Rolf and Brinkmann 1999). This must be considered if statistical variations are to be explored.
3. In earlier reports it has been stressed that DNA polymorphisms should be used only in combination with conventional markers (Ritter 1991). In the meantime hundreds of studies have explored the formal genetics and the structure and rate of irregularities. The formal genetics has been fully confirmed. The mutation behaviour of the STR systems has been extensively studied (Chakraborty and Stivers 1996; Brinkmann et al. 1998). Further irregularities such as deletions seem to be extremely rare (Watanabe et al. 1998). Therefore, this category of genetic marker systems is associated with a high level of evidential value and there no longer seems to exist any reasonable doubt why this system category should not be applied in isolation. This is especially so because of the high efficiency and more than five exclusions are on average associated with a reasonable set of marker systems with most of these residing on different chromosomes.
4. In very rare cases "double exclusions" will occur which are due to mutation but these can be identified if the structural and statistical considerations are applied as shown in our case work. Gunn et al. (1997) reported a case of an apparent double mutation in two independent STR systems, which are both very likely to be one-step mutations. In contrast to our approach these authors did not include the two mutations in the calculation of the paternity index, but calculated the combined paternity index for all remaining single-locus results which yielded, in their opinion, sufficient evidence in favour of paternity (36,000,000:1).

We also await the first case with only one exclusion in the first phase of STR application which will be confirmed by applying further STR loci. It is well known that exclusions based on a very small number of loci could be indicative of a close relative being the true father. Nevertheless, the evidential value of exclusions should not be based on usage of rigid numbers of exclusionary loci. It must be based on a statistical evaluation which has to consider the mutation rate at the given locus (or even of the particular allele) also considering the repeat structure and the number of mutational steps, etc. In doubtful cases, the number of STR loci investigated can easily be increased to reach the evidential value needed.

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Note added in proof The authors have just become aware of a recent publication by Calafell (2000), in which the author theoretically addresses the problem of STR exclusions also in paternity testing.

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