FREQUENCY AND DISTRIBUTION OF Pi, Gc, Tf AND PLG SUBTYPES BY ISOELECTRIC FOCUSING IN BARCELONA

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Introduction

The application of isoelectric focusing to the analysis of some polymorphisms has revealed considerably more genetic heterogeneity than was apparent by conventional methods.

The separation of the Pi components by isoelectric focusing has led to reliable characterization of four Pi^M alleles at the Pi gene locus (1,2).

Subtyping of TfC by isoelectric focusing has now demonstrated eight separate variants (3). The alleles commonly encountered in white people are Tf^{C1}, Tf^{C2} and Tf^{C3}.

Constans and Viau(4) in 1977 reported further subclassification of Gc by isoelectric focusing in polyacrylamide gels. This method permitted distinction of six common subtypes called 1S, 1S-1F, 1F, 2-1S, 2-1F and 2.

Finally Hobart (5) using isoelectric focusing first identified PLG variants in 1979 and named the common alleles PLG^1 and PLG^2.

Despite the vast array of population studies until now concerned with Pi, Tf and Gc, subtyping of these markers in Spanish populations has been published once in Galicians (6).

From our knownledge studies on PLG polymorphism in Spanish populations has not been reported until now.

In this paper we report the results of a survey of these serum proteins in 800 donors from the metropolitan area of Barcelona.

Material and Methods

Serum from freshly collected blood samples from around 800 healthy donors was used.

Donors were classified according to their ancestral origin as catalans or mixed populations.

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Samples were stored at -30°C prior to analysis and used without previous treatment for Pi, Gc and PLG typing. For Tf subtype determination the serum samples were diluted 1:5 with 0.5M ferrous ammonium sulphate and incubated for 18h at 4°C.

PAGIF was carried out in 0.3 mm polyacrylamide gels at a gel concentration of T=5.5% and C=3%. Ampholyte concentration was 5%. Polymerization was carried out with riboflavine and ultraviolet light.

A mixture of Ampholine and Pharmalyte (pH 2.5-5, 3.5-5, 4-6 and 4.2-4.9) were used for Pi typing. For Gc typing was used a mixture of Ampholine and Pharmalyte pH 4-6, 4-6.5 and 4.5-5.4. Ampholine pH 5-7 and 5-8 were used for Tf and PLG typing respectively.

Staining of the gels was carried out with Coomassie Blue R 250 for Pi, Tf and PLG. Gc bands were read after simple precipitation with sulphosalicylic acid.

Results and Discussion

The distribution of Pi subtypes and their allele frequencies are shown in Table 1. 14 different phenotypes were found. A fair agreement was found between observed and expected values, assuming a Hardy-Weinberg equilibrium.

The Pi^S frequency found in our population is one of the highest found within European populations in agreement with the progressive decrease of the Pi^S frequency from southwestern toward the northern European countries (the highest frequencies are found in the Galicia population). The distribution of the other alleles in European countries seems to be more uniform.

Pi subtyping offers in our population a theoretical exclusion rate of 34.64% and an EM value of 9.77, being the most useful electrophoretic marker for paternity testing in our population.

Table 2 summarizes the results of the Gc subtyping. Good agreement was noted for the Hardy-Weinberg distribution.

Gc alleles frequencies in Barcelona populations are in the range expected for caucasians.

With a theoretical chance of exclusions of non-fathers=30.12% and an EM value=9.83, the Gc polymorphism subtyped by isoelectric

Table 1. Frequencies of Pi phenotypes and Pi alleles in the Barcelona population

Phenotypes	Obs.	Exp.	Dif.	X^2
M1-M1	338	338.52	-0.52	0.00
M1-M2	219	215.06	3.93	0.07
M1-M3	111	112.33	-1.33	0.01
M1-S	116	117.74	-1.74	0.02
M1-F	3	2.40	0.59	0.14
M1-Z	2	2.40	-0.40	0.06
M2-M2	36	34.15	1.84	0.09
M2-M3	32	35.68	-3.68	0.38
M2-S	34	37.40	-3.40	0.30
M2-F	0	0.76	-0.76	0.76
M2-Z	1	0.76	0.23	0.07
M3-M3	11	9.32	1.67	0.30
M3-S	21	19.53	1.46	0.10
M3-F	1	0.39	0.60	0.90
M3-Z	0	0.39	-0.39	0.39
S-S	12	10.23	1.76	0.30
S-F	0	0.41	-0.41	0.41
S-Z	1	0.41	0.58	0.81
F-F	0	0.00	0.00	0.00
F-Z	0	0.00	0.00	0.00
Z-Z	0	0.00	0.00	0.00
Total	938	937.99		5.22

Pi M^1 = 0.6007
Pi M^2 = 0.1908
Pi M^3 = 0.0996
Pi M^S = 0.1044
Pi M^F = 0.0021
Pi M^Z = 0.0021

X^2 = 5.222971
df = 15
P > 0.99
CE = 34.64
EM value = 9.759441

Table 2. Frequencies of Gc phenotypes and Gc alleles in the Barcelona population

Phenotypes	Obs.	Exp.	Dif.	X^2
1S-1S	252	252.20	-0.20	0.00
1S-1F	125	121.16	3.83	0.12
1F-1F	15	14.55	0.44	0.01
1S-2	291	294.42	-3.42	0.03
1F-2	66	70.72	-4.72	0.31
2-2	90	85.92	4.07	0.19
Total	839	839		0.68

Gc^{1S} = 0.5482
Gc^{1F} = 0.1317
Gc^2 = 0.3200

X^2 = 0.68363 df = 3
P > 0.75
CE = 30.12 EM value = 9.830011

Table 3. Frequencies of Tf phenotypes and Tf alleles in the Barcelona population

Phenotypes	Obs.	Exp.	Dif.	X^2
C1-C1	517	516.55	0.44	0.00
C1-C2	219	223.22	-4.22	0.08
C1-C3	61	57.17	3.82	0.25
C1-B	5	5.48	-0.48	0.04
C2-C2	27	24.11	2.88	0.34
C2-C3	10	12.35	-2.35	0.44
C2-B	2	1.18	0.81	0.56
C3-C3	1	1.58	-0.58	0.21
C3-B	0	0.30	-0.30	0.30
B-B	0	0.01	-0.01	0.01
Total	842	841.99		2.26

Tf C^1 = 0.7832
Tf C^2 = 0.1692
Tf C^3 = 0.0433
Tf B = 0.0041

X^2 = 2.26526
df = 6
P > 0.75
CE = 17.05
EM value = 9.865737

Table 4. Frequencies of Plg phenotypes and Plg alleles in the Barcelona population

Phenotypes	Obs.	Exp.	Dif.	X^2
1-1	465	462.89	2.10	0.00
1-2	250	254.20	-4.20	0.06
2-2	37	34.89	2.10	0.12
Total	752	752		0.20

Plg^1 = 0.7845
Plg^2 = 0.2154

X^2 = 0.2054947
df = 1
P > 0.50
CE = 14.04
EM value = 9.896515

focusing becomes one of the most useful markers in paternity testing.

The distribution of Tf subtypes are shown in Table 3, and it can be seen that there was a good agreement between the observed distribution of phenotypes and that expected according to the Hardy-Weinberg law.

The distribution found in the sample differs slightly from the results observed in other European populations, being the Tf^{C3} allele frequency one of the lowest within the population of European origin. The same fact is found in the Galicia population (6).

The chance of exclusion using the Tf system is 17.05% and the EM value 9.87.

PLG phenotypes and gene distribution can be seen in Table 4. A good Hardy-Weinberg equilibrium was observed.

The observed allele frequencies are lower than it is observed in North-European populations and similar to the observed in italians.

The chance of exclusion using the PLG system in this population is 14.04% and the EM value=9.90.

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