TECHNICAL NOTE

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Population Study of HUMTH01, HUMVWA31/A, HUMF13A1, and HUMFES/FPS Systems in Azores

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ABSTRACT: The tetrameric short tandem repeat polymorphisms HUMTH01, HUMVWA31/A, HUMF13A1, and HUMFES/FPS were studied in blood stains obtained from a population of unrelated individuals from the Azores Archipelago (Portugal). Gene frequencies were determined and no deviation from the Hardy-Weinberg equilibrium was found. However, the allelic independence test between loci showed linkage disequilibrium between HUMVWA31 /A and HUMFES/FPS. A combined discrimination power and chance of exclusion of, respectively, 0.9999 and 0.9534, reveal the high forensic interest of the four systems. No differences with other caucasoid populations were found, but comparison with some asiatic, eskimo, and amerindian populations showed significant statistical differences.

KEYWORDS: forensic science, DNA typing, population genetics, HUMTH01, HUMVWA31/A, HUMF13A1, HUMFES/FPS, Azores, Portugal

Amplification of short tandem repeats (STRs) by polymerase chain reaction and subsequent electrophoresis of the amplified products is the most promising DNA analysis procedure for forensic investigations. A tetraplex STR amplification was developed (1–4) incorporating the polymorphisms HUMTH01 (5–7), HUMVWA31/A (8–11), HUMF13A1 (12), and HUMFES/FPS (13,10).

Interpretation of forensic STR data includes the calculation of an STR profile frequency which is dependent upon a previously generated relevant population database. Emigration from Azores to other places has been significant since the 18th century, and currently, more than 460,000 Azorians live in the United States of America. The aim of this study was to establish an Azorian population database, allowing comparisons with other populations and forensic investigations in the Azores Archipelago (Portugal) and in the U.S.A.

Materials and Methods

DNA was extracted (14) using "Chelex 100" (Sigma, St. Louis) from 3 mm^2 of cotton fabric blood stains obtained from unrelated individuals from the Azores Archipelago by venipuncture of peripheral blood. Reaction mix contained per sample 200 μm of each nucleotide (Pharmacia Biotech, Uppsala, Sweden), 100 mM tris-HCl (pH 8.3), 500 mM KCl, 15 mM $MgCl_2$, and 0.1% gelatin, 1.25 U Amplitaq polymerase (Perkin-Elmer, Roche Molecular Systems, Branchburg, New Jersey) and 0.15 μM of primers (see below) VWA/1 and VWA/2, 0.18 μM of primers TH01/1 and TH01/2, 0.16 μM of primers F13A1/1 and F13A1/2 and 0.055 μM of primers FES/1 and FES/2 (Oswell DNA Service, Southampton, UK):

TH01/1: 3′ - GTGGGCTGAAAAGCTCCCGATTAT-FAM
TH01/2: 5′ - GTGATTCCCATTGGCCTGTTCCTC
VWA/1: 5′ - CCCTAGTGGATGATAA-GAATAATCAG-TATG-JOE
VWA/2: 3′ - GGACAGATGATAAATACATAGGATG-GATGG
F13A1/1: 3′ - ATGCCATGCAGATTAGAAA-JOE
F13A1/2: 5′ - GAGGTTGCACTCCAGCCTTT
FES/1: 5′ - GGGATTTCCCTATGGATTGG-FAM
FES/2: 3′ - GCGAAAGAATGAGACTACAT

The multiplexed PCR amplification of the four loci included 5 ng of template DNA. The Perkin-Elmer 480 and 9600 (Foster City, CA) cycling parameters were as follows: 28 cycles of 95°C-1 min; 54°C-1 min, and 72°C-1 min. The samples were heat denatured at 95°C for 4 min before being loaded and electrophoresis was carried out in a 6% polyacrylamide sequencing gel on an ABI 373-A DNA Sequencer using the internal standard Genescan ROX (6-carboxyrhodamin) 2500 (Foster City, CA), during 6 h at constant power (30 W, 2500 V, and 40 mA). Fragment sizes were determined automatically using the Genescan Software (version 3.1a)

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and typed by comparison with sequenced allelic ladders - allelic designation made according to the recommendations of the DNA Commission of the International Society for Forensic Haemogenetics (15–17).

Possible divergence from the Hardy-Weinberg expectations was checked according to the exact test proposed by Guo and Thompson (18) based on the Markov chain approach. An unbiased estimate of heterozygosity was computed according to Nei (19), discrimination power according to Jones (20) following Fisher's method (21) and chance of exclusion according to Ohno (22). To test linkage disequilibrium and homozygosity excess the exact tests proposed on the Genepop program (23) were used. Comparison of population data was carried out using an exact test with the STRUC program (24).

Results and Discussion

For the four systems studied the gene frequencies in the Azores population are presented in Table 1. The most frequent alleles were the 9.3 in HUMTH01, alleles 16, 17, and 18 in HUMVWA31/A, allele 7 in HUMF13A1 and allele 11 in HUMFES/FPS like in other Caucasoid populations: Galicia-Spain (25), Italy (26), Switzerland (27,28), Germany (29,30), Austria (31,32), Britain (33), Denmark (34), North Poland (35), Zagreb-Croacia (36), and Hungary (37).

For all systems the Hardy-Weinberg equilibrium could be confirmed.

Only the systems HUMTH01 and HUMVWA31/A showed heterozygosity values >70%, one of the selection criteria proposed by Gill et al. (38) and Urquhart et al. (39) when choosing candidate loci for forensic application, although higher values (≈90%) were achieved with some (more informative) polymorphisms (D12S391, HUMFIBRA/FGA, HUMACTBP2).

The "a priori" probability that a falsely accused father will be excluded (chance of exclusion - CE) had the highest value in HUMVWA31/A and HUMTH01 systems, the four loci having a combined chance of exclusion of 95.34%, lower than the 99.9% required in paternity investigations (40).

The probability that 2 non-related random individuals do not share the same genotype, the discrimination power - DP - was >0.8 in the four systems, a selection criterion proposed by Urquhart et al. (39) but only HUMTH01 and HUMVWA31/A showed values higher than 0.9 as proposed by Gill et al. (38).

The pairwise comparisons between loci showed linkage disequilibrium ($P<0.01$) in HUMVWA31/A - HUMFES/FPS systems. Considering that these two loci are located on different chromosomes, linkage disequilibrium between these systems could be explained by genetic substructure. The Azores Archipelago was populated in the 15th century by Portuguese families from the south of the country, but some of the nine islands were populated by individuals from the north of Portugal, by the Flemish and later Arabs, Jews, French, British, and North-Americans. If there was genetic differentiation among the ancestral populations and if there are endogamous groups, genetic substructure could be a possible explanation for the linkage disequilibrium, even if all loci in the whole population meet the Hardy-Weinberg expectations. The lack of statistically significant deviations from the Hardy-Weinberg equilibrium does not imply the absence of substructure (41). If the sub-

TABLE 1—*Gene frequencies and statistical parameters of forensic interest for the HUMTH01 (n=147),* HUMVWA31/A *(n=141),* HUMF13A1 *(n=144)* and HUMFES/FPS *(n=141) systems in the Azores' population.*

Loci	TH01		VWA		F13		FES	
Allele	(N)	Prop.	(N)	Prop.	(N)	Prop.	(N)	Prop.
3.2					(33)	0.115		
4					(11)	0.038		
5					(53)	0.184		
6	(64)	0.218			(93)	0.323		
7	(45)	0.153			(95)	0.330		
8	(39)	0.133			(2)	0.007	(3)	0.011
9	(47)	0.160					(3)	0.011
9.3	(92)	0.313						
10	(7)	0.024					(89)	0.316
11							(106)	0.376
12							(63)	0.223
13							(17)	0.060
14			(23)	0.082			(1)	0.004
15			(41)	0.145				
16			(80)	0.284	(1)	0.004		
17			(67)	0.238				
18			(57)	0.202				
19			(13)	0.046				
20			(1)	0.004				
Exact test:								
P=	0.9322±0.0012		0.9032±0.0019		0.0912±0.0067		0.1420±0.0030	
h±se	0.823±0.032		0.752±0.036		0.694±0.038		0.660±0.040	
DP	0.9180		0.9262		0.8856		0.8598	
Combined							0.9999	
CE	0.5839		0.5913		0.5031		0.4489	
Combined							0.9534	

N: alleles number, Prop: proportion.
h: heterozygosity, DP: discrimination power, CE: chance of exclusion.

TABLE 2—*Genotype values comparisons between the Azores' population and other populations.*

Population	Exact Test (P±se)			
Compared	TH01	VWA	F13	FES
*Galicia-Spain (25)	0.750±0.003	0.931±0.002	0.416±0.008	0.110±0.003
*Italy (26)	–	0.517±0.005	–	0.119±0.004
*Switzerland (27)	–	0.173±0.004	–	0.050±0.002
†Basel-Switzer (28)	0.973±0.001	–	–	
†Germany (29)	–	–	–	0.030±0.001
*SW Germany (30)	0.934±0.002	0.075±0.002	–	–
†Cauc. Austria (31)	0.696±0.004	0.981±0.001	–	–
†West. Austria (32)	–	–	0.919±0.003	0.019±0.001
†Cauc. Britain (33)	0.759±0.004	0.335±0.004	–	–
*Denmark (34)	0.064±0.002	–	–	–
†North Poland (35)	0.975±0.001	0.523±0.005	–	0.805±0.003
†Zag-Croacia (36)	0.785±0.003	0.669±0.004	–	–
†Hungary (37)	0.828±0.003	0.591±0.005	–	0.012±0.001
*Japan (44)	0.000±0.000	–	–	–
†Central Japan (45)	–	0.000±0.000	0.000±0.000	–
*Toquio-Japan (46)	–	–	–	0.000±0.000
*South China (47)	0.000±0.000	0.000±0.000	–	–
*Quech.-Bolivia (48)	0.000±0.000	0.002±0.000	–	–
*Greenland Esq. (34)	0.000±0.000	–	–	–

Comparison with observed* or expected† genotype values.
Cauc.→caucasoid; Zag.→Zagreb; Quech.→Quechua Amerindians.
"–" data not published.

groups differ in their allelic frequencies at a given locus, an excess of homozygotes could be apparent in the sample (42), and therefore we tested homozygosity excess. No significant values were found; so we were also able to explain the linkage disequilibrium found as an artefact due to sampling phenomenon, as in other studies (43).

Comparisons of genotype values showed no significant differences ($P>0.01$) between population data from this study and data from other Caucasoid populations (Table 2), but there were significant statistical differences ($P<0.01$) with some asiatic (44–47), eskimo (34), and amerindian populations (48). These differences can be justified because those populations were not related with the colonization of the Azores and are located in very distant places from the Azores Archipelago. HUMFES/FPS genotype comparisons with some European Countries - Germany (29), Western Austria (32), and Hungary (37) - showed no significant differences ($P>0.01$) but P was less than 0.05.

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