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Distribution of the Pi, TfC, and Gc Subtypes in Galicia (North West Spain)

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Summary. Alpha$_1$-antitrypsin (Pi), Gc, and TfC subtypes were determined by isoelectric focusing in thin layer agarose (AGIF) and polyacrylamide gels (PAGIF) in a total of 480 individuals from Galicia. The following gene frequencies were observed:

for Pi: Pi^{M1}: 0.660; Pi^{M2}: 0.115; Pi^{M3}: 0.060; Pi^{S}: 0.149; Pi^{Z}: 0.009; Pi^{F}: 0.005; Pi^{I}: 0.001;

for Gc: Gc^{1S}: 0.572; Gc^{1F}: 0.120; Gc^{2}: 0.308;

for TfC: Tf^{C1}: 0.778; Tf^{C2}: 0.180; Tf^{C3}: 0.041; Tf^{C6}: 0.001.

A rare variant TfC6-2 was found and the intrafamilial distribution of the Tf^{C6} allele studied.

The use of these systems for forensic purposes and the peculiar distribution of some of their alleles in the Galician population are discussed.

Key words: Blood groups, Pi-TfC-Gc subtypes – Pi-TfC-Gc gene frequencies

Zusammenfassung. Die Untergruppen der Pi, Gc und TfC Serumgruppen wurden bei 480 Personen aus Galicien durch AGIF und PAGIF bestimmt. Die ermittelten Genfrequenzen sind:

für Pi: Pi^{M1}: 0,660; Pi^{M2}: 0,115; Pi^{M3}: 0,060; Pi^{S}: 0,149; Pi^{Z}: 0,009; Pi^{F}: 0,005; Pi^{I}: 0,001;

für Gc: Gc^{1S}: 0,572; Gc^{1F}: 0,120; Gc^{2}: 0,308;

für TfC: Tf^{C1}: 0,778; Tf^{C2}: 0,180; Tf^{C3}: 0,041; Tf^{C6}: 0,001.

Schlüsselwörter: Blutgruppen, Pi-TfC-Gc-Untergruppen – Pi-TfC-Gc-Genfrequenzen

Introduction

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

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The separation of the α_1-antitrypsin components by isoelectric focusing has led to reliable characterization of four Pi^M alleles at the Pi gene locus [6, 8, 9, 11, 17].

Subtyping of Tf by isoelectric focusing has now demonstrated eight separate variants, Tf^{C1}-Tf^{C8} [5, 7, 13–15]. The alleles commonly encountered in white people are Tf^{C1}, Tf^{C2}, and Tf^{C3}.

Finally, Constans and Viau [4] have reported six common Gc phenotypes which are determined by three common alleles at the Gc gene locus.

In this paper we report the results of a survey of the serum proteins α_1-antitrypsin, TfC and Gc, subtyped by isoelectric focusing in thin-Layer agarose and polyacrylamide gels on serum samples from 480 donors with four Galician grandparents.

The use of these systems for forensic purposes and the peculiar distribution of some of their alleles in the Galician population are also discussed.

Material and Methods

Serum from freshly collected blood samples from 480 healthy adults proportionally representative of regional districts of Galicia was used.

Samples were stored at −20°C prior to analysis and used without previous treatment for Gc subtyping. For TfC subtype determination, the serum samples were diluted 1 : 5 with 0.5 *M* ferrous ammonium sulfate and incubated for 18 h at 4°C. Samples were pretreated with freshly Clelland's reagent (0.05 *M* dithiothreitol) before Pi subtyping. As typing of PiM_3 proved difficult, all specimens believed to contain this allele were tested against known types across the length of the gel.

Isoelectric focusing was conducted using Pharmacia systems FBE 3000 and ECPS 2000/300 (Pharmacia Fine Chemicals, Uppsala, Sweden).

PAGIF was carried out in 0.5 mm thin-layer polyacrylamide gels at a gel concentration of T = 5'5% and cross-linking of C = 3%. Ampholine (LKB, Bromma, Sweden) concentration was 5%.

Polymerization was carried out with 0.5% (v/v) riboflavin and UV light.

A pH range 3.5–5 was used for Pi subtyping and the samples were applied by Whatman 3 MM filter papers (0.5 × 0.5 cm) at a distance of 2 cm from the cathode. The electrode solutions were aM ethanolamine for the cathode and a MH_3Po_4 for the anode. Electric focusing was carried out at 20 W for 150 min. Gels were stained with Coomassie Blue R 250.

A pH range 5–7 was used for Tf subtyping and the samples were applied a distance of 3 cm from the cathode using Whatmann 3 MM (0.5 × 0.5 cm) paper. The electrode solutions were 1 *M* ethanolamine for the cathode and 1% (v/v) acetic acid for the anode. Electric focusing was carried out at 20 W for 200 min. Gels were stained as above.

The pH range used for Gc subtyping was pH 4–6 and the samples were applied by Whatman 3 MM filter papers (0.5 × 1 cm) at a distance of 3 cm from the cathode electrode strip. The electrode solutions were 1 *M* NaOH for the cathode and 1 *M* H_3PO_4 for the anode. Electric focusing was carried out at 20 W for 200 min. The immunofixation was performed as described by Ritchie and Smith [16] with a specific 1 : 2 diluted anti Gc serum (Behring) for 5 min. The cellulose acetate strips were washed overnight before staining with Coomassie Blue.

AGIF was performed on 0.5 mm agarose gels. 1.3% agarose IEF (Pharmacia) and 13% sucrose were used. The pH gradient was prepared as for PAGIF. Samples were treated and applied as for PAGIF. The electrode solutions were 0.5 *M* NaOH for the cathode and 0.5 *M* sulfuric acid for the anode. Electric focusing was carried out at 15 W during 120 min for Gc and Tf typing and at 10 W during 160 min for Pi typing. Staining of the gels was carried out with Coomassie Blue R 250 and immunofixation of agarose gels for Gc subtype determination as above.

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

Phenotypes	Observed		Expected		Allele frequencies
	n	(%)	*n*	(%)	
Pi M1	210	43.75	209.35	43.61	Pi^{M1}: 0.6604
M1M2	67	13.96	72.65	15.14	Pi^{M2}: 0.1146
M2	9	1.87	6.30	1.31	Pi^{M3}: 0.0604
M1M3	40	8.33	38.30	7.98	Pi^{S}: 0.1490
M2M3	9	1.87	6.65	1.39	Pi^{Z}: 0.0094
M3	1	0.21	1.75	0.36	Pi^{F}: 0.0052
M1S	93	19.38	94.44	19.67	Pi^{I}: 0.0010
M2S	16	3.33	16.39	3.41	
M3S	7	1.46	8.64	1.80	
S	13	2.71	10.65	2.22	
M1Z	8	1.67	5.94	1.24	
SZ	1	0.21	1.34	0.28	
M1F	5	1.04	3.30	0.69	
M1I	1	0.21	0.66	0.14	
Others	0	0.00	3.64	0.76	
Total	480	100.00	480.00	100.00	

$\Sigma\ \chi^2 = 9.1696; df = 21;\ P > 0.95$

Table 2. TfC phenotype and gene distribution in Galicia

Phenotypes	Observed		Expected		Allele frequencies
	n	(%)	*n*	(%)	
TfC 1	286	59.58	290.63	60.55	TfC^{1}: 0.7781
2-1	142	29.58	134.61	28.05	TfC^{2}: 0.1802
2	13	2.71	15.59	3.25	TfC^{3}: 0.0406
3-1	33	6.88	30.35	6.32	TfC^{6}: 0.0010
3-2	4	0.83	7.03	1.46	
3	1	0.21	0.79	0.16	
6-2	1	0.21	0.18	0.04	
Others	0	0.00	0.82	0.17	
Total	480	100.00	480.00	100.00	

$\Sigma\ \chi^2 = 5.4505;\ df = 6;\ P > 0.30$

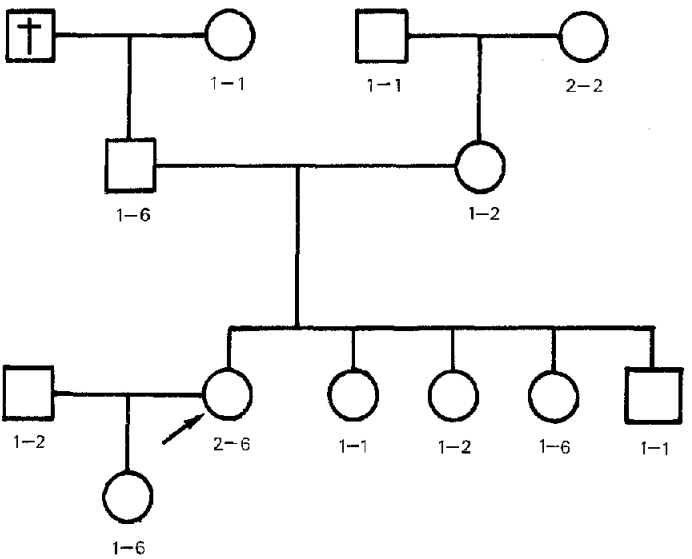

Fig. 1. Pedegree showing segregation of Tf^{C6} allele

Results and Discussion

The distribution of Pi subtypes and their allele frequencies are shown in Table 1. Eleven different phenotypes were found. Good agreement was noted for the Hardy-Weinberg distribution.

The observed frequencies for common Pi types are not significant different from the results of previous studies of the Galician population [1, 10].

The Pi^S frequency found in our Galician sample is the highest found within European populations in agreement with the progressive decrease of the Pi^S gene frequency from southwestern toward the northern European countries [1]. The distribution of Pi^M alleles in European countries seems to be more uniform.

Pi subtyping offers in our population a theoretical exclusion rate of about 31.37%. Although the unequivocal identification of the intermediary Pi^M types makes the Pi system a powerful tool in paternity testing, we think, in agreement with Weidinger et al. [18], that the experience with the isoelectric focusing procedure and the use of straight pH ranges is a necessary prerequisite for the use of this system in paternity testing.

As Carracedo and Concheiro [3] have reported, Pi types can be detected in 2-month-old bloodstains. With our recent experience we have found that it is also possible to determine Pi^M subtypes in 2-month-old bloodstains, but the pretreatment of the samples with dithiothreitol is necessary.

Table 2 summarizes the results of the TfC subtyping. A fair agreement was found between observed and expected values, assuming a Hardy-Weinberg equilibrium.

The distribution found in the sample in which four TfC subtypes were distinguished differs slightly from the results observed in other European populations.

Table 3. Gc phenotype and gene distribution in Galicia

Phenotypes	Observed		Expected		Allele frequencies
	n	(%)	*n*	(%)	
Gc 1F	4	0.83	6.89	1.43	Gc^{1S}: 0.5719
1F–1S	70	14.58	65.77	13.70	Gc^{1F}: 0.1198
1S	155	32.29	156.98	32.70	Gc^{2}: 0.3083
1F–2	37	7.71	35.46	7.39	
1S–2	169	35.21	169.27	35.27	
2	45	9.38	45.63	9.51	
Total	480	100.00	480.00	100.00	

$\Sigma\ \chi^2 = 1.5848$; $df = 3$; $P > 0.50$

This is due to the fact that frequency of the Tf^{C3} allele is one of the lowest within the population of European origin.

During the investigation the presence of the rare allele Tf^{C6} has been noted since the phenotype TfC 6-2 was found. In Fig. 1 the segregation of the Tf^{C6} allele is shown and the codominant inheritance of the Tf^{C6} allele demonstrated.

With the distinction of TfC subtypes, the system appears to be useful in paternity testing (we have calculated the theoretical exclusion rate to be 16.60%). The TfC system is also useful for the individual identification of bloodstains (bloodstains 3-month-old stored at room temperature can be typed).

The distribution of Gc 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.

With a theoretical chance of exclusions of non-fathers 29.01% in our population, the Gc polymorphism subtyped by isoelectric focusing becomes one of the most useful genetic markers in paternity testing. Futhermore, Gc subtypes can be found in 2-month-old bloodstains as reported previously [2].

Although isoelectric focusing on 0.5 mm agarose gels was found to be a reliable and cost effective technique for routine Tf and Gc subtyping, we advise thin-layer polyacrylamide gels for Pi subtyping since it is possible to use highly cross-linked gels [11] and higher voltages thus increasing the resolution of the bands.

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