TECHNICAL NOTE

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Population Frequency Distribution of HumF13A01, HumFXIIIB, and HumLIPOL Loci in the Basque Country (Northern Spain)

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ABSTRACT: A population study of unrelated individuals from the Basque Country (Northern Spain) was carried out using the GenePrint STR System. The PCR products were separated on denaturing polyacrylamide gels and visualized by silver staining. Three tetrameric loci were evaluated: HumF13A01, HumFXIIIB, and HumLIPOL. All loci fit Hardy-Weinberg expectations, and independence of alleles was found between these STR loci. A comparison with other population groups indicated allele frequencies are well conserved in Caucasians, but differ from other racial groups. The calculated parameters *a priori* probability of exclusion (Pex) and "power of discrimination" (PD) show how informative these loci are for the determination of identity and relatedness of individuals.

KEYWORDS: forensic science, forensic genetics, STR, HumF 13A01, HumFXIIIB, HumLIPOL

Commonly, ADN microsatellite loci used in forensic genetics reach values higher than 0.5 in polymorphic information content (PIC) in every studied population. However, the high PICs of these loci differ between populations. Therefore, there are differences in the PD and Pex values in different populations. Due to these variations, the allele distribution of populations is of interest before using any microsatellite locus in paternity testing and forensic casework.

We had previously analyzed the allele frequency distribution of five microsatellite loci in a Basque resident population sample (1,2). In this study, three more STR loci (HumF13A01, HumFXIIIB, and HumLIPOL) were studied. The population sample has been collected from several regions in the Basque Country, but it corresponds mainly to the Bilbao area because this is the most populated region in the Basque Country.

Materials and Methods

Sample Collection

The population sample consists of Basque Country-resident individuals (183 individuals for F13A, 306 for FXIIIB, and 212 for LIPOL). These individuals represent a pool of Northern Spain populations, with some components from west and south-west Iberian Peninsula populations, excluding Mediterranean groups.

Amplification and Genetic Typing of Amplification Products

Each locus was amplified independently in an Omn-E Hybaid (Hybaid Ltd., Teddington, UK) and Perkin-Elmer 9600 (Foster City, CA) thermalcyclers using the HumF13A01, HumFXIIIB, and HumLIPOL GenePrint™ (Promega, Madison, WI) (3) simplex systems with 1 μL of BSA (10 μg/μL) (Boehringer Mannheim, Germany) and 10 ng of DNA template per reaction. The post-amplification products were evaluated by electrophoresis in 2% agarose (Hispanlab SA, Madrid, Spain) gel with ethidium bromide (Merck, Darmstadt, Germany).

Although the amplification of the three loci was carried out independently for each one, the electrophoresis of the three amplification products thus obtained was performed in the same 6% denaturing acrylamide (Boehringer Ingelheim Bioproducts, Germany) gels (7 M urea, 46 cm long, and 0.4 mm thick) in a Cambridge Electrophoresis unit (Cambridge, UK) for 3 h and 30 min. To achieve a clear separation of the alleles, the samples were loaded twice. The first time we loaded the HumF13A01 and HumFXIIIB amplified products, which need a long migration time to separate their alleles, specially HumF13A01 because it has a high number of alleles that spread along the 283–331 bp range. The second time (30 min after) HumLIPOL was loaded, which has smaller-sized alleles than HumF13A01 and HumFXIIIB. Allelic ladders (GenePrint™) were applied every two lanes. The gels were run at 1500 V. Bands were detected by silver staining as described by Budowle et al. (1991) (4).

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Statistical Approaches

An unbiased estimate of heterozygosity was obtained following Nei (1978) (5). Polymorphism Information Content (PIC) values were obtained as Botstein et al. (1980) (6). To check possible departures from equilibrium, the Guo and Thompson exact test (7) was performed.

Genotypic linkage disequilibrium among pairs of loci (i.e., statistical independence between loci) was tested by Genepop statistical package (8).

Allele frequencies were compared by means of X^2 (chi squared) tests with those from other previous populations extracted from bibliography. For the comparison, we only took into account allelic classes whose frequencies do not make it necessary to introduce corrections in the X^2 tests. Those alleles with frequency lower than 5 were discarded.

Results and Discussion

The allele distribution of the studied population is shown in Table 1. The allele 12 of HumFXIIIB locus has not been detected in our population. It is possible to find the alleles 16 and 17 of HumF13A01 locus as well as the allele 14 of HumLIPOL locus that do not appear in all populations.

The expected and observed numbers of homozygous and heterozygous do not differ significantly. The studied population sample is in Hardy-Weinberg equilibrium for the three systems as indicated by the results of the test of Guo and Thompson (7) (Table 1). In addition, allelic independence between these loci was found.

PIC values of these loci are very similar, the HumF13A01 locus being the most informative in our population (0.690), while the HumFXIIIB locus shows the lowest value (0.658). The PIC parameter is related with the power of discrimination (PD) being the highest PD also for the HumF13A01 locus (0.879), while the lowest discriminative locus is HumFXIIIB (0.857). As for paternity testing, HumF13A HumF1 locus shows the highest Pex (0.503), while HumFXIIIB locus shows the lowest (0.459) (Table 1).

The results of the comparisons between our population sample and other populations are given in Table 2. HumF13A01 locus has been extensively studied; therefore, we have compared many populations. The only statistically significant differences found in the comparisons with Caucasian populations have been for Central Spain, France, and Italy. Of these, only Central Spain and Italian population data could be compared for HumFXIIIB locus. The result of this comparison is again statistically significant for the Italian data, though no statistically significant difference has been detected between our sample and Central Spain. The differences observed could be due to the lack of Mediterranean component in the Basque resident sample, though they could also be spureous differences. An exhaustive comparison of a greater number of loci between these populations could give a solution.

The HumLIPOL locus has been reported in very few populations. We have found differences in three of the four comparisons between Caucasian populations and our sample (Galicia, Austria, and Hungary) (Table 2). The most surprising difference observed takes place in the Galician population, which demonstrates no differences in the majority of the previously studied loci (9,10). However, the low number of Caucasian populations published for this locus, does not allow us to infer if the HumLIPOL locus has a high level of variation between Caucasian populations.

Finally, the comparisons between our population sample and other racial groups (Black USA, Hispanic USA, and Japanese) show statistically significant differences ($p < 0.001$) in every studied loci.

TABLE 1—*Allele distribution of HumF13A01, HumFXIIIB, and HumLIPOL loci.*

Allele	HumF13A01 ($N = 183$) Frequencies ± sd		HumFXIIIB ($N = 306$) Frequencies ± sd		HumLIPOL ($N = 212$) Frequencies ± sd	
3.2	0.04645	0.011				
4	0.05191	0.012				
5	0.18579	0.020				
6	0.33880	0.025	0.09314	0.012		
7	0.33333	0.025	0.01471	0.005	0.00236	0.002
8	0.00273	0.003	0.25980	0.018		
9			0.22712	0.017	0.04009	0.009
10			0.40359	0.020	0.33255	0.023
11			0.00163	0.002	0.31604	0.023
12					0.23349	0.021
13	0.00273	0.003			0.07075	0.012
14	0.00546	0.004			0.00472	0.003
15	0.01913	0.007				
16	0.00820	0.005				
17	0.00546	0.004				
Homozygotes obs/exp	42/48.268		103/88.649		62/57.219	
Heterozygotes obs/exp	141/134.731		203/217.350		150/154.780	
Heterozygosity obs	0.770		0.663		0.707	
Heterozygosity exp	0.736 ± 0.011		0.710 ± 0.008		0.730 ± 0.009	
Guo and Thompson test	$p = 0.106$		$p = 0.443$		$p = 0.740$	
PIC	0.690		0.658		0.680	
PD	0.879		0.857		0.874	
Pex	0.503		0.459		0.484	

N: Number of individuals.
PIC: Polymorphic Information Content.
PD: "power of discrimination."
Pex: Probability of exclusion.

TABLE 2—*Inter-population comparisons.*

	HumF13A01					HumFXIIIB					HumLIPOL				
Population	*N*	X^2	df	*p*	Ref	*N*	X^2	df	*p*	Ref	*N*	X^2	df	*p*	Ref
Galicia	143	7.05	5	0.217	(9)						113	9.64	4	0.047	(9)
Catalonia						141	3.46	5	0.629	(11)					
Central Spain	199	10.27	4	0.036	(12)	196	3.66	4	0.454	(12)					
North Portugal	232	10.06	6	0.122	(13)										
Central Portugal	344	9.46	4	0.051	(14)										
South Portugal	108	5.22	5	0.390	(15)										
France	234	13.69	5	0.018	(16)										
Italy	221	13.36	6	0.038	(17)	119	11.55	4	0.021	(18)					
Holland	195	5.36	5	0.374	(19)										
Germany	163	6.53	6	0.367	(20)	289	1.17	4	0.884	(21)					
Switzerland	425	3.38	5	0.642	(22)	205	2.96	4	0.564	(23)					
Austria	382	2.16	4	0.707	(24)	216	0.42	4	0.981	(25)	550	20.78	4	0.0003	(26)
Hungary	223	3.45	5	0.632	(27)	223	3.02	4	0.554	(27)	223	12.98	4	0.011	(27)
Norway	300	2.73	5	0.7416	(28)										
USA Caucasians	209	3.63	4	0.459	(3)	207	0.53	4	0.971	(3)	204	6.79	4	0.147	(3)
Black USA	218	112.83	9	0.0001	(3)	220	281.00	4	0.0001	(3)	219	53.69	4	0.0001	(3)
Hispanic USA	222	93.43	9	0.0001	(3)	217	44.30	4	0.0001	(3)	210	28.38	4	0.0001	(3)
Japan	392	478.41	9	0.0001	(29)	367	302.60	4	0.0001	(29)	337	519.17	4	0.0001	(29)

NOTE: Ref: (9)Pestoni et al. (1995); (11) Gené et al. (1997); (12) Martín et al. (1995); (13) Pinheiro et al. (1997); (14) Souto et al. (1996); (15) Geada et al. (1996); (16) Rousselet et al. (1996); (17) Dobosz et al. (1996); (18) Piccinini et al. (1996); (19) Sjerps et al. (1995); (20) Puers et al. (1993); (21) Augustin et al. (1996); (22) Kratzer et al (1996); (23) Dimo-Simonin et al. (1997); (24) Ambach et al. (1996); (25) Neuhuber et al. (1996); (26) Glock et al. (1996); (27) Füredi et al. (1997); (28) Dupuy et al. (1994); (3) Promega Corporation (1996); and (29) Nakamura et al. (1996).

In conclusion, HumF13A01 and HumFXIIIB loci do not show significant differences between Caucasian populations. It is clear that the use of particular genetic databases for each population is the most appropriate tool to calculate probabilities in forensic casework. However, in some casework, this is not possible. In this case, the use of other Caucasian databases does not have great influence in the final result of Pex and PD probabilities whenever Caucasian individuals are involved. The HumLIPOL locus shows some differences between Caucasian populations. Thus, the aforementioned possibility of other caucasian database extrapolation should be avoided.

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