TECHNICAL NOTE

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Population Study of the HUMTH01, HUMVWA31A, HUMF13A1, and HUMFES/FPS STR Polymorphisms in the North of Portugal

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ABSTRACT: Allele and genotype frequencies of four short tandem-repeat loci were determined in a population sample from the North of Portugal using the polymerase chain reaction (PCR). After denaturing PAGE, 6 alleles were identified for HUMTH01 (n = 419), 9 alleles for HUMVWA31A (n = 376), 12 alleles for HUMF13A1 (n = 232), and 5 alleles for HUMFES/FPS (n = 409). No deviation from Hardy-Weinberg equilibrium was found. The allele frequencies observed are similar to those of the European populations compared. The combined power of discrimination is 0.999.

KEYWORDS: forensic science, DNA typing, polymerase chain reaction, short tandem repeat, HUMTH01, HUMVWA31A, HUMF13A1, HUMFES/FPS, population genetics, Portugal

Tetrameric short tandem repeats (STRs) represent a rich source of highly polymorphic markers in the human genome (1,2), which may be studied by polymerase chain reaction (PCR) (3). They can be used for individual identification in forensic sciences and in population studies. In order to obtain data from the western Iberian Peninsula population, the allele frequencies and genotype distribution of HUMTH01 (4), HUMVWA31A (5), HUMFI3A1 (6), and HUMFES/FPS (7) polymorphisms were studied in a sample population from the North of Portugal. The data obtained in the present study were compared with Spanish and European population data. In addition, the theoretical values of these markers for paternity testing and forensic casework was evaluated.

Materials and Methods

Blood samples were obtained from healthy unrelated individuals from the North of Portugal. DNA was extracted from fresh peripheral blood leukocytes using phenol-chloroform-isoamyl alcohol according to the protocol described by Auxbel et al. (8). The PCR reagant was: 1–10 ng template DNA; 10 × PCR buffer (50 mM KCl, 10 mM Tris-HCl pH 9, 0.1% Triton X-100); 1.5 mM $MgCl_2$, 200 μM each dNTP; 10 pmol each primer; and about 1 U Taq polymerase, diluted to a total volume of 25 μL with distilled water. Singleplex PCR amplifications were performed in a Perkin Elmer PE1 Thermocycler; negative and positive control samples were treated likewise.

Amplifications were achieved using the primers described by Edwards et al. (2) for HUMTH01, Kimpton et al. (5) for HUMVWA31A, and Polymeropoulos et al. (6,7) for HUMF13A1, and HUMFES/FPS respectively, with the following conditions: 95°C 1 min; 60°C 1 min; 72°C 2 min; 30 cycles. Forward primers were labeled with fluorescein amidite in 5′ position, in order to analyze the PCR product by a Laser Fluorescent Sequencer.

Electrophoresis was performed in 6% (w/v acrylamide/bisacrylamide) polyacrylamide denaturing high performance DNA sequencing gels (Ready Mix Gel ALF grade Pharmacia™). The electrophoresis was carried out on the Automated Laser Fluorescent (ALF) DNA Sequencer (Pharmacia) at 1450 V, 38 mA, 45 W, 50°C and laser power at 3 mW for 220 min.

Aliquots of 0.5 μL of the amplified DNA were mixed with internal fluorescent labeled standard sizers and diluted in formamide sample buffer. External lane ladders with 50 to 500 bp (Pharmacia) were also used. Fragment sizes were determined automatically using Fragment Manager v. 1.2 Software (Pharmacia). Allelic ladders from Promega™ were used. We used a nomenclature for DNA fragments based on the number of repeats.

A standard Chi-square goodness-of-fit statistic was calculated to assess Hardy-Weinberg expectations. Where expected genotype frequencies were less than 5, these were pooled collectively according to the system outlined by Dickinson-Gibbons (9). Possible divergence from Hardy-Weinberg equilibrium (HWE) was also determined by calculating the exact test proposed by Guo and Thompson (1992) (10). The power of discrimination (PD) was calculated from the genotype data on the basis of an equation derived by Fisher ($PD = 1 - \Sigma P_j^2$, where P_j is the frequency of each genotype) (11). The Portugal data were compared with other European sample populations using an RxC contingency table χ^2 test for homogeneity.

Results

In a blind trial, DNA samples from 50 individuals were typed by us in Portugal and in Spain at the Forensic Genetics Laboratory

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(University of Barcelona). Concordant HUMTH01, HUMVWA31A, HUMF13A1, and HUMFES/FPS types were found in all cases.

For the four systems the genotype and allele frequencies in the population analyzed are shown in Tables 1, 2, 3, and 4. The HUMTH01 analysis demonstrated 18 variant genotypes, representing products of 6 alleles. Alleles 6 and 9.3 present the highest frequencies. HUMVWA31A polymorphism presents 29 genotypes representing products of 9 alleles, of which alleles 17 and 16 present the highest frequency. At the locus HUM13A1, 31 genotypes were observed corresponding to 12 alleles. Alleles 5, 6, and 7 have the highest frequencies. The HUMFES/FPS system presents 17 genotypes, product of 7 alleles, with the highest frequencies in alleles 10, 11, and 12. For all the polymorphisms analyzed the distribution of the genotypes are in Hardy-Weinberg equilibrium.

TABLE 1—*Genotype and allele frequency distributions at the HUMTH01 locus in a random sample of 419 individuals from the North of Portugal.*

Allele	6	7	8	9	9.3	10	Frequency
6	11	32	28	37	54	2	0.209
7		11	22	23	37	—	0.162
8			4	22	33	2	0.137
9				14	41	2	0.182
9.3					44	—	0.302
10						—	0.007

$\chi^2 = 9.48$, DF = 11, $P = 0.575$.
Exact test: $P = 0.3123$.

TABLE 2—*Genotype and allele frequency distributions at the HUMVWA31A locus in a random sample of 376 individuals from the North of Portugal.*

Allele	13	14	15	16	17	18	19	20	21	Frequency
13	—	1	—	2	1	—	—	—	—	0.005
14		2	17	12	20	14	11	1	—	0.106
15			9	27	20	19	5	—	—	0.141
16				15	45	28	10	5	—	0.211
17					29	31	18	1	2	0.261
18						17	9	3	—	0.183
19							2	—	—	0.076
20								—	—	0.013
21									—	0.002

$\chi^2 = 17.56$, DF = 14, $P = 0.225$.
Exact test: $P = 0.3093$.

TABLE 3—*Genotype and allele frequency distributions at the HUMF13A1 locus in a random sample of 232 individuals from the North of Portugal.*

Allele	3.2	4	5	6	7	8	9	13	14	15	16	17	Frequency
3.2	4	—	2	13	13	1	—	—	1	—	1	—	0.084
4		1	4	3	9	—	—	1	—	—	—	1	0.043
5			9	29	21	—	—	—	—	1	2	1	0.168
6				16	59	1	—	1	—	1	3	—	0.306
7					22	5	1	—	1	2	3	—	0.340
8						—	—	—	—	—	—	—	0.015
9							—	—	—	—	—	—	0.002
13								—	—	—	—	—	0.004
14									—	—	—	—	0.004
15										—	—	—	0.008
16											—	—	0.019
17												—	0.004

$\chi^2 = 13.70$, DF = 7, $P = 0.075$.
Exact test: $P = 0.1085$.

TABLE 4—*Genotype and allele frequency distributions at the HUMFES/FPS locus in a random sample of 409 individuals from the North of Portugal.*

Allele	8	9	10	11	12	13	14	Frequency
8	—	—	4	1	2	1	—	0.010
9		—	—	1	1	—	—	0.002
10			42	84	65	10	2	0.304
11				67	64	18	—	0.369
12					38	8	1	0.265
13						—	—	0.045
14							—	0.004

$\chi^2 = 11.50$, DF = 6, $P = 0.075$.
Exact test: $P = 0.1134$.

Discussion

The data from Portugal (allele counts) for the HUMTH01 locus were compared with other European populations, each of which is in HWE. Statistically significant differences were observed with Catalonia (NE Spain) (12) and Zagreb (13). Nevertheless, more data on other Mediterranean populations are needed to justify any conclusions. On the other hand, no significant differences were observed with the populations of: Galicia (NW Spain) (14), Basque Country (N Spain) (15), Italy (16), Switzerland (17), or Hungary (18) (Fig. 1).

For HUMVWA31A (Fig. 2), HUMF13A1 (Fig. 3), and HUMFES/FPS (Fig. 4) systems, Portugal does not differ significantly from other European populations (12–15,19–21).

From these results, it can be concluded that there is a general uniformity for the four systems in the European populations compared. From a forensic point of view, theoretical values were calculated from gene frequencies obtained in our population (Table 5). The four systems demonstrated a highly polymorphic allele

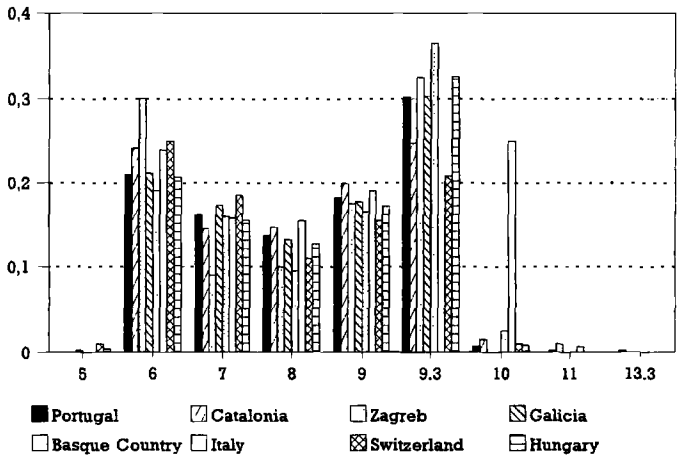

FIG. 1—*HUMTH01 allele frequencies in eight different Caucasian populations.*

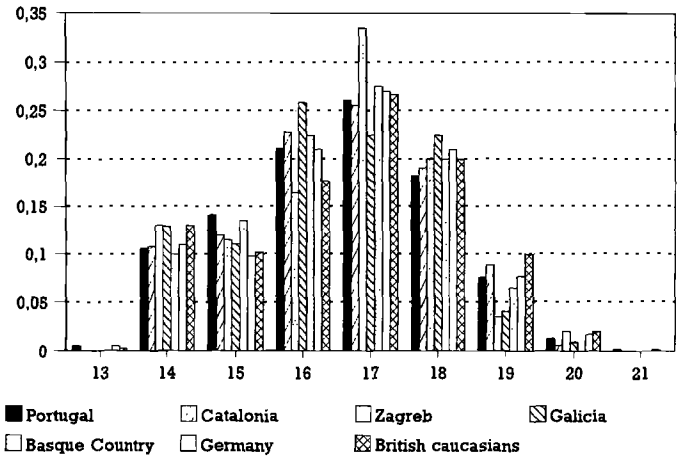

FIG. 2—*HUMVWA31A allele frequencies in seven different Caucasian populations.*

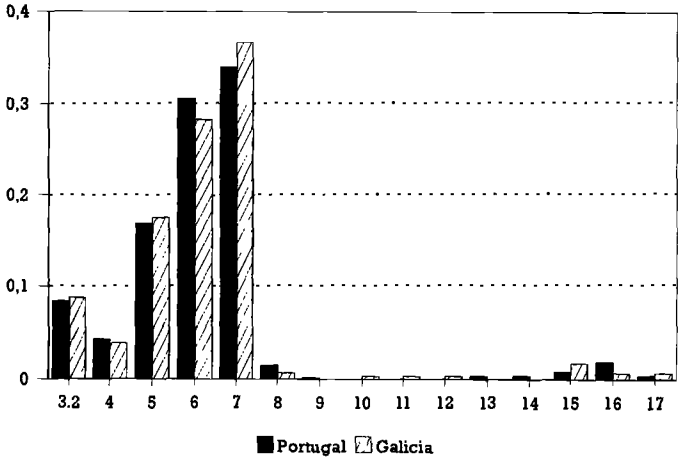

FIG. 3—*HUMF13A1 allele frequencies in two different Caucasian populations.*

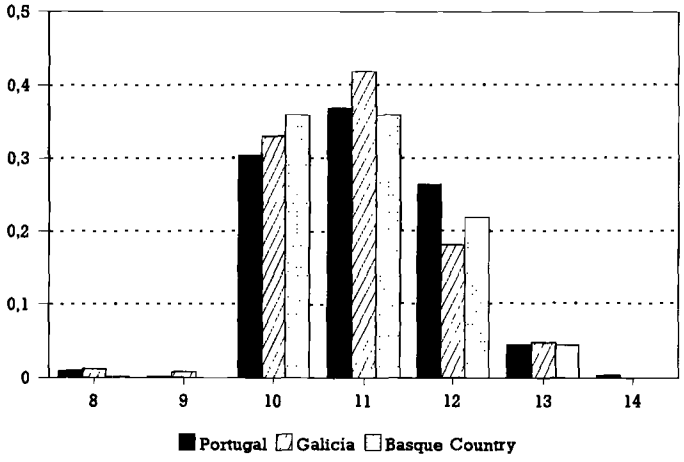

FIG. 4—*HUMFES/FPS allele frequencies in three different Caucasian populations.*

TABLE 5—*Statistical parameters of medico-legal interest for the STR systems HUMTH01, HUMVWA31A, HUMF13A1, and HUMFES/FPS.*

System	H	PD	CE	EB
HUMTH01	79.95	0.921	0.578	9.578
HUMVWA31A	80.31	0.940	0.635	9.496
HUMF13A1	77.58	0.900	0.534	9.547
HUMFES/FPS	64.06	0.850	0.432	9.684

H: Heterozygosity value.
PD: Power of discrimination.
CE: Chance of exclusion.
EB: Biostatistical efficiency.

distribution leading to a high forensic efficiency with a combined power of discrimination of 0.999.

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