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SHORT COMMUNICATION

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The STR system hTPO: population and segregation data

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Abstract A population study was carried out on a random sample of 164 individuals from North Portugal using the short tandem repeat (STR) system hTPO (locus: 2p23–2pter). After electrophoresis, 7 alleles were identified of which 6 had been previously described and a new one, estimated to be 134 bp long. The observed genotype distribution is in Hardy-Weinberg equilibrium. In order to assess the forensic applicability of the system, namely for paternity investigations, 109 mother-child pairs were analysed. No exclusions were found and the observed distribution did not deviate from the expected. Since hTPO has a relatively high information content (PIC = 0.60; H = 0.65) this system can be very useful in paternity investigations.

Key words hTPO · STR · Population genetics · Paternity investigations

Introduction

The STR polymorphism hTPO was first described by Anker et al. (1992) and shows a variation in the number of (AATG) repeats inside intron 10 of the thyroid peroxidase gene (locus: 2p23–2pter). The only published population data for this system are from Galicia (Luis and Caeiro 1995) and China (Huang et al. 1995).

In this work we present the first results on the forensic validation of hTPO, using mother-child pair analyses, and further population data for this system.

Material and methods

Samples (blood and buccal swabs) were collected from unrelated individuals from North Portugal and from mother/child pairs involved in paternity investigations. Blood samples were obtained by venipuncture and 7 µl of whole blood were incubated for 30 min with distilled sterile water and centrifuged at 12000 g for 4 min. Pellets or buccal swabs were used directly in DNA extraction using the chelating resin method (200 µl of 5% Chelex 100 BioRad) described by Lareu et al. (1994). The primers used were those described by Anker et al. (1992). The PCR mixture (pH 9.0) was made to a final volume of 25 µl and included 10 mM Tris/HCl, 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM of each nucleotide, 0.25 µM of each primer, 1 U Taq polymerase (Pharmacia) and 6 µl of the extracted sample.

Amplification conditions were 5 min at 93° C, followed by 94° C–1 min, 63° C–0.5 min, 72° C–1.5 min, for 27–35 cycles (thermocycler: SPCR1 Gene-Tech, Stuart Scientific, UK). PCR amplifications were always performed with negative and positive (from previously typed individuals) controls and at least 2 independent amplification products (from different samples) were obtained from all individuals and subsequently typed.

Amplified DNA fragments were separated by horizontal electrophoresis in polyacrylamide gels according to Luis and Caeiro (1995). Bands were visualised by the silver staining method of Budowle et al. (1991).

Allele nomenclature is according to Huang et al. (1995), following the recommendations of the DNA Commission of the International Society of Forensic Haemogenetics (1994). Genotyping was performed by side-by-side comparison, using previously typed samples (Luis and Caeiro 1995).

Allele frequencies were estimated by gene counting; population conformity with Hardy -Weinberg expectations was tested by χ^2 analysis according to Guo and Thompson (1992). Mother-child segregation analysis was performed by classical χ^2 tests separately for each allele.

Results

Amplification results

Amplifications under the conditions described were regularly successful and insignificant extra bands were obtained only in cases of excess DNA template. Depending on DNA content and quality amplifications free from shadow bands could be obtained in the range of 27–35 cycles. All duplicate typings from the same individuals using either buccal swabs or blood as the DNA source were concordant. Furthermore, successful amplifications were also obtained from glycerol preserved erythrocyte preparations (containing remains of leukocytes) stored at –20° C for at least 3 years, using the same DNA extraction method as for whole blood.

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Population genetics

All previously described alleles were detected in our sample. A new allele 13 was also found (Fig. 1), which from its mobility, we have inferred to be 134 bp in size. Further characterisation is in progress. Table 1 shows the observed distribution of hTPO genotypes in the studied sample. The population is in Hardy-Weinberg equilibrium (P = 0.777). Allele frequencies are displayed in Table 2. In our sample the observed heterozygosity (H = 0.69) is slightly higher than the corresponding expected value (0.65). The polymorphic information content (PIC) is 0.60, and the "a priori" exclusion probability 0.38.

No mother-child exclusions were found by studying 109 pairs. The segregation analysis performed for each allele separately demonstrated no segregation distortions and no heterogeneity between male and female gene pools.

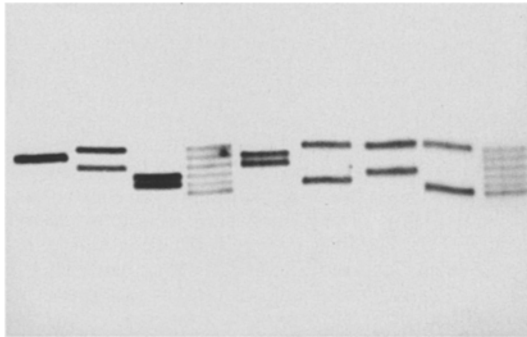

Fig. 1 Some hTPO phenotypes and allelic ladders (alleles 8 to 13). From left to right: 9–9; 8–10; 11–12; allelic ladder; 9–10; 8–12; 8–11; 8–13; allelic ladder

Table 1 hTPO genotype distribution in a population sample from North Portugal

Genotype	Observed	Expected
6–8	1	0.00
6–11	1	0.62
8–8	37	41.00
8–9	11	11.50
8–10	13	11.50
8–11	58	51.00
8–12	6	6.50
8–13	1	0.50
9–10	2	1.61
9–11	7	7.15
9–12	3	0.91
10–10	1	0.81
10–11	6	7.15
11 –11	13	15.86
11 –12	4	4.04
Others	0	2.85
Total	164	164.00

Discussion

The amplification and electrophoretic genotyping of hTPO proved to be very reproducible. The fact that only a simple tetranucleotide repeat motif was observed, is specially favourable for unambiguous genotyping, particularly because the overall size of the amplified fragments is relatively small (106–134 bp) when compared to other routinely used tetranucleotide STRs (e.g. FES: 211–235 bp; F13A01: 183–235 bp; Urquhart et al. 1994).

Concerning the population genetics of the system, the allele distribution in our sample was in Hardy-Weinberg equilibrium and allele frequencies observed (Table 2) can only be compared with those from 3 previously reported studies. Population heterogeneity in the hTPO system among Caucasians (suggested by the moderately significant differences found between our results and those from Galicia) needs to be substantiated with further data.

Mother/child analysis revealed that the system behaves in a regular codominant mendelian fashion, since neither mother/child exclusions nor segregation distortions were found.

The values of information content parameters for hTPO are slightly lower than some currently used STRs. However the number of alleles and their distribution are less complex allowing a much more accurate estimate of their true frequencies.

Therefore, considering (i) the simplicity of PCR amplification, that can be easily combined in multiplex procedures with currently used STRs, as recently demonstrated by Huang et al. (1995), (ii) the relatively high information content and (iii) the reliable genotyping without technical problems, hTPO can be a very useful system for forensic applications, particularly in paternity investigations.

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Table 2 Frequencies (%) of hTPO alleles in some Caucasian and Asian populations

Population	N	6	8	9	10	11	12	13	14	Reference
N.Portugal	164	0.6	50.0	7.0	7.0	31.1	4.0	0.3	0.0	This work
Galicia	260	0.0	53.8	11.9	5.42	26.0	2.9	0.0	0.0	Luis and Caeiro (1995)
Caucasian	73	1.0	44.0	10.0	10.0	34.0	1.0	0.0	0.0	Anker et al. (1992)
Chinese	116	0.0	54.3	9.9	1.3	33.2	0.9	0.0	0.4	Huang et al. (1995)

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