ELSEVIER

Forensic Science International
110 (2000) 167–177

Forensic Science International

www.elsevier.com/locate/forsciint

A study on ten short tandem repeat systems: African immigrant and Spanish population data

J.J. Gamero[a,*], J.L. Romero[a], J.L. Gonzalez[a], M.I. Arufe[a], M.I. Cuesta[a], F. Corte-Real[b], M. Carvalho[b], M.J. Anjos[b], D.N. Vieira[b], M.C. Vide[b]

[a]*Department of Legal Medicine, Faculty of Medicine, University of Cadiz, Plz. Fragela s/n, Cadiz 11003, Spain*
[b]*Institute of Legal Medicine of Coimbra, 3000 Coimbra, Portugal*

Received 28 August 1999; received in revised form 20 February 2000; accepted 24 February 2000

Abstract

This work presents the results obtained from a genetic–population study for the D1S1656 system in the population of Southwest Spain (Huelva, Cádiz and Sevilla), Spaniards of Caucasian origin from North Africa (Ceuta), as well as in the black Central West African and Moroccan immigrant populations in Spain. The results of a study of the autochtonous population of the Canary Islands ($n=138$), and immigrant Central West African populations in Spain ($n=132$), obtained for nine short tandem repeat (STR) loci (D3S1358, VWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820), as well as the amelogenin locus, all contained in Profiler Plus™ (Perkin-Elmer) PCR amplification kits, are also presented. Except for the FGA and VWA data on immigrant Central West African populations in Spain, no deviations from the Hardy–Weinberg equilibrium were detected. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Short tandem repeats; Polymorphism; Population genetics; Forensic analysis

1. Introduction

The goal of this paper was to study a recently introduced STR, D1S1656 [1], in four different populations from Spain, North Africa and Central West Africa (the latter being

*Corresponding author. Fax: +34-56-223-139.
E-mail address: joaquin.gamero@uca.es (J.J. Gamero)

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PII: S0379-0738(00)00166-3

a small population sample of 108). These populations are specifically those from Southwest Spain (Cadiz, Huelva and Sevilla), the Spanish population of Caucasian origin in North Africa as well as the Moroccan and Central West African immigrant populations that use the south of Spain as a point of entry into the European Community (EC). Likewise, an automated fluorescence-based multiplex DNA profiling system using the Profiler Plus™ PCR amplification kit (Perkin-Elmer, Norwalk, CT, USA), in which the repeat regions of the nine STR loci are co-amplified: D3S1358, VWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820 and the amelogenin gene, was used on two populations. One population sample comprised individuals from the autochtonous population of the Island of Gran Canaria and the other of the black immigrant population of Central West Africa.

2. Materials and methods

Whole EDTA blood was obtained by venipuncture from 198 Spaniards from the south-west of Andalusia (Cadiz, Huelva and Seville), 88 unrelated Spaniards of Caucasian origin from North Africa (Ceuta), 101 unrelated immigrants from Morocco, 132 unrelated black Central West African immigrants from different countries situated to the south of the Sahara (Cameroon, Mali, Liberia, Sierra Leona, Gambia, Ruanda, Guinea, Guinea Bissau, Burkina Faso, Ivory Coast, Senegal), and 138 unrelated individuals from the autochtonous population of the Canary Islands. For this latter group only those individuals who descend from at least three generations born in the Canary Islands qualified for selection. It must also be pointed out that, of the 132 West African immigrants studied with the Profiler Plus™ amplification kit, 108 immigrants were also studied for the D1S1656 system.

The DNA was extracted using Chelex 100 protocol as described by Walsh et al. [2]. The quantity of recovered DNA was determined using QuantiBlot® human DNA quantitation kit (Perkin-Elmer).

Amplification by PCR of the STR loci was performed using the Profiler Plus™ Kit (Perkin-Elmer) using 1.0–2.5 ng DNA, according to the manufacturer's recommendations. For the D1S1656 locus, 5-μl aliquots of the extracts with a DNA content of approximately 5 ng/μl were used for amplification. Primers: forward primer 5′ GTG TTG CTC AAG GGT CAA CT, reverse primer 5′ GAG AAA TAG AAT CAC TAG GGA ACC (* Fluorescein labelled at the 5′ end). The D1S1656 locus was amplified as described by Lareu et al. [1]. The PCR was performed in a Perkin-Elmer 9600 thermocycler.

Electrophoresis was carried out on 4% polyacrylamide denaturing sequencing gels on a 377 automated system (Applied Biosystems Division/Perkin-Elmer). The length of the amplified DNA fragments was determined by using internal lane standard Genescan-500 ROX (Perkin-Elmer) and Genescan-2500 ROX (Perkin-Elmer), when Profiler Plus™ Kit (Perkin-Elmer) and D1S1656 were analyzed, respectively.

For the amelogenin locus and nine STR loci: D3S1358, VWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317 and D7S820 (Profiler Plus™ Kit, Perkin-Elmer), allele designations were made using Genescan PCR analysis software with local

Southern method and by comparison with allelic ladders provided by the manufacturer. For the D1S1656 locus, allele designations were made using Genescan PCR analysis software with local Southern method and by comparison with allelic ladders kindly provided by Lareu et al. [1] (Santiago de Compostela, Spain).

Statistical analysis of the results was performed using the following test. Hardy–Weinberg equilibrium was performed using the exact test according to Guo and Thompson [3] involving the GENEPOP programme (version 3.1b, 1997) [4]. An unbiased estimate of heterozygosity was computed according to Nei [5]. The chance exclusion (CE) for paternity was calculated according to Ohno et al. [6] and the discrimination power was calculated according to Jones [7]. Comparisons of the allele frequencies between different populations were performed by χ^2 statistic for two-way contingency tables (BMDP programme) [8] and the STRUC program [9]. Furthermore, using the PHYLIP 3.5c package [10], neighbor-joining trees [11] were obtained from the genetic distances of Reynolds et al. [12] and also Nei [13]. The statistical robustness of their nodes were tested through a bootstrap approach [14,15]. Loci were resampled with replacement 1000 times.

3. Results and discussion

We have analyzed the STR loci D1S1656 in samples from two Spanish populations and two African immigrant populations to obtain allele and genotype frequencies and hence ascertain the utility of this loci in forensic stain analysis and paternity testing in the populations studied. The accurate identification for the D1S1656 system of the neighbouring alleles such as the 15.3 and 16 did not cause any problems, being clearly differentiated by means of the methodology recommended by Lareu et al. [1].

Allele frequencies and parameters of forensic efficiency for the locus D1S1656 studied in each population are shown in Table 1.

After the statistical analysis for the D1S1656 system (Table 1), the studied populations were observed to be in Hardy–Weinberg equilibrium for the analyzed marker. The other statistical parameters (Table 1), show that this loci is a robust and discriminating system, very useful in routine forensic casework, above all when used together with other markers.

In addition, when a comparative study between the populations studied for the D1S1656 system included in this paper and those analyzed in the Iberian Peninsula (Portuguese, Galician, Aragonese and Andalusian) for the same marker was carried out using two-way contingency tables [1,16–18], highly significant differences were observed between the Moroccan immigrant population and the black West African population as well as between these two populations and the rest of the analyzed populations in Table 2. Similarly, the significant differences between the Aragonese population (North East Spain) [17] and almost all the other Spanish and Portuguese populations analyzed (Andalusia [18], South West Spain, the Spanish population of North Africa and Central Portugal [16]) must also be pointed out.

Likewise, Fig. 1 shows the neighbor-joining tree [11] obtained from the genetic distances of Reynolds et al. [12] for a group of eight populations, six of Caucasian

Table 1
Allele frequencies, forensic efficiency parameters and *P* values for the exact test for Hardy–Weinberg equilibrium (HWE) for D1S1656 in the four populations studied.

Allele	South West (Spain)	North African (Spain)	Moroccan immigrant	Central West African black immigrant	Galicians N.W. Spain [1]	Aragon N.E. Spain [17]	Andalusia South Spain [18]	Central Portugal [16]
n	198	88	101	108	125	104	126	257
10	0.003	0.000	0.010	0.032	0.004	0.000	0.000	0.004
11	0.058	0.062	0.039	0.028	0.064	0.058	0.063	0.072
12	0.143	0.153	0.178	0.046	0.160	0.144	0.147	0.140
13	0.048	0.068	0.094	0.116	0.076	0.053	0.071	0.054
13.3	0.003	0.000	0.005	0.018	0.000	0.000	0.000	0.000
14	0.124	0.114	0.079	0.338	0.092	0.087	0.091	0.126
14.3	0.010	0.006	0.044	0.005	0.000	0.000	0.000	0.006
15	0.188	0.148	0.139	0.162	0.164	0.159	0.175	0.160
15.3	0.038	0.057	0.094	0.014	0.080	0.063	0.056	0.053
16	0.106	0.153	0.079	0.088	0.088	0.087	0.091	0.124
16.3	0.048	0.028	0.094	0.074	0.016	0.000	0.060	0.025
17	0.061	0.040	0.030	0.018	0.088	0.149	0.044	0.053
17.3	0.104	0.125	0.054	0.046	0.128	0.120	0.143	0.119
18	0.010	0.000	0.005	0.000	0.000	0.000	0.000	0.004
18.3	0.045	0.040	0.035	0.014	0.036	0.072	0.036	0.041
19.3	0.008	0.006	0.020	0.000	0.004	0.010	0.020	0.017
20.3	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.002
H[a]	0.891	0.887	0.901	0.826				
PD[a]	0.978	0.977	0.982	0.953				
CE[a]	0.781	0.771	0.800	0.675				
P[a]	0.344	0.667	0.751	0.283				

[a] H, observed heterozygosity; PD, power of discrimination; CE, a priori chance of exclusion; *P*, Hardy–Weinberg equilibrium exact test.

Table 2
χ^2 comparisons between different populations for D1S1656 locus

Populations[a]	χ^2	P
Moroc. I. — W. Afric. I.	82.883	0.0000
Moroc I. — N. Afric. (Spain)	31.939	0.0066
Moroc I. — S-W Spain	41.952	0.0004
Moroc. I. — Galicia [1]	45.537	0.0001
Moroc. I. — C. Portugal [16]	57.111	0.0000
Moroc. I. — Aragon [17]	63.087	0.0000
Moroc. I. — Andalusia [18]	32.811	0.0050
W. Afric. I. — N. Afric. (Spain)	71.889	0.0000
W. Afric. I. — S-W Spain	95.186	0.0000
W. Afric. I. — Galicia [1]	105.430	0.0000
W. Afric. I. — C. Portugal [16]	117.567	0.0000
W. Afric. I. — Aragon [17]	122.172	0.0000
W. Afric. I. — Andalusia [18]	91.496	0.0000
N. Afric. (Spain) — S-W Spain	11.731	0.7623
N. Afric.(Spain) — Galicia [1]	11.917	0.5345
N. Afric.(Spain) — C. Portugal [16]	5.431	0.9878
N. Afric.(Spain) — Aragon [17]	25.474	0.0127
N. Afric.(Spain) — Andalusia [18]	10.289	0.5906
S-W Spain — Galicia [1]	23.750	0.0951
S-W Spain — C. Portugal [16]	12.797	0.6875
S-W Spain — Aragon [17]	34.453	0.0047
S-W Spain — Andalusia [18]	17.359	0.3627
Galicia [1] — C. Portugal [16]	17.124	0.3115
Galicia [1] — Aragon [17]	12.972	0.3711
Galicia [1] — Andalusia [18]	16.092	0.1871
C. Portugal [16] — Aragon [17]	33.847	0.0036
C. Portugal [16] — Andalusia [18]	16.424	0.3544
Aragon [17] — Andalusia [18]	32.199	0.0007

[a] Moroc. I.: Moroccan immigrant; W. Afric. I.: Central West African black immigrant; N. Afric. (Spain): North African (Spain); S-W Spain: Southwest (Spain); Galicia [1]: Northwest (Spain); C. Portugal [16]: Central Portugal; Aragon [17]: Northeast Spain; Andalusia [18]: South Spain.

origin, one Arab and one Negroid, in which the D1S1656 STR loci were analyzed. A very similar topology was obtained with the Nei distance [13]. The values of the analyzed genetic distances [12] varied between 0.0011 and 0.0616, the minimum (0.0011) observed being between the populations of Central Portugal [16] and the Spanish population of North Africa. The maximum values were obtained between the genetic distances of the Negroid population and the rest of the studied populations. Within the group of analyzed Caucasian populations, the maximum distance (0.0106) was observed between the Spanish population of North Africa and Aragon (North East Spain) [17].

The different observed groupings in Fig. 1 must be singled out. The first one consists of the populations of Aragon (Northeast Spain) [17] and Northwest Spain [1]. The second grouping consists of the populations of Southwest Spain, West Africa (immigrants), Central Portugal [16] and the Spanish population of North Africa. The third one consists of the populations of Morocco (immigrants) and South Spain [18]. The

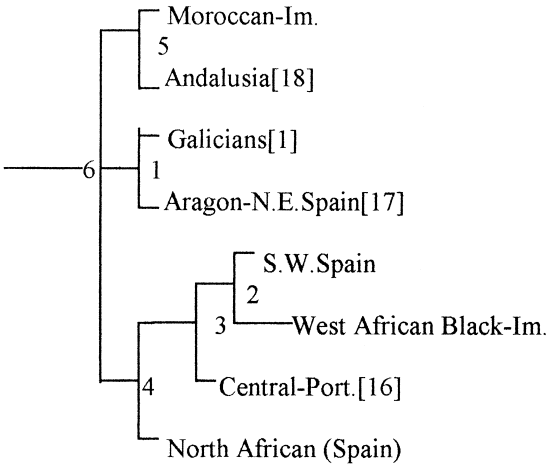

Fig. 1. Dendrogram by neighbor-joining method, for the distance of Reynolds et al. [12] for D1S1656 locus.

statistical robustness [14,15] of the neighbor-joining tree nodes is quite clear after the same topology was obtained in 100% of the replacements carried out.

On the other hand, the distribution of observed allelic frequencies and the statistical inferences in the autochthonous population of the Canary Islands (ACI) and the other of the black immigrant population of West Africa (BIWA) analyzed for the nine loci (Profiler Plus™ kit, Perkin-Elmer) are shown in Tables 3 and 4.

After the statistical analysis, the autochthonous Canary Islands population (Table 3) was observed to be in Hardy–Weinberg equilibrium for the nine analyzed systems ($P>0.01$ in the nine systems). On the other hand, when the corresponding statistical analysis was carried out for the black immigrant population of West Africa sample (Table 4), this population was observed to be in Hardy–Weinberg equilibrium for almost all the analyzed markers ($P>0.01$ in the nine systems), except for the FGA and VWA systems. Possible reasons could include inbreeding, population substructure and selection [19]. Given the influence on the genepool of the structure or composition of the West African black immigrant population (BIWA) in the south and centre of Europe, currently made up of individuals from different geographical areas of West Africa, population substructure appears to be the most likely explanation for this deviation.

When a comparative study between the populations studied (ACI and BIWA) for the nine loci included in Profiler Plus™ Kit, (Perkin-Elmer) as well as between the latter populations and other populations from Spain and Africa was carried out (Table 5), no significant differences were observed ($P>0.01$) between the West African immigrant population and the populations of Angola and Guinea Bissau [20] for the total number of markers studied. The non-existence of significant differences between the Canary Islands and Andalusian populations [21] for all the analyzed markers (except for the locus D5S818) must be mentioned.

Likewise, Fig. 2 shows the neighbor-joining tree [11] obtained from the genetic distances of Reynolds et al. [12] for a group of five populations, two of Caucasian origin

Table 3
Allele frequencies, forensic efficiency parameters and *P* values for the exact test for Hardy–Weinberg equilibrium (HWE) for nine loci (Profiler Plus™) in an autochthonous Canary Islands population sample, Spain

Allele	D3S1358	VWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
n	138	138	138	138	138	138	138	138	138
7									0.0254
8				0.0072			0.0109	0.1340	0.1123
9				0.0181		0.0072	0.0398	0.0725	0.1449
10				0.0978		0.0326	0.0688	0.0725	0.2572
11				0.1087		0.0145	0.3043	0.2754	0.2246
12				0.0978		0.1811	0.3732	0.2971	0.1739
13	0.0109			0.2645		0.0978	0.1956	0.1159	0.0580
14	0.0797	0.1159		0.2391		0.1522	0.0072	0.0326	0.0036
15	0.2283	0.1667		0.1014		0.1196			
16	0.2427	0.2681		0.0543		0.1304			
17	0.2174	0.1739		0.0109		0.1232			
18	0.1993	0.1739	0.0145			0.0471			
19	0.0217	0.0761	0.0688			0.0507			
20		0.0254	0.1413			0.0217			
21			0.1522			0.0145			
22			0.1848			0.0072			
23			0.1703						
24			0.1667						
25			0.0652						
26			0.0290						
27			0.0036		0.0072				
28			0.0036		0.1667				
29					0.2246				
30					0.2826				
30.2					0.0145				
31					0.0688				
31.2					0.0833				
32					0.0145				
32.2					0.0906				
33					0.0072				
33.2					0.0398				
H[a]	0.7951	0.8200	0.8559	0.8282	0.8199	0.7601	0.7233	0.7929	0.8155
PD[a]	0.9260	0.9432	0.9622	0.9495	0.9450	0.9742	0.8764	0.9283	0.9404
CE[a]	0.5919	0.6416	0.7086	0.6635	0.6490	0.7601	0.4832	0.6004	0.6334
P[a]	0.2340	0.0606	0.0823	0.0157	0.0101	0.0268	0.0536	0.0131	0.0892

[a] H, observed heterozygosity; PD, power of discrimination; CE, a priori chance of exclusion; *P*, Hardy–Weinberg equilibrium exact test.

and three of Negroid origin, in which nine loci were analyzed (Profiler Plus kit, Perkin-Elmer). A very similar topology was obtained with the Nei distance [13]. The values of the studied genetic distances [12] varied between 0.0041 and 0.0474. The minimum observed value (0.0041) between the Canary Islands (Spain) and Andalusian (Spain) [21] populations must be highlighted. The maximum value (0.0474) was

Table 4
Allele frequencies, forensic efficiency parameters and *P* values for the exact test for Hardy–Weinberg equilibrium (HWE) for nine loci (Profiler Plus™) in a black immigrant population sample

Allele	D3S1358	VWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
n	132	132	132	132	132	132	132	132	132
7									0.0076
8							0.1666	0.0114	0.1780
9							0.0114	0.0038	0.1136
10				0.0076		0.0076	0.0606	0.0265	0.3220
11		0.0038		0.0379			0.1553	0.2652	0.2614
12		0.0038		0.1250		0.0606	0.3561	0.4507	0.0796
13	0.0152	0.0151		0.1667		0.0492	0.2311	0.1780	0.0379
13.2						0.0038			
14	0.0606	0.0795		0.3447		0.0416	0.0151	0.0606	
14.2						0.0076			
15	0.3106	0.1894		0.2462		0.0909	0.0038	0.0038	
16	0.3901	0.2614		0.0492		0.2576			
17	0.1742	0.2121		0.0189		0.1932			
18	0.0417	0.1250	0.0189	0.0038		0.1174			
18.2			0.0038						
19	0.0076	0.0644	0.0720			0.0682			
19.2			0.0076						
20		0.0341	0.0455			0.0492			
21		0.0114	0.0833			0.0379			
22			0.2348			0.0114			
23			0.1553			0.0038			
24			0.1970						
25			0.0758						
26			0.0454						
27			0.0227		0.0568				
28			0.0189		0.2348				
29			0.0038		0.2045				
30			0.0038		0.1439				
30.2			0.0038		0.0076				
31					0.0871				
31.2			0.0038		0.0492				
32			0.0038		0.0152				
32.2					0.0947				
33									
33.2					0.0492				
34					0.0151				
34.2					0.0076				
35					0.0151				
35.2					0.0038				
36					0.0114				
38					0.0038				
H[a]	0.7152	0.8232	0.8586	0.7729	0.8567	0.8577	0.7639	0.6903	0.7756
PD[a]	0.8713	0.9457	0.9652	0.9156	0.9643	0.9656	0.9088	0.8554	0.9160
CE[a]	0.4749	0.6512	0.7212	0.5677	0.7174	0.7221	0.5499	0.4469	0.5676
P[a]	0.5493	0.0052	0.0000	0.2823	0.0738	0.0413	0.2369	0.3581	0.0428

[a] H, observed heterozygosity; PD, power of discrimination; CE, a priori chance of exclusion; *P*, Hardy–Weinberg equilibrium exact test.

Table 5
Genotype value comparisons for the nine loci (Profiler Plus™) between the populations analyzed and other populations: exact test ($P \pm$S.E.)

Populations compared[a]	D3S1358	VWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820
Canary I. — African B. I.	0.000±0.000	0.494±0.004	0.197±0.009	0.000±0.000	0.041±0.003	0.000±0.000	0.000±0.000	0.000±0.000	0.097±0.002
Canary I. — Andalusia [21]	0.124±0.004	0.782±0.005	0.974±0.002	0.856±0.003	0.862±0.005	0.702±0.006	0.000±0.000	0.852±0.003	0.653±0.005
Canary I. — G. Bissau [20]	0.010±0.002	0.684±0.004	0.005±0.001	0.009±0.000	0.519±0.008	0.001±0.000	0.000±0.000	0.003±0.000	0.111±0.003
Canary I. — Angola [20]	0.062±0.008	0.271±0.006	0.073±0.005	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	0.000±0.000	0.025±0.001
African B. I. — Andalusia [21]	0.000±0.000	0.193±0.003	0.027±0.003	0.000±0.000	0.006±0.001	0.000±0.000	0.000±0.000	0.000±0.000	0.236±0.004
African B. I. — G.Bissau [20]	0.633±0.003	0.993±0.001	0.837±0.004	0.618±0.006	0.252±0.007	0.597±0.005	0.226±0.009	0.914±0.001	0.737±0.001
African B. I. — Angola [20]	0.828±0.002	0.934±0.005	0.997±0.000	0.976±0.000	0.719±0.005	0.210±0.005	0.486±0.007	0.919±0.001	0.440±0.004

[a] Canary I., autochthonous Canary Islands population, Spain; African B. I., black immigrant from West Africa.

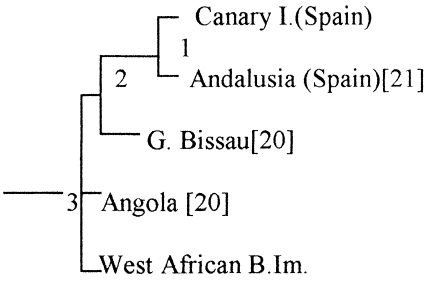

Fig. 2. Dendrogram by neighbor-joining method, for the distance of Reynolds et al. [12] for nine loci (Profiler Plus™).

obtained between the genetic distance of the Andalusian population (Spain) [21] and Guinea Bissau [20].

Finally, the different groupings observed in Fig. 2 must be considered. The grouping consisting of the populations of the Canary Islands (Spain) and Andalusia (Spain) [21] is separate from the analyzed populations of Negroid origin.The statistical robustness [14,15] of the neighbor-joining tree nodes (Fig. 2) reached 92.3% in node 1, 98.6% in 2 and 78% in 3.

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