



## Population database of STRs in West Africa: a genetic study of TPOX, HUMVWA31/A, HUMTH01, and CYP19

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### Abstract

Four tetrameric STRs (TPOX, HUMVWA31/A, HUMTH01, and CYP19) were analysed in a West African population (Cabo Verde). No significant deviations from Hardy–Weinberg proportions were observed, either in conventional or exact tests. Pairwise comparisons confirmed allelic independence for all the combinations of loci. Data is provided for the first time about CYP19 in Black populations. In comparisons between African and Afro-American populations, significant frequency differences for several alleles at the TH01 and VWA31/A loci were observed. The allele frequencies provided in this study contribute to a better knowledge of the variability of these markers among the main human groups, especially in the context of Sub-Saharan African populations.

### Introduction

Interspersed throughout the human genome, tandem repetitions of DNA sequences constitute a feature of the organisation of hereditary material. Short tandem repeats (STRs) of sequences of 2–6 bp are particularly well represented (Weber & May, 1989; Tautz, 1989; Litt & Luty, 1989). STRs display a statistical distribution in discrete entities, which, coupled to their highly polymorphic character, make these markers one of the most useful instruments in population studies and individual genetic identification.

In accordance with the population database reported up to now, attention has been principally focused on Caucasian populations, and so the available data on other human groups may, for the moment, be reduced. This is particularly true in the case of African populations. In this study, a genetic analysis of four tetranucleotide repeats (TPOX, HUMVWA31/A, HUMTH01, and CYP19) in a West African population was carried out. The goal is to extend our knowledge about the genetic profile of the populations of this geographical area and thus provide a database for fur-

ther specific analyses for anthropological and forensic purposes.

### Materials and methods

#### *Samples*

The population of Cabo Verde (West Africa, 14.43 N, 17.31 W) was established by settlers from Senegal and Gambia (Carreira, 1983), and therefore corresponds to the Guinean racial group. 244 healthy unrelated individuals randomly selected from the population of Cabo Verde were analysed. Only autochthonous individuals for at least two generations were taken into account in this study. Blood was collected in EDTA-Na<sub>2</sub> as anticoagulant and DNA was extracted by the phenol-chloroform method (Maniatis, Fritsch & Sambrook, 1982) and stored at –20°C not longer than 2 years pending analysis.

#### *DNA amplification*

The amplification of TPOX, HUMVWA31/A, HUMTH01, and CYP19 was performed individually.

Table 1. Allele frequencies of the 4 STR systems in a West Africa population from Cabo Verde

TPOX N = 488		HUMVWA31/A N = 428		HUMTH01 N = 442		CYP19 N = 384	
Allele	Frequency	Allele	Frequency	Allele	Frequency	Allele	Frequency
6	0.0553	12	0.0047	6	0.1787	7-3	0.3594
7	0.0246	13	0.0093	7	0.2624	7	0.3567
8	0.2746	14	0.0864	8	0.2330	8	0.0729
9	0.1639	15	0.1565	9	0.1787	11	0.1667
10	0.1147	16	0.2921	9.3	0.1448	12	0.0443
11	0.3422	17	0.2289	10	0.0023		
12	0.0246	18	0.1378				
		19	0.0561				
		20	0.0234				
		21	0.0047				

N: No. alleles analysed.

Table 2. Heterozygosities, PIC, PD, and CE values for STR loci in West Africa (Cabo Verde)

	TPOX	HUMVWA31/A	HUMTH01	CYP19
$H_o$	77.87 ± 2.80	80.84 ± 2.69	80.54 ± 2.66	71.35 ± 3.26
$H_e$	76.48 ± 2.72	80.94 ± 2.68	79.38 ± 2.72	71.22 ± 3.27
PIC	72.74	78.15	75.88	66.49
PD	90.81	93.33	92.36	86.38
CE	55.00	62.39	58.40	46.05

$H_o$ : observed heterozygosity,  $H_e$ : expected heterozygosity, PIC: Polymorphic Information Content, PD: Power of Discrimination, CE: Chance of Exclusion.

PCR took place in 12.5  $\mu$ L reaction volumes in 20 mM Tris-HCl at pH 8.4, 50 mM KCl, 20–100 ng of template DNA, 200  $\mu$ M each deoxynucleotide, 0.5 U Taq DNA polymerase (Gibco-BRL), MgCl<sub>2</sub> 1.5 mM for VWA and CYP19, and 3 mM for TPOX and TH01. Primer sequences were as described: TPOX (Anker, Steinbrueck & Donnis-Keller, 1992), TH01 (Gill, Kimpton & Sullivan, 1992), VWA31/A (Kimpton, Walton & Gill, 1992) and CYP19 (Polymeropoulos et al., 1991).

Amplification parameters were as follows: TH01: an initial cycle at 93 °C for 5 min, followed by 30 cycles at 94 °C–1 min, 54 °C–1 min, 72 °C–1 min, CYP19: 94 °C–1 min, 62 °C–1 min, 72 °C – 1 min, for 30 cycles. For both, a further elongation cycle was carried out at 72 °C for 10 min. The conditions for VWA and TPOX were as previously detailed (Luis & Caeiro, 1995)

### Molecular separation

Amplified products were electrophoresed in horizontal polyacrylamide gels (120 mm × 190 mm × 0.4 mm) in discontinuous systems (Bridge 125 mM Tris-Glycine pH 8.8, and 375 mM Tris-HCl pH 8.8 for the gel). Gel composition was adjusted for each STR: VWA (9%T, 4%C), TPOX (10%T, 5%C), CYP19 (9%T, 4%C). For TH01, denaturing electrophoresis in continuous 0.5×TBE buffer containing 8 M Urea in the gel was carried out. After electrophoresis, silver staining for DNA band detection was accomplished. Molecular phenotypes were determined using reference ladders obtained from alleles of known sizes. The alleles comprising each ladder were 8, 9, 10, 11, and 12 for TPOX; 14, 15, 16, 17, 18, 19, and 20 for VWA; 6, 7, 8, 9, 9.3, and 10 for TH01 and 7–3, 7, 8, 11, and 12 for CYP19.

Table 3. Hardy–Weinberg equilibrium analysis for STR loci in West Africa (Cabo Verde)

	TPOX	HUMVWA31/A	HUMTH01	CYP19
Heterozygosity test	0.0767	0.0002	0.0502	0.0068
$p$	$0.70 < p < 0.80$	$0.975 < p < 0.990$	$0.80 < p < 0.90$	$0.90 < p < 0.95$
$\chi^2$	17.31	51.37	12.19	13.00
$p$	$0.60 < p < 0.70$	$0.20 < p < 0.30$	$0.60 < p < 0.70$	$0.20 < p < 0.30$
Exact test <sup>a</sup>	0.7222	0.0550	0.6384	0.1150

<sup>a</sup>:  $p$  values

Table 4. Pairwise test of allelic independence between loci by means of Markov chains

Combination	Probability	$\pm$ s.e.
VWA/TPOX	0.2377	$\pm$ 0.0259
VWA/TH01	0.8707	$\pm$ 0.0360
TPOX/TH01	0.3819	$\pm$ 0.0540
VWA/CYP19	0.7021	$\pm$ 0.0589
TPOX/CYP19	0.7820	$\pm$ 0.0470
TH01/CYP19	0.6170	$\pm$ 0.0413

### Statistical analysis

Allele frequencies for each STR were obtained from the phenotype values by gene counting. Expected and unbiased values of heterozygosity were obtained as proposed by Nei (1978), and PIC values, according to Botstein et al. (1980). Chance of exclusion (CE) and power of discrimination (PD) were calculated according to Hummel and Gerchow (1981) and Fisher (1951), respectively. Evaluation of the Hardy–Weinberg equilibrium was performed using three different methods: Pearson's Chi-square ( $\chi^2$ ), the unbiased estimate of homozygote/heterozygote values, and exact test (Guo & Thompson, 1992) consisting of 50 batches, each with a size of 1000, for a dememorisation period of 1000, the latter being performed by means of GENEPOP program (Raymond & Rousset, 1995).

Interpopulation comparisons were made with 2 way  $R \times C$  contingency tables using the  $G$  statistics (Sokal & Rohlf, 1969), and the  $t_s$  algorithm for assessing specific allele comparisons. In order to test the independence of segregation of alleles across the loci (i.e. genetic disequilibrium), an exact test based on Markov chains was carried out.

### Results and discussion

Consecutive alleles differ in size by four base pairs for each of the systems under analysis. However, the allele 9.3 for TH01 has a deletion in the 5th repeat (Puers et al., 1993; Urquhart et al., 1994). Reliable results were obtained after analysis of TH01 in denaturing polyacrylamide gels followed by silver staining. A correlation of electrophoretic patterns was observed in native and denaturing gels for CYP19.

### Allele frequencies

The sizes of the alleles found in this study were: 130–166 bp for VWA31/A, 158–174 bp for HUMTH01, 106–130 bp for TPOX, and 154–178 bp for CYP19. Table 1 shows the distribution of allele frequencies from autochthonous individuals from Cabo Verde (West Africa). The most frequent alleles were: TPOX 11 (0.342), HUMTH01 7 (0.262), HUMVWA 16 (0.292), and CYP19 7-3 (0.359). The presence of the allele 12 of VWA31/A is noteworthy (with a frequency of 0.0047), for it has only been found so far in Caucasian populations in Portugal with frequencies of 0.002 (Amorim, Gusmao & Prata, 1996), and in Germany with frequencies of 0.0006 (Huckenbeck et al., 1996). This seems to indicate that this is an allele shared both in Caucasian and Black populations. The degree of variability of such allele distribution is summarised by the values of  $H$  and PIC (Table 2). The values obtained for VWA31/A are similar to those observed among the Caucasoid and Mongoloid populations so far reported. Nevertheless, for TPOX, the population of Cabo Verde exhibits the highest degree of variability registered to date (PIC = 0.727 and  $H$  = 0.765), leading to significant differences with the populations belonging to these major groups ( $p$  values with a range of 0.0005–0.001 for Caucasians and 0.0002–0.0006 for Mongoloids). Thus, in this West African population, TPOX exhibits a biostatistical efficiency similar to VWA and TH01,

Table 5.  $G$  and  $t_s$  allele frequency comparisons between samples from Cabo Verde and African and Afro-American samples in HUMVWA31/A

	Alleles										
	11	12	13	14	15	16	17	18	19	20	21
<b>Ovambos<sup>a</sup></b>											
$t_s$	5.587***	1.653	2.117*	0.883	1.316	1.849	0.944	0.848	1.335	0.228	1.653
$p$	< 0.001	0.514	0.030	0.378	0.188	0.066	0.347	0.396	0.184	0.773	0.099
$G = 33.440$ $p < 0.001$											
<b>S. Tome &amp; Principe<sup>b</sup></b>											
$t_s$		1.816	1.722	0.137	0.924	0.171	0.940	0.114	0.000	0.486	1.816
$p$		0.070	0.086	0.090	0.358	0.864	0.3740	0.910	1	0.628	0.071
$G = 5.377$ $0.7 < p < 0.8$											
<b>Afrocaribbeans<sup>c</sup></b>											
$t_s$		1.985*	0.896	0.356	2.203*	1.859	0.610	0.905	0.704	0.532	0.002
$p$		0.048	0.188	0.278	0.042	0.066	0.542	0.368	0.484	0.587	0.984
$G = 14.365$ $0.1 < p < 0.2$											
<b>Black USA<sup>d</sup></b>											
$t_s$		1.659	0.568	0.746	1.983*	1.301	0.742	0.661	1.298	2.216*	1.660
$p$		0.097	0.572	0.460	0.033	0.194	0.459	0.512	0.197	0.028	0.097
$G = 8.776$ $0.3 < p < 0.5$											

<sup>a</sup>Data from Brinkmann et al. (1996).

<sup>b</sup>Data from Amorim et al. (1996).

<sup>c</sup>Data from Evett et al. (1997).

<sup>d</sup>Data from Sajantila et al. (1994).

\*Significant at 0.05 level.

\*\*\*Significant at 0.001 level.

in contrast to Caucasian and Mongoloid populations, in which TPOX is less useful than the two other STRs. Significant differences for TH01 were found compared to Asian samples from China (Huang, Schumm & Budowle, 1995) ( $t_s = 2.31$ ,  $p = 0.021$ ), which is in line with lower values of TH01 polymorphism generally observed among Mongoloid populations.

#### *Genotype frequencies and genetic equilibrium*

In order to analyse the genetic structure of the population for the loci studied here, three statistical approaches were used. The analysis of biased and unbiased heterozygosities gives very conservative estimates. Given that the analysis of the differences of genotype frequencies is condensed into a single value, important differences may go unnoticed. This may be due to mutual compensations of excesses and deficits between observed and expected values for the different genotype classes. Thus, the assessment of

genetic equilibrium by exact test (Guo & Thompson, 1992) seems to be more suitable. No significant deviations from expected values under the Hardy–Weinberg equilibrium were found in any of the STRs (Table 3).

An independence test for the phenotype expression of the four STRs was performed after estimating pairwise linkage disequilibrium by means of an exact test, and no statistical evidence of association between the loci was observed in any of the combinations (Table 4). The  $p$  values range between 0.871 (combination VWA/TH01) and 0.238 (combination VWA/TPOX).

#### *Population comparisons*

Available data from Black populations are, at present, relatively scarce. For this reason this analysis can only refer to VWA and TH01 for the populations listed in Tables 5 and 6.

Table 6.  $G$  and  $t_s$  allele frequency comparisons between samples from Cabo Verde and African and Afro-American samples in HUMTH01

	Alleles						
	5	6	7	8	9	9.3	10
<b>Ovambos<sup>a</sup></b>							
$t_s$		5.752***	3.326**	3.874***	0.883	5.653***	1.412
$p$		< 0.001	< 0.01	< 0.001	0.396	< 0.001	0.156
				$G = 67.570$			
				$p < 0.001$			
<b>S. Tome &amp; Principe<sup>b</sup></b>							
$t_s$		3.428***	2.707**	3.289***	1.029	4.399***	5.863***
$p$		< 0.001	< 0.01	< 0.001	0.303	< 0.001	< 0.001
				$G = 39.870$			
				$p < 0.001$			
<b>Afrocaribbeans<sup>c</sup></b>							
$t_s$	2.017*	1.439	3.733***	1.035	2.109*	0.662	1.721
$p$	0.424	0.153	< 0.001	0.304	0.036	0.508	0.086
				$G = 19.058$			
				$0.001 < p < 0.01$			
<b>Black USA<sup>d</sup></b>							
$t_s$		2.798**	5.235***	1.553	1.289	1.698	2.003*
$p$		< 0.01	< 0.001	0.085	1.008	0.091	0.042
				$G = 31.412$			
				$p < 0.001$			
<b>Black USA<sup>e</sup></b>							
$t_s$		1.513	3.480***	0.577	2.704**	1.089	0.287
$p$		0.131	< 0.001	0.566	< 0.01	0.276	0.773
				$G = 12.475$			
				$0.002 < p < 0.005$			

<sup>a</sup>Data from Brinkmann et al. (1996).

<sup>b</sup>Data from Amorim et al. (1996).

<sup>c</sup>Data from Evett et al. (1997).

<sup>d</sup>Data from Budowle et al. (1997).

<sup>e</sup>Data from Puers et al. (1993).

\*Significant at 0.05 level.

\*\*Significant at 0.01 level.

\*\*\*Significant at 0.001 level.

The application of the  $G$  statistics to compare the allelic frequencies of VWA in Cabo Verde with others is displayed in Table 5. No significant differences were found except with the Ovambos ( $G = 33.440$ ,  $p < 0.001$ , 10 d.f.), where differences are largely due to the frequency distribution of the alleles 11 and 13. Since the  $G$  value provides a global evaluation of differences for all the alleles of the locus, the lack of evidence of significant differences for  $G$  does not allow us to liken the distribution of allelic classes between the populations being compared. Therefore, a detailed analysis for specific allele classes using the  $t_s$

algorithm was also carried out. Significant differences for alleles 15 and 20 (with Black USA) and alleles 12 and 15 (with Afrocaribbeans) were found. The number of observed significant differences is four times higher than that expected by chance as a consequence of the number of repetitions. These results lend support to the above-mentioned considerations, given that such allele differences go unnoticed after the application of a global analysis such as the  $G$  test.

$G$  and  $t_s$  values generated from the comparison of TH01 allelic frequency in Cabo Verde with other Black populations are summarised in Table 6. Signif-

ificant differences within each population were found after the application of *G*. Once again, as previously observed for VWA, the biggest differences among the populations under study were found when the Ovambos sample was compared with the other samples. Significant differences were also observed after comparing the different populations with each other. The genetic heterogeneity for TH01 among the populations of this major human group would therefore be coherent with the high degree of diversity reported for this human group (Cavalli-Sforza, Menozzi & Piazza, 1994).

The ratio of the TH01 alleles 9/9.3 ranges between values of 30 and 50 in Mongoloid populations, whereas in Caucasians these values are typically of around 0.5, and are always less than 1. Balanced values for both alleles are observed in African and Afro-American populations, and the value of this ratio is thus around 1. Nonetheless, the sample of Ovambos from Namibia with values of 6.86 is an exception. This is mainly due to the particularly low value of the allele 9.3 (0.022), which chiefly accounts for the previously indicated significant differences with the other populations.

In closing, a specific knowledge of the genetic characteristics of the population is required in order to carry out precise analyses of STRs for forensic purposes (Meyer, Wiegand & Brinkmann, 1995). In this study, a West African population database is provided for the systems HUMVWA31/A, HUMTH01, TPOX, and CYP19, the latter being the first data so far reported for African populations.

As a consequence of the biological variability of human populations, the PIC value of an STR may display substantial differences depending on the population context. Therefore, the most informative systems in a human biological group may not be the most relevant in another, as was previously observed for TPOX in Black populations and TH01 in Mongoloid populations, for example. This suggests the convenience in assessing specific batteries of markers for the main human races. The high degree of variability and usefulness of these four loci, as well as their accordance with genetic equilibrium and independence in segregation, would seem to reveal the relevance of these STRs in this African population, and their suitability in forensic studies.

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