Int J Legal Med (2002) 116:255–257
DOI 10.1007/s00414-001-0280-9

ORIGINAL ARTICLE

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The identification of war victims by reverse paternity is associated with significant risks of false inclusion

Received: 8 August 2001 / Accepted: 23 November 2001 / Published online: 12 June 2002

Abstract Since February 2001 the process of DNA identification of war victims in Croatia relies on the database of over 3,000 9-locus (D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317 and D7S820) STR genotypes of relatives of missing persons. Instead of a targeted approach to DNA typing, the genotype of each skeletal remains analysed is compared to all genotypes in the database to identify potential parents and children. Although this approach has significantly increased the pace of identification by DNA typing, non-targeted matching in a database containing several thousand genotypes considerably decreases the significance of inclusion, especially when identification is based on reverse paternity analysis. To support this statistical prediction we present 3 cases of 10 STR loci matches and 1 case of 11 STR loci matches between a child, child's mother and skeletal remains that did not originate from a father of that child.

Keywords STR sequences · Amplified fragment length polymorphism · DNA typing · Human identity · Paternity

Introduction

The process of human identification by DNA typing in Croatia dates back to 1994 when no reliable methods were available. The development of STR profiling kits was a great boost and promoted DNA typing to a significant segment of the overall process of identification of war victims. However, due to limited laboratory capacity, until recently DNA profiling was used only in selected targeted cases to confirm or reject the proposed identity (Primorac et al. 1996). In 2000 the capacity of three Croatian DNA laboratories was significantly increased and a nearly complete database of genotypes from relatives of missing persons was compiled. Due to the need to remain compatible with genotypes determined during previous years, the database still relies on the 9 loci covered by the AmpFlSTR Profiler kit (D3S1358, vWA, FGA, TH01, TPOX, CSF1PO, D5S818, D13S317 and D7S820) and as of July 2001 contains over 3,000 individual STR genotypes.

Hundreds of skeletal remains have been successfully identified in Croatia using this system, but with the increasing number of relatives in the database, there is an increased risk of false positive identification, especially when identification is based on reverse paternity. Here we present 4 examples where matches in 10 or 11 STR loci were observed between a child and a spouse of a missing person and skeletal remains that did not originate from that missing person.

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Materials and methods

Powdered samples of skeletal remains (tooth or bone) were decalcified for 48 h at 56°C in 50 mM Tris/HCl buffer pH 8.0 containing 50 mM EDTA, 100 mM NaCl and 0.7 mg/ml proteinase K. DNA was isolated by extraction with phenol/chloroform/isoamyl alcohol (25:24:1), followed by *n*-butanol extraction. Isolated DNA was concentrated and further purified by ultrafiltration on Centricon-100 concentrators (Millipore, Bedford, Mass.). Blood samples from relatives of missing persons were collected on FTA cards (Whatman Bioscience, Cambridge, UK) and DNA was purified by Chelex extraction (Walsh et al. 1991). Purified DNA was amplified using the AmpFlSTR Profiler and AmpFlSTR Profiler Plus kits (Applied Biosystems, Foster City, Calif.) according to the manufacturer's instructions. The probability of parenthood was calculated as described earlier (Primorac and Schanfield 2000) using local population data.

Case examples

Case „Borovo“

Skeletal remains X were exhumed in the Croatian village of Borovo near the Serbian border. STR genotypes from a bone sample were compared to all genotypes in the database and 69 potential parents or children that matched in all 9 STR loci analysed were identified. After individually comparing the genotypes of the skeletal remains X with genotypes of other relatives for each possible parent or child we were able to identify one that was a potential child of the skeletal remains X (son of the missing person A). There was a full nine-locus match between the genotypes of the spouse of the missing person A, the son of the missing person A and the skeletal remains X (Table 1). The calculated probability of parenthood was 99.94% (LR=1,368). The AmpFlSTR Profiler Plus kit was used to obtain genotypes on the additional three STR loci, D8S1179, D21S11, and D18S51. One of the loci was consistent with the hypothesis that the skeletal remains X belonged to the missing person A (cumulative LR=4,699), but the other two loci excluded skeletal remains X as a father of the son of missing person A (Table 1).

Case “Dalj”

Skeletal remains Y were exhumed in the Croatian village Dalj near the Serbian border. By comparing the nine-locus STR genotype to all genotypes in the database, 89 potential parents or children were identified but comparison with other relatives eliminated all but two of them (daughter of missing person B, and son of missing person C). The calculated probabilities of parenthood were 99.83% (LR=579) and 99.54% (LR=221) for missing persons B and C, respectively. Additional loci included in the AmpFlSTR Profiler Plus kit excluded missing person B in one and missing person C in two loci. When calculated for the matching 11 loci the probability of parenthood for the missing person B was relatively high (LR=26,658), but the exclusion at the D21S11 locus was further supported by disparities in age (estimated age of skeletal remains) and the location of disappearance between missing person C and skeletal remains Y. Subsequently skeletal remains Y were identified as another missing person whose age (24 years old) practically excluded him as a possible father of the daughter of missing person C who was 9 years old.

Case “Nijemci”

Skeletal remains Z were exhumed in the Croatian village Nijemci. The nine-locus STR genotype determined matched with 30 potential parents or children in the database. After comparing the genotype of the skeletal remains Z with other relatives for the corresponding missing persons, we excluded all but one potential child of skeletal remains Z (son of missing person D). There was a full nine-locus match between genotypes of missing person Z, spouse and a child of missing person D (Table 1). The calculated probability of parenthood was 99.90% (LR=960). We used the AmpFlSTR Profiler Plus kit to obtain genotypes on three additional STR loci (Table 1) and of these, one (D18S51) was in accordance with the proposed identity, while the other two showed exclusions.

Results and discussion

The increase in the number of analysed loci has significantly increased the evidential value of STR typing and this system is now generally considered sufficient for the determination of paternity (Calafell 2000; Brinkmann et

Table 1 STR genotypes of the skeletal remains „X“, „Y“, and „Z“, and relatives of missing persons „A“, „B“, „C“, and „D“. In all cases there was a full match in all nine loci covered by the AmpFlSTR Profiler kit. The AmpFlSTR Profiler plus kit was used to obtain information for three additional STR loci

Case examples	D3S1358		vWA		FGA		Amelogenin		TH01		TPOX		CSF1PO		D5S818		D13S317		D7S820		D8S1179		D21S11		D18S51	
Case 1																										
Remains X	14	17	15	17	21	23	X	Y	6	9.3	8	8	10	12	11	12	12	13	9	12	*13*	*15*	*31*	*33*	14	14
Child A	14	17	15	17	23	23	X	Y	8	9.3	8	8	11	12	11	13	11	12	9	11	*12*	*13*	*28*	*28*	14	16
Spouse A	17	17	17	17	21	23	X	X	6	8	8	8	11	12	12	13	11	13	10	11	*13*	*14*	*28*	*29*	14	16
Case 2																										
Remains Y	15	18	17	18	20	22	X	Y	7	9	8	11	11	12	11	12	12	12	10	11	11	14	*28*	*30*	15	15
Child B	15	15	17	18	22	22	X	X	7	9	8	8	11	12	10	11	8	12	10	10	11	12	*29*	*32*	14	15
Spouse B	15	16	14	17	20	22	X	X	7	9.3	8	10	11	13	10	11	8	8	9	10	12	12	*30*	*32*	14	16
Remains Y	15	18	17	18	20	22	X	Y	7	9	8	11	11	12	11	12	12	12	10	11	*11*	*14*	*28*	*30*	15	15
Child C	15	16	16	18	20	23	X	Y	9	9.3	8	8	10	12	11	12	12	12	8	10	*12*	*14*	*31*	*31*	15	16
Spouse C	16	16	16	16	22	23	X	X	9	9.3	8	8	10	10	12	12	9	12	8	10	*14*	*14*	*31*	*31*	15	16
Case 3																										
Remains Z	16	16	16	18	23	24	X	Y	6	9	11	11	11	12	12	13	12	12	9	10	*12*	*13*	*27*	*31*	17	19
Child D	15	16	16	18	23	23	X	Y	9	9	8	11	10	11	11	12	11	12	10	10	*14*	*14*	*28*	*32*	16	17
Spouse D	15	15	16	18	22	23	X	X	8	9	8	11	10	11	11	11	11	13	10	11	*13*	*14*	*32*	*32*	16	17

Loci that led to exclusion of proposed identity are printed in italics

al. 2001). In addition to resolving fatherhood issues, paternity determination is frequently used for human identification. However, in the case of mass disasters when thousands of people are missing, the number of potential fathers in the database needs to be included in the calculation to correctly interpret the significance of the paternity index determined.

Here we presented 3 cases of a 10-STR-locus match and one case of an 11-STR-locus match between a child and a person who was not a father of that child. In all 4 cases the paternity index calculated for the matching loci was quite high (up to 26,000), but the fact that the matching genotype was found by a random search in a database of over 1,000 potential fathers, considerably decreased the significance. Using the Croatian population data we calculated the mean probability of paternity exclusion in trio (mother/child/alleged father) cases (Evett and Weir 1998; Lee et al. 2000) to be 99.96% for the set of 9 STR loci covered by AmpFlSTR Profiler kit and 99.9986% for the combined AmpFlSTR Profiler and Profiler Plus kits. When this probability is applied to a pool of over 1,000 genotypes of potential fathers in the database, even the combined AmpFlSTR Profiler and Profiler Plus kits are expected to result in false inclusion in more than 1% of cases. This is of course only an average estimate that can vary by more than one order of magnitude depending on the individual genotypes.

Conclusions

As predicted by statistical genetics and demonstrated by the cases presented, the application of reverse paternity analysis in large databases is associated with a significant risk of false inclusion. If the genotypes of parents of the missing person are not available to support the identification, multiple children should be analysed whenever possible. In addition, other evidence based on the information about time, place and other conditions of disappearance, as well as anthropological and other "classical" forensic data should always be compiled and compared before any final conclusion is made. The introduction of novel procedures that cover more STR loci will decrease the risk of false inclusion, but in situations where old databases of 9 or 12-locus genotypes already exist this might require locating and/or resampling of old biological material.

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