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SHORT COMMUNICATION

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Study on the STR TPOX in an Italian and an Austrian population using two different primer pairs and three different electrophoretic methods

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Abstract The short tandem repeat TPOX was studied using two different pairs of primers and three different electrophoretic methods with the aim of optimizing and standardizing the typing conditions for this locus. A genetic population study was subsequently conducted on two population samples from Central Italy (151 individuals) and from Austria (153 individuals) and compared using an $R \times C$ contingency table. With the aim of using this system for forensic samples, differences in sensitivity between the methods utilized were studied and several parameters of forensic interest for the two populations (PD, MEC, MEP, pM, PIC) were calculated. A new multiplex system for the loci CSF1PO, TPOX and CD4 is also presented.

Key words Short tandem repeats · TPOX · Central Italy · Austria

Introduction

Some articles have been published for the TPOX locus (Anker et al. 1992), but none on populations from Central Italy and Austria. Using the primers proposed by Anker et al. (1992) fragments ranging from 106 and 130 bp are obtained; with the primers described by Huang et al. (1995) the range of amplification products is between 232 and 248 bp. Manual methods for the resolution of STR-PCR products require the use of native or denaturing vertical PAGE and native horizontal PAGE followed by silver staining (Allen et al. 1989). Recently a method has been published which permits analysis with denaturing horizontal PAGE (Neuhuber et al. 1996). Various authors report that the use of different systems of resolution can cause problems in typing AT-rich STRs due to different electrophoretic mobilities (Eng et al. 1994; Lareu et al. 1994; Möller and Brinkmann 1994). The aim of this study was to establish whether they give identical results and to present allele/genotype frequency data in two samples from the population of Central Italy (Tuscany) and Austria (Graz and Salzburg).

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Materials and methods

Blood samples were taken from resident unrelated donors DNA was extracted from the Italian samples with a saline procedure (Miller et al. 1988) and from the Austrian samples as described previously (Klintschar et al. 1997).

Control samples consisting of four bloodstains and DNA samples were distributed among the three laboratories. For sensitivity studies serial dilutions of cell line DNA were prepared (K562, Promega, USA) from 10 ng/µl to 0.1 ng/µl and an amplified diluted TPOX ladder (1/2, 1/5, 1/10, 1/20, 1/50).

PCR analysis using primers suggested by Huang et al. (1995)

Amplification of TPOX locus was first optimized with a monoplex system, under the conditions described, using 1 µM of each primer and 30–34 cycles of amplification. The Italian samples were typed using this protocol. Then a multiplex analysis was performed using the STR CSF1PO (Hammond et al. 1994), TPOX (Huang et al. 1995) and CD4 (Hammond et al. 1994). The ranges of amplification for these STRs do not result in overlapping between alleles: CSF1PO (299–323 bp), TPOX (232–248 bp), CD4 (125–175 bp). Amplification was conducted in a thermal cycler (MJ Research) in a 25 µl reaction volume, using 10 ng of DNA, reaction buffer (75 mM of Tris-HCl pH 9.0 at 25° C, 20 mM (NH4)2 SO4, 0.01% Tween, 1.5 mM MgCl2), 200 µM of dNTP for every mix and for each primer 0.75 µM for CSF1PO, 0.70 µM for TPOX and 2 µM for CD4, using 0.25 units of polymerase (Red Hot Thermostable, Advanced Biotechnologies) with the hot-start method. The PCR conditions were 96° C for 2 min, followed by 30 to 34 cycles at 94° C for 1 min, 64° C for 1 min and 72° C for 1.5 min. A final extension for 10 min at 72° C was also done.

Of the PCR products 6 µl was loaded in 6% denaturing polyacrylamide gels containing 7 M urea and 0.5 × Tris-borate-EDTA. Electrophoresis was carried out for 3–4 h at a constant voltage of 1000 V. After electrophoresis, the bands were visualised by the silver staining technique (Allen et al. 1989) with minor modifications.

Classification of alleles was performed using a sequenced allelic ladder supplied by Promega GenePrint System for locus CSF1PO and TPOX. For CD4, a mix of known and sequenced alleles was used. Comparison with cell line DNA K562 (Promega, USA) was used as a positive control.

PCR analysis by primers suggested by Anker et al. (1992)

The Austrian samples were typed using a monoplex amplification system and primers described by Anker et al. (1992). Amplification conditions in a thermocycler TRIO-thermoblock were 3 min at 94° C, followed by 94° C for 1 min, 63° C for 1 min, and 72° C for 2 min for 30 cycles. Typing was performed using horizontal native polyacrylamide electrophoresis according to Wiegand et al. (1993) (7% T instead of 5% T).

A sequenced allelic ladder was kindly provided by B. Brinkmann, Munster.

Denaturing horizontal PAGE

The control samples which were amplified with both primer pairs were used to evaluate the suitability of the horizontal high-resolution denaturing PAGE (Neuhuber et al. 1994).

Sensitivity studies

Two sensitivity studies were performed. Firstly, serial dilutions of cell line K562 DNA (10, 5, 0.5, 0.2, 0.1 ng/ µl) were amplified in an attempt to compare the sensitivity of both primer pairs. Secondly, a diluted TPOX ladder (1/2, 1/5, 1/10, 1/20, 1/50) was run on the three types of PAGE to compare the sensitivities of these methods.

Statistics

The statistical analyses were performed using the HWE-Analysis program (C. Puers, Münster, Germany). The frequency profile comparison between different populations was carried out using a test for genetic heterogeneity (R × C contingency test; G. Carmody, Ottawa, Canada).

Results and discussion

Both the primers used and the three electrophoretic systems applied gave identical results with control bloodstains and DNA samples distributed among the three laboratories. Moreover the two PCR systems had the same sensitivity: the detection limit for K562 DNA was 0.5 ng. The three electrophoretic systems evaluated, however, had a different detection limit. While it was possible to visualize TPOX ladder up to a dilution of 1/10 on the horizontal native gel, the vertical denaturing PAGE also permitted the visualization of the 1/20 diluted ladder. The horizontal denaturing PAGE was the most sensitive method and was possible to visualize the 1/50 dilution. The advantage of the native horizontal PAGE, however, was that it was least labour intensive. The vertical denaturing PAGE, on the other hand, permitted typing of the multiplex system and repeat sample loading which was not possible using the other methods. The horizontal denaturing electrophoresis is an interesting alternative for small laboratories with limited equipment.

In the study of the population sample from Central Italy, 5 alleles were identified and designated from 8 to 12 as suggested by the DNA Commission of the International Society of Forensic Haemogenetics (1994), corresponding

Table 1 Allele and genotype frequencies of the TPOX system in Central Italian and Austrian population

Genotype	Central Italy (n = 151)	Austria (n = 153)
7– 8	–	1
8– 8	40	52
8– 9	13	20
8–10	13	7
8–11	43	34
8–12	7	3
9– 9	–	2
9–10	3	1
9–11	9	9
9–12	2	2
10–11	7	7
10–12	1	1
11–11	6	12
11–12	2	2

Table 2 Allele frequencies of TPOX system in both populations studied

Allele	Central Italy frequency	Austria frequency
7	–	0.003
8	0.533	0.552
9	0.106	0.117
10	0.079	0.052
11	0.242	0.248
12	0.039	0.026

Table 3 Forensic values for TPOX system in Central Italy and Austria populations

	Italian	Austrian
PD[a]	0.816	0.798
MEC[b]	0.400	0.369
MEP[c]	0.342	0.308
pM[d]	0.183	0.201
H obs.	0.695	0.562
H exp ± SE[e]	0.640 ± 0.0765	0.614 ± 0.0771
PIC[f]	0.591	0.561

PD[a] = discrimination power (Fisher 1951)
MEC[b] = mean paternity exclusion chance (Kruger et al. 1968)
MEP[c] = mean exclusion probability
pM[d] = probability of match (Jones 1972)
H obs. = observed heterozygous phenotypes
H exp ± SE[e] = expected heterozygous phenotypes (Nei and Roychoudhury 1974)
PIC[f] = polymorphism information content (Botstein et al. 1980)

to 12 genotypes. In the Austrian sample, allele 7 was also identified. A total of 6 alleles corresponding to 14 genotypes were identified (Tables 1 and 2).

The two populations were compared using an R × C contingency table: no significant difference in the frequency distribution of alleles was found ($X^2 = 1.537$; df = 5; $0.95 > P > 0.90$). No significant deviations from Hardy-Weinberg equilibrium were detected ($P > 0.95$). Likewise, the study of forensic parameters did not reveal significant differences between the two populations (Table 3). TPOX locus showed relatively high PIC values (average 0.576), similar to those reported in other studies (Gusmao et al. 1995). Average MEC values were 0.384.

The multiplex system used in this study proved to be also suitable for forensic samples (e.g. blood, saliva etc.) and allows significant reductions in time and materials. The limits of detection of all three systems was the same and comparable to that of the singleplex reaction i.e. 0.5 ng for K562 DNA were sufficient for typing.

The analyses carried out using PCR with a monoplex or multiplex system, with two pairs of different primers and with three different systems of resolution, demonstrate that the results are reproducible and comparable. Of the electrophoretic methods tested one was more sensitive but, all three were suitable and can be recomended. The two samples examined, taken from Caucasian populations from Central Italy and Austria, can be considered similar for STR TPOX. In conclusion, the data from this study of STR TPOX confirm its validity for the analysis of forensic samples and for paternity testing.

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