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A study of four short tandem repeat systems: African immigrant, Portuguese and Spanish population data

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1. Introduction

The ability to amplify multiple short tandem repeat (STR) loci in a simple reaction, coupled with the development of fluorescence-based PCR technology allowing the successful co-amplification of loci with overlapping size ranges, has enabled the acquisition of greater levels of information and productivity in laboratories where large numbers of samples are analyzed [1].

Tetrameric short tandem repeat (STR) typing systems are a reliable, rapid and sensitive method for forensic identification, paternity testing [2,3] and population genetic studies [4].

In this study the allele frequencies for four traditional tetrameric STR loci: HUMTH01, HUMVWA31/A, HUMFESFPS and HUMF13A01, in five different populations were analyzed. These populations are specifically those from Portugal, South West Spain, the Spanish population of Caucasian origin in North Africa as well as the Moroccan and West African immigrant populations that use the South of Spain as a point of entry into the European Community (EC).

2. Materials and methods

Whole EDTA blood was obtained by venipuncture from 344 Portuguese, 201 Spaniards from the southwest of Spain, 110 unrelated Spaniards of Caucasian origin from North

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Africa (Ceuta), 108 unrelated immigrants from Morocco and 110 unrelated Central West African black immigrants from different countries situated to the South of the Sahara. The DNA was extracted using a Chelex 100 protocol as described by Walsh et al. [5].

Table 1
Allele frequency distribution for four STR loci in five populations investigated

Locus	Allele	Portugal [*n*=344]	Moroccan Im. [*n*=108]	North African (Spain) [*n*=110]	S.W. Spain [*n*=201]	W. African Black Im. [*n*=110]
HUMTHO1	5					0.0045
	6	0.2311	0.1157	0.1636	0.2363	0.0995
	7	0.1265	0.2037	0.1863	0.1617	0.4091
	8	0.1352	0.1805	0.1363	0.1269	0.2136
	9	0.1933	0.3240	0.2182	0.2015	0.1955
	9.3	0.3052	0.1342	0.2763	0.2662	0.0682
	10	0.0087	0.0370	0.0182	0.0074	0.0136
	11		0.0046			
HUMFES	8	0.0189	0.0324	0.0182	0.0050	0.1111
	9		0.0509	0.0318	0.0025	0.1065
	10	0.3110	0.3611	0.3182	0.3184	0.2269
	11	0.3881	0.3148	0.3455	0.3682	0.3009
	12	0.2471	0.1759	0.2409	0.2487	0.1667
	13	0.0349	0.0648	0.0454	0.0572	0.0833
	14					0.0046
HUMVWA31/A	4					0.0046
	5					0.0046
	6					0.0046
	8					0.0046
	12					0.0046
	13	0.0014	0.0046		0.0050	0.0091
	14	0.1221	0.1343	0.1045	0.0920	0.0773
	15	0.1148	0.1991	0.1273	0.1070	0.2182
	16	0.2224	0.2222	0.2364	0.2264	0.2318
	17	0.2762	0.2037	0.2409	0.3134	0.2227
	18	0.1817	0.1389	0.2000	0.1368	0.1227
	19	0.0669	0.0741	0.0682	0.0945	0.0500
	20	0.0102	0.0231	0.0182	0.0224	0.0364
	21	0.0043		0.0045	0.0025	0.0091
HUMF13A1	3.2	0.0843	0.1620	0.0818	0.0721	0.1667
	4	0.0291	0.0602	0.0454	0.0299	0.1435
	5	0.1817	0.2500	0.2136	0.2338	0.3426
	6	0.3052	0.1805	0.2364	0.3109	0.0972
	7	0.3575	0.2731	0.3818	0.3259	0.1157
	8	0.0116	0.0046	0.0136	0.0149	0.0880
	9			0.0091		
	10					0.0046
	11		0.0046			0.0046
	12		0.0092			0.0046
	13			0.0045	0.0025	0.0139
	14	0.0102		0.0091	0.0025	0.0093
	15		0.0278		0.0025	0.0046
	16	0.0160	0.0093	0.0045	0.0025	
	17	0.0044			0.0025	0.0046

Five-microliter aliquots of the extracts with a DNA content of approximately 5 ng/μl were used for amplification. The THO1, VWA, F13A1 and FES/FPS loci were amplified as described by Corte Real et al. [6]. Electrophoresis was carried out on 4% polyacrylamide denaturing sequencing gels on a 377 automated sequencer (Applied Biosystems Division, Perkin-Elmer). The length of the amplified DNA fragments were determined by using internal lane standard Genescan-2500 ROX (Perkin-Elmer). Allele designations were made using Genescan PCR analysis software with local Southern Method and by comparison with allelic ladders. Statistical analysis of the results was performed using the following tests. Hardy–Weinberg equilibrium was tested using the exact test according to Guo and Thompson [7] involving the GENEPOP programme (version 3.1b, 1997) [8]. The chance of exclusion (CE) for paternity was calculated according to Ohno et al. [9] and the discrimination power was calculated according to Jones [10].

3. Results

The distribution of observed allelic frequencies in samples from one Portuguese population, two Spanish populations and two African immigrant populations to obtain allele and genotype frequencies for the four loci (THO1, VWA, F13A1 and FES/FPS) is shown in Table 1.

Statistical inferences for each locus in the five analyzed populations are shown in Table 2. After the statistical analysis, the Portuguese, Spaniards from the South West of Spain and unrelated immigrants from Morocco (Table 2) were observed to be in Hardy–Weinberg equilibrium for the four analyzed systems ($p > 0.01$ in all four systems). On the other hand, when the corresponding statistical analysis was carried out on samples for the Spaniards of Caucasian origin from North Africa (Ceuta) and Central West African black immigrants sample (Table 2), these populations were observed to be in Hardy–Weinberg equilibrium for almost all the analyzed markers ($p>0.01$ in the four systems) except for the HUMVWA31A system. Possible reasons include inbreeding, population substructure and selection [11]. In the specific case of the population of Spaniards of Caucasian origin from North Africa, the disequilibrium observed for this marker (HUMVWA31A) may be the consequence of the migratory movements that constantly take place in the zone. Likewise, given the influence on the genepool of the structure or composition of the Central and West African black immigrant population in the South and centre of Europe, currently made up of individuals from different geographical areas of Central and West Africa,

Table 2
P values for the exact test for Hardy–Weinberg equilibrium

	TH01	FES	VWA	F13A1
Portugal	0.8600	0.8200	0.8400	0.2400
Moroccan Im.	0.2500	0.4100	0.2200	0.0400
North African (Spain)	0.7700	0.4100	0.0002	0.2000
S.W. Spain	0.1500	0.7310	0.1400	0.0700
West African Black Im.	0.8661	0.1083	0.0004	0.0127

population substructure appears to be the most likely explanation for this deviation for the HUMVWA31A loci.

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