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SHORT COMMUNICATION

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Allele frequency distributions of 13 PCR-based systems in a population from North-East Spain

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Abstract Population data studies were carried out on a Caucasian population from North-East Spain (n = 129–292 individuals) for 13 PCR-based polymorphic DNA loci: six short tandem repeat loci (HumTH01, HumTPOX, HumCSF1PO, HumF13A01, HumFES/FPS, HumvWFA31), the six PM loci (HLA-DQα, LDLR, GYPA, HBGG, D7S8, GC) and one variable number tandem repeat locus (D1S80).The genotypes distributions were in accordance with Hardy-Weinberg expectations. The combined use of the 13 polymorphic systems provides a high power of discrimination and power of exclusion for use in forensic casework and paternity testing.

Key words PCR · Short tandem repeat · D1S80 · HLA-DQα · PM · Spanish population

Introduction

During the last years important advances in molecular biology have provided forensic science with new and powerful tools to help solve criminal and paternity issues. In particular the polymerase chain reaction (PCR) (Saiki et al. 1985) has been an important advance in DNA technology wich enables samples with small amounts or even degraded DNA to be typed.

There are several genetic loci that can be amplified by PCR, such as STR (Edwards et al. 1991, 1992; Urquhart et al. 1994) or VNTR loci (Budowle et al. 1991). In order to use genetic loci in forensic casework, population databases are needed for statistical purposes. This report presents the allele frequency distribution of 13 PCR-based systems in a Spanish population.

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Materials and methods

Sample preparation

Whole blood samples were collected in EDTA by venipuncture from 292 unrelated Caucasian individuals living in the North of Spain. Genomic DNA was extracted by standard phenol-chloroform method (Sambrook et al. 1989).

PCR amplification and typing

HumTH01, HumTPOX and HumCSF1PO were amplified using a multiplex system. The other three STR loci (HumFES/FPS, HumvWFA31, HumF13A01) were amplified in singleplex. The reaction assay and the amplification conditions were performed according to the manufacturer's recommendations using the Gene Print STR systems (Promega Corporation, Madison WI) in a Linus dualcycler thermocycler. The PCR products were typed by vertical electrophoresis on 0.75 mm thick 4% denaturing polyacrylamide gels (19:1 acrylamide:bisacrylamide, 7 M urea) and silver staining (Bassam et al. 1991). Electrophoresis was carried out for 1–1.5 h on a Hoefer SE 620 vertical slab gel electrophoresis unit (Hoefer Scientific Instruments, Calif.) at a constant voltage of 1000 V with a fixed temperature of 51° C.

The amplification and typing of D1S80 system were performed using the Forensic DNA Amplification Reagent set following the manufacture's protocol (Perkin-Elmer Corporation, Norwalk CT). The D1S80 locus was typed by vertical electrophoresis on 0.75 mm thick native polyacrylamide gels with GeneAmp Detection Gel (Perkin-Elmer Corporation, Norwalk CT) and silver staining (Bassam et al. 1991).The electrophoresis was carried out for 1 h on a Hoefer SE 620 vertical slab gel electrophoresis unit at a constant voltage of 500 V.

For the systems LDLR, GYPA, HBGG, D7S8, GC and HLA-DQα, amplification and typing were carried out using the Amplitype PM and HLA-DQα Forensic DNA Amplification and Typing Kit (Perkin-Elmer Corporation, Norwalk CT) respectively in a Linus dualcycler thermocycler, according to the manufacture's protocol.

Statistical methods

The frequencies of each allele for each polymorphic locus were estimated by the traditional counting method. For the D1S80 locus the alleles were binned into five groups (I: 16–18; II: 19–23; III: 24; IV: 25–28; V: 29–40) to estimate Hardy-Weinberg equilibrium (H-W) as suggested by Brenner and Morris (1990). Unbiased ex-

Table 1 Observed allele frequencies for six STR systems in a Spanish population (North-East Spain)

TH01* (*n* = 584)		vWA (*n* = 258)		TPOX (*n* = 316)		CSF1PO (*n* = 316)		F13A** (*n* = 288)		FES/FPS (*n* = 290)	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
5	0.0017	13	0.0039	8	0.5317	8	0.0032	3.2	0.0868	8	0.0172
6	0.1952	14	0.1434	9	0.0981	9	0.0190	4	0.0347	9	0.0035
7	0.1421	15	0.1202	10	0.0411	10	0.3196	5	0.2083	10	0.2586
8	0.1250	16	0.1473	11	0.2911	11	0.3101	6	0.2813	11	0.4586
9	0.2380	17	0.2403	12	0.0317	12	0.2722	7	0.3576	12	0.2138
9.3	0.2962	18	0.2248	13	0.0063	13	0.0759	8	0.0069	13	0.0448
11	0.0017	19	0.1046					9	0.0035	14	0.0035
		20	0.155					14	0.0139		
								15	0.0035		
								16	0.0035		

* Alleles 10 was not observed
** Alleles 10, 11, 12 and 13 were not observed

Table 2 Observed allele frequencies for LDLR, GYPA, HBGG, D7S8, GC and HLA-DQα loci in a Spanish population (North-East Spain)

LDLR (*n* = 394)		GYPA (*n* = 394)		HPGG (*n* = 394)		D7S8 (*n* = 394)		GC (*n* = 394)		HLA-DQα (*n* = 488)	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
A	0.4568	A	0.5279	A	0.4467	A	0.5508	A	0.3071	1.1	0.1619
B	0.5432	B	0.4721	B	0.5381	B	0.4492	B	0.1675	1.2	0.1434
				C	0.0152			C	0.5254	1.3	0.0656
										2	0.2029
										3	0.1250
										4	0.3012

Table 3 Observed allele frequencies for D1S80 locus in a Spanish population (North-East Spain)

D1S80* (*n* = 498)					
Allele	Freq	Allele	Freq	Allele	Freq
16	0.0020	24	0.3695	32	0.0060
17	0.0040	25	0.0562	33	0.0020
18	0.2129	26	0.0241	34	0.0040
19	0.0101	27	0.0141	35	0.0020
20	0.0221	28	0.0542	36	0.0020
21	0.0301	29	0.0442	37	0.0060
22	0.0542	30	0.0080	40	0.0020
23	0.0101	31	0.0602		

* Alleles 38 and 39 were not observed

pected heterozygosity of the loci and the standard error were computed as described by Nei and Roychoudhury (1974). The power of discrimination was calculated according to Jones (1972) and the power of exclusion as described by Garber and Morris (1983). Possible divergence from H-W expectations was determined by calculating a χ^2-test and the umbiased estimate of the expected homozygote/heterozygote frequencies (Chakraborty et al. 1988; Nei and Roychoudhury 1978, 1994).

Results

The allele frequencies for each polymorphic locus are displayed in Tables 1–3. The allele distributions for the 13 PCR-based systems showed no significant deviation from H-W equilibrium ($p > 0.05$). However, LDLR system gave

Table 4 Statistical data for 13 genetic systems in a Spanish population (North-East Spain)

System	Power of discrimination PD	Power of exclusion PE	Allelic diversity h (s.e.)	Chi square test*	Homozygosity test*
HUMTH01	0.918	0.569	0.783 ± 0.024	0.27	0.85
HUMTPOX	0.799	0.369	0.662 ± 0.038	0.77	0.70
HUMCSF1PO	0.870	0.467	0.724 ± 0.035	0.90	0.44
HUMvWFA31	0.945	0.467	0.827 ± 0.033	0.21	0.76
HUMFES/FPS	0.834	0.418	0.677 ± 0.039	0.46	0.50
HUMF13A1	0.890	0.510	0.743 ± 0.036	0.13	0.09
D1S80	0.906	0.541	0.760 ± 0.027	0.28	0.22
HLA-DQα	0.932	0.609	0.803 ± 0.025	0.89	0.08
LDLR	0.623	0.186	0.497 ± 0.036	0.09	0.09
GYPA	0.624	0.187	0.500 ± 0.036	0.38	0.36
HBGG	0.645	0.206	0.512 ± 0.036	0.70	0.65
D7S8	0.622	0.186	0.496 ± 0.036	0.83	0.86
GC	0.768	0.321	0.603 ± 0.036	0.19	0.25

* These values are probability values

a low p value for the χ^2t-test ($p = 0.09$) and the homozygosity test ($p = 0.09$), HLA-DQα and HumF13A01 systems also gave low p values for the homozygosity test i.e. 0.08 and 0.09 respectively. The results of the χ^2-test and the homozygosity test, power of discrimination and power of exclusion are shown in Table 4. The distribution of allele frequencies presented here are similar to those observed in other Spanish populations (Alonso et al. 1993; Martin et al. 1996; Pestoni et al. 1996). The combined use of these systems provides a high power of discrimination and exclusion that can be used as an important tool in forensic and paternity testing.

In conclusion we consider that this study provides a statistical basis for use of these sytems in forensic casework.

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