SOUTHERN SPAIN POPULATION FREQUENCIES OF THE LOCI LDLR, GYPA, HBGG, D7S8 AND Gc. A COMPARISON BETWEEN ANDALUSIAN AND CANARY ISLANDS FREQUENCIES.

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Introduction

The AmpliType® PM PCR Amplification and Typing Kit was developed by Roche Molecular Systems USA. It simultaneously coamplifies HLA DQα, LDLR (Low density lipoprotein receptor), GYPA (Glycophorin A), HBGG (hemoglobin G gammaglobin), D7S8 and Gc (group specific component) and types the last five loci in a single reverse dot blot strip. Its use is widespread and it was proved to be specially useful due to its high sensitivity, very close to that obtained with the HLA-DQα system (Espinheira 1994; Pinheiro 1994; Reena 1995; Budowle 1995).

Before a genetic marker can be used for the analysis of evidentiary samples its forensic applicability should be evaluated. This multiplex Amplification and Typing kit has been widely validated (Herrin 1994; Budowle 1995; Reena 1995; Fildes 1995; Sanz 1994). In addition, the routine use in identity testing, both in forensic casework and paternity testing, needs statistical evaluation, therefore a reference population database must be established by each laboratory. Since the routine work in our laboratory, located in Southern Spain, involves populations of Andalusia and the Canary Islands, we have undertaken a comparison between the two populations in order to assess the applicability of common or separate databases. The correspondence between PCR genotyping and IEF phenotyping results for Gc, including individuals showing rare migrating protein patterns, is also reported.

Material and Methods

Blood samples were taken from 158 individuals (106 Andalusian and 52 from the Canary Islands) from our casework and staff. DNA extraction was achieved using Chelating Agent (Sigma) according to Perkin Elmer Cetus Corporation protocols. Amplification and Typing was performed with the AmpliType PM kit (Perkin Elmer®) and according to protocols provided by the manufacturer. The cycling reaction was carried out in a Perkin Elmer 480 Thermocycler.

Statistical Analysis

The frequency of each allele for each locus was calculated from the numbers of each genotype in the sample set. Possible divergence from Hardy-Weinberg equilibrium was determined by the Chi-square test. Comparison between populations were also carried out by the Chi-square test. Estimates of heterozygosity and combined power of discrimination (PD) and a priori chance of exclusion (CE) were calculated.

Results

Table 1

Locus	Genotype	ANDALUSIA			CANARY ISLANDS		
		Observed		All. Freq.	Observed		All. Freq.
		N	%		N	%	
LDLR	AA	28	26.41	A=0.4953	12	23.08	A=0.4231
	AB	49	46.23	B=0.5047	20	38.46	B=0.5769
	BB	29	27.36	X^2=0.6039	20	38.46	X^2=2.3357
				df=1 p=0.4371			df=1 p=0.1264
GYPA	AA	28	26.41	A=0.5094	12	23.08	A=0.4615
	AB	52	49.06	B=0.4906	24	46.15	B=0.5385
	BB	26	24.53	X^2=0.0362	16	30.77	X^2=0.2649
				df=1 p=0.8490			df=1 p=0.6068
D7S8	AA	35	33.02	A=0.5849	9	17.31	A=0.4712
	AB	54	50.94	B=0.4151	31	59.61	B=0.5288
	BB	17	16.04	X^2=0.2551	12	23.08	X^2=2.0027
				df=1 p=0.6135			df=1 p=0.1570
HBGG	AA	27	25.47	A=0.4953	14	26.92	A=0.5481
	AB	51	48.11	B=0.4906	27	51.92	B=0.4327
	AC	0	- *	C=0.0142	2	3.58*	C=0.0192
	BB	25	23.58	X^2=0.0538	9	17.31	X^2=1.1435
	BC	3	2.83*	df=1 p=0.8167	0	- *	df=1 p=0.2849
	CC	0	- *		0	- *	
Gc	AA	12	11.32*	A=0.3160	3	5.77*	A=0.2212
	AB	12	11.32	B=0.1840	4	7.69*	B=0.2115
	AC	31	29.24	C=0.5000	13	25.00	C=0.5673
	BB	2	1.89*	X^2=0.8353	3	5.77*	X^2=0.0296
	BC	23	21.70	df=2 p=0.6586	12	23.08	df=1 p=0.8634
	CC	26	24.53		17	32.69	

*pooled classes

Table 2 Comparison between the two populations

	LDLR	GYPA	D7S8	HBGG	Gc
X^2	2.9396	0.3011	2.2578	1.1973	0.8649
df	4	4	4	6	6
p	0.5680	0.9897	0.6885	0.9770	0.9902

No statistically significant differences were found between them

Table 3 Allelic frequencies and heterozygosity of both populations

	LDLR	GYPA	D7S8	HBGG	Gc
Allele Frequency	A=0.4715 B=0.5285	A=0.4937 B=0.5063	A=0.5475 B=0.4525	A=0.5127 B=0.4715 C=0.0158	A=0.2848 B=0.1930 C=0.5221
Heterozygosity (%)	43.67	48.10	53.80	52.53	60.13

Combined Power of Discrimination= 0.9957

Combined Power of Exclusion= 0.7472

Genotyping of Gc variants

Rare migrating IEF patterns, occasionally encountered in our casework and typed according to the recommendations of the International Workshop of the Gc system held in 1978 (Westwood 1986), were submitted to genotyping with the PM AmpliType® kit which was considered to unambiguously type 2, 1F and 1S Gc alleles (Reynolds 1990). The following results were obtained:

Gc IEF phenotyping	1S1C	1F1C	1S1A	1S1C
Gc PM genotyping	BC	BB	CC	CC

Comments:

1.- Cathodic variants of Gc-1 were genotyped as B allele (1F) or C allele (1S). The first example corresponds to a paternity case where the child received the rare allele from the alleged father. PM genotyping alone would reduce paternity index.

2.- Anodic variant of Gc-1 resulted as C allele (1S) or, presumably, was undetected (not amplified or not hybridated).

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