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Population distribution of six PCR-amplified loci in Madeira Archipelago (Portugal)

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Abstract

Frequency data of the short tandem repeat (STR) loci HUMTH01, HUMVWA31/A, HUMF13A1, HUMFES/FPS, D12S391 and HUMFIBRA/FGA were determined in blood stains obtained from a population of unrelated individuals from the Madeira Archipelago. The observed genotype distribution showed no significant deviation from the Hardy–Weinberg equilibrium and there was no evidence for association of alleles among the six loci. Population data showed a combined discrimination power of 0.9999998 and a chance of exclusion of 0.99597. The frequencies are similar to those of other compared caucasian populations but significant differences were found between the Madeira population and Japanese, Chinese, Greenland Eskimos and Quechua Amerindians. The six loci studied, together proved to be highly discriminating and valuable for forensic cases.

Keywords: Short tandem repeats; HUMTH01; HUMVWA31/A; HUMF13A1; HUMFES/FPS; D12S391; HUMFIBRA/FGA; Population genetics; Madeira

1. Introduction

The STR polymorphisms HUMTH01 [1,2], HUMVWA31/A [3,4], HUMF13A1 [5], HUMFES/FPS [6], D12S391 [7], HUMFIBRA/FGA [8] are increasingly used for

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paternity testing and forensic identification. All of these systems consist of tetranucleotide repeat units with some variants.

Allele frequency data from the relevant populations must accompany the use of genetic markers.

The Madeira population originates mainly from south of Portugal, Morocco and Algeria, since the 15th century. Emigration from Madeira to other places has been significant and more than 750 000 individuals live in South Africa and Venezuela, where population databases can be useful to forensic investigations.

2. Materials and methods

DNA extraction was carried out from air-dried blood stains on cotton fabric obtained from unrelated individuals from Madeira Archipelago using Chelex method [9].

Multiplex PCR amplification of the TH01, VWA, F13A1 and FES/FPS [10] and singleplex amplification of the D12S391 and FIBRA/FGA loci used, per sample, 5 ng of DNA, 200 μm of each nucleotide (Pharmacia), 5 μl of 10× buffer (Perkin-Elmer), 1.25 U Amplitaq polymerase (Perkin-Elmer and Dynazyme-Finn Zymes Oy), 0.15 μM of primers 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, 0.055 μM of primers FES/1 and FES/2, 0.25 μM of primers D12/1 and D12/2, 0.25 μM of primers FGA/1 and FGA/2 (Oswell DNA Service).

The PCR cycling conditions were (PE 480, PE 9600 thermocyclers):

TH01, VWA, F13A1, FES/FPS: 28 cycles of 95°C, 1 min; 54°C, 1 min; 72°C, 1 min.

D12S391, FIBRA/FGA: 30 cycles of 94°C, 45 s; 60°C, 1 min; 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 (multiplex detection) and 350 (to the D12S391 and FIBRA/FGA systems), during 6 h at constant power (30 W, 2500 V and 40 mA). Fragment sizes were determined automatically using the Genescan Software 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).

Hardy–Weinberg equilibrium was tested with the exact test proposed by Guo and Thompson [11]. An unbiased estimate of heterozygosity was computed according to Nei [12], discrimination power according to Jones [13] and chance of exclusion according to Ohno et al. [14]. To test linkage disequilibrium it was used an exact test proposed on the Genepop program [15] and comparison of population data was carried out using an exact test with the STRUC program [16].

3. Results and discussion

The gene frequencies at the HUMTH01, HUMVWA31/A, HUMF13A1, HUMFES/FPS, D12S391 and HUMFIBRA/FGA systems and the evaluation of the Hardy–

Table 1
Allelic frequencies at the HUMTH01 system ($n=137$)

Allele	Frequency	Allele	Frequency
6	0.2226±0.0251	9	0.1642±0.0224
7	0.1533±0.0218	9.3	0.3029±0.0278
8	0.1533±0.0218	10	0.0037±0.0037

Exact test: $P=0.0691\pm0.0019$.

Table 2
Allelic frequencies at the HUMVWA31/A system ($n=137$)

Allele	Frequency	Allele	Frequency
14	0.1277±0.0202	18	0.1423±0.0211
15	0.1131±0.0191	19	0.0730±0.0157
16	0.2591±0.0265	20	0.0110±0.0063
17	0.2737±0.0269		

Exact test: $P=0.0343\pm0.0012$.

Weinberg equilibrium in the Madeira population are presented in Tables 1–6. There is agreement between the observed genotype values and those expected under Hardy–Weinberg equilibrium ($P>0.01$ in the six systems).

With the exception of HUMFES/FPS, all the systems showed heterozygosity values >70% (Table 7), the highest value being observed in the D12S391 marker (87.32%). The six loci showed a combined chance of exclusion (CE) of 0.99597 and a combined discrimination power (DP) of 0.9999998, the systems D12S391 and HUMFIBRA/FGA being the most informative.

Table 3
Allelic frequencies at the HUMF13A1 system ($n=137$)

Allele	Frequency	Allele	Frequency
3.2	0.0657±0.0150	12	0.0037±0.0037
4	0.0402±0.0119	13	0.0037±0.0037
5	0.1861±0.0235	14	0.0037±0.0037
6	0.2409±0.0258	16	0.0073±0.0051
7	0.4197±0.0298	17	0.0073±0.0051
8	0.0219±0.0088		

Exact test: $P=0.4539\pm0.0083$.

Table 4
Allelic frequencies at the HUMFES/FPS system ($n=140$)

Allele	Frequency	Allele	Frequency
8	0.0071±0.0050	12	0.2679±0.0265
10	0.2893±0.0271	13	0.0321±0.0105
11	0.4036±0.0293		

Exact test: $P=0.9726\pm0.0010$.

Table 5
Allelic frequencies at the D12S391 system ($n=142$)

Allele	Frequency	Allele	Frequency
15	0.0282±0.0098	21	0.1127±0.0188
16	0.0141±0.0070	22	0.0810±0.0162
17	0.1127±0.0188	23	0.0810±0.0162
18	0.2289±0.0249	24	0.0247±0.0092
19	0.1479±0.0210	25	0.0141±0.0070
20	0.1549±0.0215		

Exact test: $P=0.1811\pm0.0043$.

Table 6
Allelic frequencies at the HUMFIBRA/FGA system ($n=145$)

Allele	Frequency	Allele	Frequency
18	0.0103±0.0059	23	0.1552±0.0213
19	0.0414±0.0117	24	0.1276±0.0196
20	0.1379±0.0203	25	0.1207±0.0191
21	0.2345±0.0249	26	0.0241±0.0090
22	0.1310±0.0198	27	0.0035±0.0035
22.2	0.0069±0.0049	28	0.0069±0.0049

Exact test: $P=0.1996\pm0.0056$.

The pairwise comparisons between loci showed no linkage disequilibrium ($P>0.01$). Only pairwise test between HUMTH01–HUMF13A1 ($P=0.0387\pm0.0044$) and HUMTH01–HUMFES/FPS ($P=0.0329\pm0.0031$) showed P values <0.05.

Comparisons of genotype values showed no significant differences ($P>0.01$) between population data from this study and data from other caucasoid populations (Table 8), but we found statistical differences between Madeira population and Japanese, Chinese, Greenland Eskimos and Quechua Amerindians.

Table 7
Statistical parameters of forensic interest for the STRs studied

Systems	$h\pm$S.E.	DP	CE
HUMTH01	0.7810±0.0353	0.91063	0.57505
HUMVWA31/A	0.7883±0.0349	0.92801	0.61333
HUMF13A1	0.7664±0.0362	0.87365	0.49983
HUMFES/FPS	0.6857±0.0392	0.83379	0.40596
D12S391	0.8732±0.0279	0.95674	0.72299
HUMFIBRA/FGA	0.7862±0.0341	0.95749	0.70201
Combined		0.9999998	0.99597

h, heterozygosity; DP, discrimination power; CE, chance of exclusion.

Table 8
Genotype values comparisons between Madeira and other populations: exact test ($P \pm$S.E.)

Population compared	TH01	VWA	F13	FES	D12	FGA
Galicia, Spain* [17]	0.605±0.005	0.114±0.003	0.695±0.006	0.229±0.005	–	–
Galicia, Spain* [7]	–	–	–	–	0.376±0.004	–
Catalonia, Spain† [18]	–	–	–	–	0.863±0.003	–
Italy* [19]	–	0.085±0.003	–	0.990±0.001	–	–
North Italy† [20]	–	–	–	–	–	0.996±0.000
Switzerland* [21]	–	0.066±0.002	–	0.942±0.002	–	–
Basel, Switzerland† [22]	0.632±0.004	–	–	–	–	–
Netherlands* [23]	–	–	–	–	–	0.316±0.005
Germany† [24]	–	–	–	0.867±0.003	–	–
SW Germany* [25]	0.158±0.003	0.029±0.002	–	–	–	–
Münster, Germany* [7]	–	–	–	–	0.569±0.005	–
Germany† [26]	–	–	–	–	–	0.587±0.006
Caucasian, Austria† [27]	0.299±0.004	0.433±0.005	–	–	–	–
Western Austria† [28]	–	–	0.637±0.006	0.598±0.006	–	–
Vienna, Austria* [29]	–	–	–	–	0.626±0.005	–
Caucasian, Austria* [30]	–	–	–	–	–	0.040±0.002
Caucasian, Britain† [31]	0.284±0.004	0.063±0.003	–	–	–	–
Denmark* [32]	0.026±0.001	–	–	–	–	–
North Poland† [33]	0.618±0.004	0.171±0.004	–	0.718±0.004	–	–
Zagreb, Croatia† [34]	0.371±0.004	0.363±0.004	–	–	–	–
Hungary† [35]	0.534±0.005	0.167±0.004	–	0.928±0.002	–	–
Barany., Hungary† [36]	–	–	–	–	–	0.253±0.004
Turkey† [26]	–	–	–	–	–	0.558±0.005
Morocco† [26]	–	–	–	–	–	0.162±0.004
Japan* [37]	0.000±0.000	–	–	–	–	–
Central Japan† [38]	–	0.002±0.000	0.000±0.000	–	–	–
Tokyo, Japan* [39]	–	–	–	0.000±0.000	–	–
Japan† [26]	–	–	–	–	–	0.232±0.004
South China* [40]	0.000±0.000	0.000±0.000	–	–	–	–
Quechua Amerindians, Bolivia* [41]	0.000±0.000	0.000±0.000	–	–	–	–
Greenland Eskimos* [32]	0.000±0.000	–	–	–	–	–

Comparison with observed* or expected† genotype values.

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