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SHORT COMMUNICATION

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Population study of the short tandem repeat polymorphisms HumTH01, HumvWA31, HumFESFPS and HumF13A01 in Sicily (Southern Italy)

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Abstract Population genetic studies were carried out on randomly selected and unrelated healthy individuals from Sicily (n = 140–150 individuals) using the short tandem repeat (STR) systems HumTH01, HumvWA31, HumFESFPS and HumF13A01. After vertical electrophoresis on polyacrylamide denaturing gels 6 alleles could be identified for TH01, 9 for vWA31, 7 for FESFPS and 11 for F13A01. No significant deviations from Hardy-Weinberg were observed.

Key words Short tandem repeats · HumTH01 · HumvWA31 · HumFESFPS · HumF13A01 · PCR · Population studies · Sicily · Southern Italy

Introduction

Short tandem repeat (STR) systems are highly polymorphic loci consisting of simple, compound, or complex [1] tandemly repeated sequences (2–7 bp) extremely short in length (< 400 bp), which are amenable to amplification by the polymerase chain reaction (PCR). Because of these characteristics they are very useful for the forensic analysis of biological material containing small quantities of DNA and/or highly degraded DNA. Also, STR amplification products differing in size by as little as one base can be clearly resolved by denaturing polyacrylamide gel elecrophoresis when an appropriate standardization protocol is employed. This allows exact identification of alleles and the possibility to construct population databases on allele frequencies and genotype distributions, essential for identity or paternity testing. This paper presents allele frequency data in a southern Italian population sample for the following STR loci:

HUMTH01: Human tyrosine hydroxylase gene, intron 1 [2]; chromosomal location: 11p15.5; repeat: AATG

HUMVWA31: Human von Willebrand factor gene, intron 40 [3]; chromosomal location: 12p12-pter; repeat: TCTA, TCTG

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HUMFESFPS: Human C-FES/FPS proto-oncogene, intron 5 [4]; chromosomal location: 15q25-qter; repeat: ATTT

HUMF13A01: Human coagulation factor XIII A subunit gene, [5]; chromosomal location: 6p24-25; repeat: GAAA.

Materials and methods

Blood samples were obtained from randomly selected and unrelated healthy individuals from the province of Messina (Eastern Sicily). DNA was extracted by standard Chelex 100 extraction procedure [6]. The PCR amplifications of HumTH01, HumvWA31, HumFESFPS and HumF13A01 were performed separately accord-

Table 1 Allele frequencies for HumTH01 (n = 140), HumvWA31 (n = 150), HumFES/FPS (n = 150) and HumF13A01 (n = 148) in a Sicilian population

Allele	HumTH01	HumvWA31	HumFES/FPS	HumF13A01
3.2				0.094
4				0.023
5				0.253
6	0.217			0.260
7	0.185			0.293
8	0.142		0.003	0.006
9	0.203		0.006	
9.3	0.207			
10	0.042		0.253	
11		0.003	0.436	
12			0.226	0.003
13		0.003	0.066	0.013
14		0.106	0.006	0.010
15		0.083		0.030
16		0.206		0.010
17		0.330		
18		0.173		
19		0.086		
20		0.006		

ing to the manufacturer's recommendations using GenePrint STR system kits (Promega). PCR was carried out in 25 µl reaction volumes containing 10–100 ng template DNA and 0.5 units of AmpliTaq DNA polymerase (Perkin-Elmer) in a 480 or 9600 Perkin Elmer thermal cycler.

PCR products were separated in vertical standard 6% polyacrylamide denaturing sequencing gels (7 M urea, 5.7% acrylamide, 0.3% bisacrylamide, 100 mM tris-borate pH 8.3 and 1 mM Na_2EDTA), 32 cm long and 0.1-0.2 mm thick. After electrophoresis gels were stained with silver nitrate [7]. Alleles were classified by comparison with the allelic ladders supplied by Promega GenePrint STR systems and the allelic designation was made according to the recommendations of the DNA Commission of the International Society of Forensic Haemogenetics [8].

Allele and genotype frequencies were determined and Hardy-Weinberg equilibrium was tested by a simple χ^2 method and an exact test [9]. From the genotype data, the power of discrimination (PD) according to the equation suggested by Fisher [10] was calculated. The heterozygosity value (OH) as described by Nei and Roychoudhury [11] was determined.

Results and discussions

Allelic frequency distributions for HumTH01, HumvWA31, HumFES/FPS and HumF13A01 in a eastern Sicilian population (Southern Italy) are shown in Table 1. All alleles differed in size by one repeat unit (i.e. 4 basepairs) for all loci, except for HumTH01 allele 9.3 and HumF13A01 allele 3.2. The "non consensus" allele 9.3 at the HumTH01 locus is represented by a 173 bp sized allele originating from a single-base deletion of a thymidine residue in the fifth of 10 TCAT repeats [1, 12]. The "non consensus" allele 3.2 at the HumF13A01 locus is the smallest known allele for this system and is represented by a 181 bp sized allele involving a deletion of a hexanucleotide (GAGTAA) outside the repeat region, immediately 3′ to the repeat [1]. The ability to type these alleles unequivocally demonstrated the high resolution power of the electrophoretic system used in the present study.

The results of the χ^2-test to analyse the correspondence of the genotype frequencies with Hardy-Weinberg expectations are shown in Table 2. They demonstrate that no significant deviations from HW equilibrium were found for all loci. The degrees of freedom were calculated on the difference between the number of contributions to the χ^2 (total number of quantities) and the number of observed alleles in the list of genotypes not grouped together (number of bonds imposed at the quantities) considering that observed and expected genotypes with expected < 2 were grouped in a single class of contribution. Table 2 shows the *P*-values obtained performing the exact test to confirm the χ^2–test results, the power of discrimination and heterozygosity calculated for each system.

In conclusion, an Eastern Sicilian population database has been determined for the first time for HumTH01, HumvWA31, HumFESFPS and HumF13A01 and no sig-

Table 2 Statistical data

System	χ^2	P	Ex.Test	PD	OH
TH01	24.543	0.05–0.02	0.02	0.92	0.80
vWA31	19.458	0.10–0.05	0.10	0.91	0.86
FES/FPS	9.056	0.70–0.20	0.50	0.83	0.74
F13A01	16.274	0.20–0.10	0.53	0.91	0.74

Table 3 Population data comparison for TH01

Allele	Messina *n* = 140	Tuscany [13] *n* = 232	Italy [14] *n* = 232	Germany [15] *n* = 195	Portugal [16] *n* = 419	Poland [17] *n* = 203
5				0.010		0.002
6	0.217	0.263	0.265	0.230	0.209	0.248
7	0.185	0.159	0.140	0.210	0.162	0.125
8	0.142	0.144	0.153	0.110	0.137	0.120
9	0.203	0.138	0.178	0.140	0.182	0.184
9.3	0.207	0.274	0.252	0.310*	0.302	0.305
10	0.042		0.010		0.007	0.012
Rare		0.022				

*9.3/10

Table 4 Population data comparison for vWA31

Allele	Messina *n* = 150	Tuscany [13] *n* = 200	Milan [18] *n* = 118	Ancona [19] *n* = 114	Germany [15] *n* = 195	Portugal [16] *n* = 376	Poland [17] *n* = 185
11	0.003						
12							
13	0.003					0.005	0.005
14	0.106	0.085	0.097	0.096	0.060	0.106	0.064
15	0.083	0.115	0.122	0.074	0.080	0.141	0.097
16	0.206	0.250	0.224	0.197	0.200	0.211	0.194
17	0.330	0.260	0.267	0.280	0.300	0.261	0.286
18	0.173	0.217	0.182	0.223	0.220	0.183	0.232
19	0.086	0.065	0.089	0.096	0.110	0.076	0.094
20	0.006		0.016	0.030	0.020	0.013	0.024
21					0.010	0.002	
Rare		0.008					

Table 5 Population data comparison for FES/FPS

Allele	Messina *n* = 150	Tuscany [13] *n* = 234	Milan [20] *n* = 115	Florence [21] *n* = 64	Germany [15] *n* = 195	Portugal [16] *n* = 409	Poland [17] *n* = 106
7			0.004				
8	0.003		0.008		0.010	0.010	0.018
9	0.006			0.010	0.010	0.002	0.009
10	0.253	0.276	0.295	0.230	0.250	0.304	0.264
11	0.436	0.395	0.413	0.390	0.490	0.369	0.419
12	0.226	0.256	0.226	0.310	0.220	0.265	0.221
13	0.066	0.066	0.052	0.060	0.030	0.045	0.066
14	0.006					0.004	
Rare		0.007					

Table 6 Population data comparison for F13A01

	Messina *n* = 148	Tuscany [13] *n* = 174	Germany [15] *n* = 195	Portugal [16] *n* = 232
3.2	0.094	0.065	0.060	0.084
4	0.023	0.044	0.040	0.043
5	0.253	0.189	0.190	0.168
6	0.260	0.278	0.280	0.306
7	0.293	0.319	0.390	0.340
8	0.006		0.010	0.015
9				0.002
12	0.003			
13	0.013	0.018		0.004
14	0.010	0.024	0.010	0.004
15	0.030	0.015	0.010	0.008
16	0.010	0.018	0.010	0.019
17				0.004
Rare		0.030		

nificant differences were found in comparison with data from other Italian (and Caucasian) populations (Tables 3–6). Therefore our data could be considered representative of the whole Italian population. Sicily is an island with a discrete degree of emigration to the rest of Italy and also immigration from other regions of the Country. We consider that this study provides additional statistical basis to use these loci in routine forensic casework.

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