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TECHNICAL NOTES

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Evaluation of the Reliability of DNA Typing in the Process of Identification of War Victims in Croatia

ABSTRACT: Aiming to estimate frequency of various types of errors that can occur in large scale process of identification, we identified and compared genotypes of 911 parent–child pairs in the database of 3498 relatives of people that disappeared during 1991/1992 war in Croatia. Genotypes of 891 pairs (97.8%) were matching, while 20 pairs did not match in one or more loci. Reanalysis of these samples revealed that out of 1822 analyzed genotypes, one genotype was completely wrong, and two genotypes had one wrong allele because of human errors. Five genotypes had a single wrong allele due to either PCR or electrophoresis errors. In five genotypes mutations were the cause of mismatch. Genetic inconsistencies with parentage were found in four ''fathers'' (4.2%) and three ''mothers'' (0.36%). As the majority of observed single-locus errors were caused by nonhuman errors, all databases produced with similar technology would probably have comparable level of errors.

KEYWORDS: forensic science, DNA typing, human identification, quality control, databasing, paternity

DNA typing is a powerful tool for forensic analysis and it is being routinely used in casework, paternity analysis and the identification of victims of mass fatality events (1–3). At least 15 short tandem repeat (STR) loci are being analyzed in nearly all laboratories, and this system is now generally considered sufficient to determine identity or paternity with very high probability of inclusion or exclusion (4–6). However, calculated probabilities reflect only biostatistical relationships between different genotypes, and other factors that might affect the results are rarely evaluated. In a simple paternity or identity cases the situation is very straightforward and all errors (sample replacement, allele dropout, false reading, typing errors, etc.) can be easily identified and resolved by repeated analysis of samples, but in a large identification projects that involve thousands of samples this is not the case (7–9).

Nearly 3500 relatives of missing persons were analyzed during the process of identification of war victims in Croatia, leading to the identification of over 1000 skeletal remains. Aiming to evaluate reliability of the whole identification process, we utilized the fact that individuals with known biological relationships (pairs mother–child, or father–child) exist within the pool of relatives to determine frequency of various types of errors that may have occurred during the process of sample collection, DNA analysis and databasing.

Materials and Methods

Blood taken from 3498 family members of missing persons was collected on FTA® cards (Whatman Bioscience, Cambridge, UK), including 1822 samples of 911 parent–child pairs that reported missing relatives and came to give blood sample. All samples were coded and FTA® cards were stored at room temperature. DNA was obtained by Chelex® 100 (Bio-Rad Laboratories, CA) extraction (10). PCR amplification was performed in the period of 1998–2007 using AmpFlSTR Profiler, AmpFlSTR Profiler Plus and AmpFlSTR Identifiler kit (Applied Biosystems, Foster City, CA), according to the manufacturer's instructions. All samples that were initially analyzed by AmpFlSTR Profiler and AmpFlSTR Profiler Plus kits were subsequently reanalyzed using AmpFlSTR Identifiler kit (covering D8S1179, D21S11, D7S820, CSF1PO, D3S1358, TH01, D13S317, D16S539, D2S138, D19S433, vWA, TPOX, D18S51, D5S818, and FGA loci). For 40 reanalyzed samples AmpFlSTR Identifiler kit was used. Samples were loaded on the ABI PRISM® 310 Genetic Analyzer with injection time of 5 sec at 15 kV. Data were analyzed with ABI PRISM Genotyper 2.5 software, or by manual comparison to allelic ladder (automated allele calling gradually replaced manual reading between 2002 and 2004). Profiles were hand entered into database by two independent analysts.

Results and Discussion

At the moment when this analysis was performed Croatian database of relatives of missing people contained 1822 individual genotypes of 911 parent–child pairs, 815 of them were mother–child pairs and 96 were father–child pairs. Comparison of these pairs revealed that genotypes of 891 pairs (97.8%) were fully matching, while 20 pairs did not match in one or more of the analyzed loci. Aiming to identify causes for these deviations from expected results, we reanalyzed DNA in these 40 blood samples using AmpFlSTR Identifiler.

Out of 40 reanalyzed samples, results for 32 were found to be identical as in the database and for eight samples genotypes entered in the database were found to be erroneous. In one case, the genotype was completely wrong, due to inadvertent sample switching.

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TABLE 1—*Identified errors in the database.*

No	Locus	Database	Actual	Type of Error	Cause of Error
1	D2S1338	17, 17	27, 32.2	Alleles assigned to wrong locus	Manual transcription error
2	D2S1338	23, 23	23, 24	Allelic drop-out	PCR flaw
3	D2S1338	17, 17	17, 26	Allelic drop-out	PCR flaw
4	D2S1338	17, 17	17, 23	Allelic drop-out	PCR flaw
5	D19S433	14, 14	14.2, 14.2	Partial repeat miscall	Genotyper software or electrophoretic aberration
6	D19S433	13.2, 13.2	13, 14	Insufficient allele resolution	Electrophoresis flaw
7	D13S317	9, 12	9, 10	Miscall	Human error

Other observed errors are listed in Table 1. The most frequent error was allelic drop-out which occurred three times. Interestingly all three observed cases occurred at the D2S1338 locus, which is the largest fragment amplified by the AmpFlSTR Identifiler kit. Larger alleles at this locus frequently amplify with lower efficiency, sometimes not reaching the detection level indicating that additional proofreading procedures should be performed when homozygous D2S1338 locus is being reported.

In addition to identified errors in the process of DNA that caused mismatch, this study revealed another 12 genetic inconsistencies with parentage. Mismatches in a single locus were identified in four cases and mismatch in two loci in one case, presumably due to meiotic mutations. For the last seven genotypes parentage was excluded, after excluding both experimental mistakes (the same genotype was obtained by two independent analyses in two laboratories) and mutations (they differed in more than three loci). In four cases "fathers" were not biological fathers (4.2%, 4/96), while in three cases "mothers" were found not to be biological mothers (0.36%, 3/815) of their presumptive children. However, as the design of this study prevented us from identifying real persons behind the genotypes, it should be noted that although "mothers" reported "children" to be their biological children, they might have also been adopted children, children from husband's previous marriage or something similar.

Conclusions

By comparing 911 pairs of parents and children in the database, we evaluated reliability of DNA typing (consisting of sample collection, DNA analysis and databasing) as a part of the process of identification of war victims in Croatia. Out of total of 1822 genotypes, one genotype (0.05%) was completely wrong as a result of clear human error. Two additional genotypes (0.1%) had one wrong locus, again because of a human error. Five additional genotypes (0.27%) had a single wrong locus due to either PCR or electrophoretic errors. It is important to note that technology-associated imperfections caused over 70% of all observed single-locus errors, indicating that all databases produced with similar technology would probably have comparable level of errors.

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