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SHORT COMMUNICATION

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Frequencies of the five PCR-based genetic markers LDLR, GYPA, HBGG, D7S8 and GC in the population of Asturias (North Spain)

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Abstract Allele and genotype frequencies of the loci LDLR, GYPA, HBGG, D7S8 and GC (PM loci) were investigated in a population sample of 215 unrelated individuals from Asturias (North Spain). Multiplex amplification and simultaneous typing of the five loci was carried out using the polymarker PCR amplification and typing kit. All loci met Hardy-Weinberg expectations. The Asturian sample does not differ significantly from other Caucasians, but significant differences were observed between this population and SW Hispanic, Afro-american and Korean populations.

Key words DNA · Polymorphism · Genetic markers · PCR · LDLR · GYPA · HBGG · D7S8 · GC · Asturian population

Introduction

Multiplex amplification and typing of Low Density Lipoprotein Receptor (LDLR) [1], Glycophorin A (GYPA) [2], Hemoglobin G gammaglobin (HBGG) [3], D7S8 [4] and Group Specific Component (GC) [5] has been proved to be very useful for forensic identification [6–15]. The polymarker kit (Amplitype PM PCR Amplification and Typing Kit, Perkin Elmer) is based on reverse dot blot technology using sequence specific oligonucleotide probes. This paper presents data on the frequencies of LDLR, GYPA, HBGG, D7S8 and GC alleles in a population sample from Asturias (North Spain).

Figure 1 shows the location of Asturias on the Spanish map. Asturias is a Northern Spanish region next to Galicia and not far from the Basque area. The Galician and Basque populations are well defined populations from both the cultural and genetic point of view. Asturias has had some migrations which do not include Arabic invasions of the Iberian peninsula and a better characterization of this region from a genetic point of view could be interesting.

Fig. 1 Location of Asturias on the Spanish map

Material and methods

Blood samples from 215 healthy unrelated individuals from Asturias were collected. DNA was extracted with Chelex 100 using the method described by Walsh et al. [16]. The five loci were simultaneously amplified and typed using the AmpliType PM PCR Amplification Typing Kit (Perkin Elmer Corporation, Norwalk, CT). Amplification was carried out in a Perkin-Elmer DNA Thermal Cycler 480. The conditions were those recommended by the manufacturer (Roche Molecular Systems, Branchburg, N. J.).

Results and discussion

The distributions of observed genotype and allele frequencies for the five loci are summarized in Table 1. No deviation of the Hardy-Weinberg equilibrium was detected for any of the five loci investigated. Comparative studies were carried out with other populations studied up to date for these five loci [6, 8–15] (Table 2). The As-

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Table 1 Gene and phenotype frequencies of LDLR, GYPA, D7S8, HBGG and Gc in Asturias, showing χ^2 figures from Hardy-Weinberg equilibrium

System	Phenotypes	Number observed	Number expected	Allele frequencies	χ^2
LDLR	AA	45	44.22	A = 0.4535	$\chi^2 = 0.0466$
	AB	105	106.57	B = 0.5465	d.f. = 1
	BB	65	64.22		
GYPA	AA	61	59.92	A = 0.5279	$\chi^2 = 0.0877$
	AB	105	107.17	B = 0.4721	d.f. = 1
	BB	49	47.92		
HBGG	AA	53	53.25	A = 0.4977	$\chi^2 = 0.0094$
	AB	107	106.50	B = 0.4977	d.f. = 3
	AC	1	1.00	C = 0.0046	
	BB	53	53.25		
	BC	1	1.00		
	CC	0	0.00		
D7S8	AA	76	75.02	A = 0.5907	$\chi^2 = 0.0766$
	AB	102	103.96	B = 0.4093	d.f. = 1
	BB	37	36.02		
Gc	AA	21	20.26	A = 0.3070	$\chi^2 = 0.2552$
	AB	20	21.80	B = 0.1651	d.f. = 3
	AC	70	69.68	C = 0.5279	
	BB	6	5.86		
	BC	39	37.48		
	CC	59	59.92		

Table 2 Comparison of allele frequencies for LDLR, GYPA, HBGG, D7S8 and GC loci between Asturias and other populations (χ^2 values are placed above and p values below)

Populations	LDLR	GYPA	HBGG	D7S8	GC
Asturian-Basque Country (Spain) [12]	0.085 0.7703	0.077 0.7808	0.682 0.7109	0.101 0.7509	2.969 0.2266
Asturian Galicia (Spain) [14]	1.239 0.2657	0.807 0.3689	4.206 0.1221	0.408 0.5232	0.485 0.7856
Asturian Saragosse (Spain)*	4.499E4 0.9831	0.742 0.3889	0.06 0.9703	1.243 0.265	0.687 0.7092
Asturian North Portugal [11]	0.104 0.7468	0.011 0.9178	0.334 0.563	1.552 0.4602	0.05 0.9754
Asturian South Portugal [13]	2.02 0.1552	0.089 0.7655	1.419 0.492	1.516E4 0.9902	1.59 0.4515
Asturian Coimbra (Portugal) [14]	0.171 0.6791	0.392 0.5311	0.985 0.611	0.158 0.9606	1.028 0.5982
Asturian North Bavarian [8]	2.491 0.1145	1.44 0.2301	0.944 0.6236	0.036 0.8484	0.197 0.906
Asturian Arab [10]	0.005 0.9438	3.359 0.0668	6.255 0.0438	1.492 0.2219	7.824 0.02
Asturian USA Caucasian [6]	9.627E5 0.9922	1.295 0.2551	0.374 0.8293	0.249 0.6174	1.324 0.5157
Asturian USA Afroamerican [6]	30.298 0.0001	0.958 0.3276	74.675 0.0001	0.229 0.6321	142.018 0.0001
Asturian USA S-E Hispanic [6]	0.61 0.4347	0.007 0.9346	3.519 0.1722	0.013 0.908	1.982 0.3711
Asturian USA S-W Hispanic [6]	4.818 0.0282	7.271 0.007	12.717 0.0017	3.844 0.0499	1.371 0.5039
Asturian Korean [9]	61.124 0.0001	0.015 0.9028	2324.502 0.0001	2.416 0.1201	54.469 0.0001
Asturian Japanese [15]	62.124 0.0001	1.868 0.1717	2751.312 0.0001	0.126 0.7231	53.638 0.0001

* Unpublished data

turian sample does not differ significantly from other Caucasians, but significant differences were observed between this population and SW Hispanic, Afro-american, Arab, Korean and Japanese populations.

In order that there can be confidence that DNA profile frequency estimates are meaningful even with small size databases, two methods have been recently proposed for estimating minimum allele frequency bounds for PCR-

Table 3 Statistical parameters for polymarker loci in Asturias (*h* allelic diversity; *PD* power of discrimination; *CE* chance of exclusion)

	h%	PD%	CE%
LDLR	49.80	62.7	18.64
GYPA	50.08	63.00	18.71
HBGG	50.69	63.03	19.37
D7S8	48.58	62.1	18.33
Gc	60.26	76.71	31.96

based loci [17]. One of these methods is appropriate for PM loci and is based only on the sample size. According to this the minimum allele frequency in our sample is 0.00694.

Other statistics of genetic and medico-legal interest are shown in Table 3. The combined chance of exclusion (CE) is 0.7035 in the Asturian population. The combined discrimination power (PD) is 0.99 calculated following the method of Fisher [18].The allelic diversity values (h) were calculated as described by Nei and Roychoudhury [19] (Table 3). The observed heterozygosity of each genetic marker did not deviate from the expected value (h). These results are similar to those obtained for other populations studied.

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