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# Plasma Protein and Red Cell Enzyme Groups in Galicia (North West Spain)*

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**Summary.** Galactose-1-phosphate uridyl transferase (GALT), esterase D (EsD), and plasminogen (PLG) phenotypes were determined by isoelectric focusing in thin-layer polyacrylamide gels (PAGIF) in a random sample from Galicia. Haptoglobins (Hp) were determined by conventional electrophoresis. The following gene frequencies were observed: for GALT: $GALT^N$: 0.930; $GALT^{D1}$: 0.044; $GALT^{D2}$: 0.025; for EsD: $EsD^1$: 0.874; $EsD^2$: 0.104; $EsD^3$: 0.021; for PLG: $PLG^1$: 0.800; $PLG^2$: 0.199; for Hp: $Hp^1$: 0.426; $Hp^2$: 0.573. Population data results of all electrophoretic markers typed until now in Galician population are also included.

**Key words:** Blood groups, GALT-EsD-PLG-Hp – GALT-EsD-PLG-Hp gene frequencies, Galicia (Spain)

**Zusammenfassung.** Die Phänotypen der GALT, EsD und PLG wurden durch PAGIF in einer Stichprobe aus Galizien bestimmt. Hp wurden durch konventionelle Elektrophorese bestimmt. Folgende Genfrequenzen wurden ermittelt: für GALT: $GALT^N$: 0,930; $GALT^{D1}$: 0,044; $GALT^{D2}$: 0,025; für EsD: $EsD^1$: 0,874; $EsD^2$: 0,104; $EsD^3$: 0,021; für PLG: $PLG^1$: 0,800; $PLG^2$: 0,199; für Hp: $Hp^1$: 0,426; $Hp^2$: 0,573. Die Ergebnisse der Bevölkerungsdaten aller elektrophoretischen Charakteristika (Markers), die bisher innerhalb der Galizischen Bevölkerung ermittelt wurden, wurden ebenfalls berücksichtigt.

**Schlüsselwörter:** Blutgruppen, GALT-EsD-PLG-Hp – GALT-EsD-PLG-Hp Genfrequenzen, Galizien (Spanien)

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The Iberian peninsula is home to several different populations, each with a clearly identifiable culture reflecting the different historical influences experienced by each group. This is occasionally reflected in genetic traits, but unfortunately there have been published only few studies on the blood group distribution of Spanish populations up to now.

The aim of this paper is to offer the previously unpublished results of testing for certain protein and enzyme polymorphisms (GALT, EsD, PLG, and Hp) in the Galician population.

Population data results of all markers typed until now in this population are also included.

These data are of particular interest in the investigation of paternity, since the Galician population has one of the highest rates of emigration in Europe, and, as a consequence, Galicians are frequently involved in investigations to establish paternity.

## Material and Methods

Freshly collected blood samples from healthy adults proportionally representative of Galician regional districts were used.

Blood samples were collected by syringe into 5-ml tubes containing heparin or EDTA as anticoagulant. The plasma was removed and stored at −20°C for subsequent analysis for PLG and Hp. Red cells were washed three times in physiologic saline solution. Packed cells were stored at −20°C until used for determination of GALT and EsD.

For GALT and EsD subtype determination the samples were pretreated with fresh Clelland's reagent (0.05 dithiothreitol). Before PLG typing the serum samples were pretreated with a neuraminidase (SIGMA type V) solution (1 U/ml) for at least 12 h at −4°C. For Hp typing, samples were pretreated as follows: 10 g sucrose was added to 10 ml of a 0.1% (w/v) bromophenol blue solution in distilled water. Then a freshly prepared hemolysate was obtained from packed red cells that were washed three times and diluted 1:100 with distilled water. Sucrose solution (100 µl) and hemolysate (40 µl) were added to 100 µl serum and incubated for at least 15 min at room temperature. Samples were then ready for use.

GALT, EsD subtypes, and PLG types were determined by isoelectric focusing in polyacrylamide gels (PAGIF). Hp typing was carried out by vertical polyacrylamide gel electrophoresis.

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 of 5–7 was used for GALT subtyping, and the samples were applied at a distance of 1 cm from the anode using Whatman 3 MM (0.5 × 0.5 cm) paper. The electrode solutions were 1 *M* ethanolamine for the cathode and 1 *M* phosphoric acid for the anode. Electric focusing was carried out at 5 W for 200 min, with a limited voltage of 2500 V. Gels were stained using the procedures described by Vaccaro et al. [1].

A pH range of 4–6 was used for EsD subtyping, and the samples were applied using Whatman 3 MM filter papers (1 × 0.5 cm) at a distance of 1 cm from the cathode. The electrode solutions were as above. Electric focusing was carried out at 15 W for 150 min. Gels were stained with 4-methylumbelliferyl-acetate, according to the method of Hopkinson et al. [2].

The pH range used for PLG typing was pH 4–8, and the samples were applied with Whatman 3 MM filter papers (0.5 × 0.8 cm) at a distance of 4 cm from the anode electrode strip. The electrode solutions were 11% ethanolamine for the cathode and 0.04 *M* glutamic acid for

the anode. Electric focusing was carried out at 5 W for 270 min, with a voltage limited to 1500 V. Gels were stained with Coomassie blue R-250 according to the method of Pascali [3].

Haptoglobin phenotypes were determined by vertical polyacrylamide gel electrophoresis according to the technique of Baxter et al. [4].

## Results and Discussion

Table 1 summarizes the results of GALT subtypes. Good agreement with the Hardy-Weinberg law was found, and the results are in the expected range for white people.

The distribution of EsD subtypes is listed in Table 2. The agreement with the Hardy-Weinberg law is not perfect because a EsD 5-5 homozygote was observed in the sample. The distribution found in the sample differs slightly from results observed in other European populations, since the the $EsD^5$ allele has one of the highest frequencies seen in populations of European origin.

Table 3 shows the results of the PLG typing. Good agreement with the Hardy-Weinberg law was found. The distribution found in the sample, in which some rare alleles were distinguished, differs slightly from that observed in other

**Table 1.** GALT phenotype and gene distribution in Galicia

| Phenotypes | Observed | | Expected | | Allele frequencies |
|---|---|---|---|---|---|
| | *n* | (%) | *n* | (%) | |
| GALT N | 154 | 86.03 | 154.873 | 86.52 | $GALT^N$: 0.9302 |
| N-D1 | 16 | 8.94 | 14.883 | 8.31 | $GALT^{D1}$: 0.0447 |
| N-D2 | 9 | 5.03 | 8.372 | 4.68 | $GALT^{D2}$: 0.0251 |
| Others | 0 | 0 | 0.872 | 0.49 | |
| Total | 179 | 100.00 | 179 | 100.00 | |

$\sum \chi^2 = 1.008891$; $df = 3$; $0.80 > P > 0.70$

**Table 2.** Frequencies of EsD phenotypes and EsD alleles in the Galicia population

| Phenotypes | Observed | | Expected | | Allele frequencies |
|---|---|---|---|---|---|
| | *n* | (%) | *n* | (%) | |
| EsD 1 | 302 | 76.65 | 301.24 | 76.45 | $EsD^1$: 0.8744 |
| 2-1 | 70 | 17.77 | 71.76 | 18.20 | $EsD^2$: 0.1040 |
| 2 | 6 | 1.52 | 4.26 | 1.08 | $EsD^3$: 0.0216 |
| 5-1 | 15 | 3.81 | 14.89 | 3.77 | |
| 5-2 | 0 | 0 | 1.77 | 0.46 | |
| 5-5 | 1 | 0.25 | 0.18 | 0.04 | |
| Total | 394 | 100.00 | 394 | 100.00 | |

$\sum \chi^2 = 6.15350$; $df = 3$; $0.20 > P > 0.10$

**Table 3.** PLG phenotypes and gene distribution in Galicia

| Phenotypes | Observed | | Expected | | Allele frequencies |
|---|---|---|---|---|---|
| | *n* | (%) | *n* | (%) | |
| PLG 1 | 451 | 64.15 | 450.08 | 64.023 | $PLG^1$: 0.8001 |
| 2-1 | 223 | 31.72 | 224.84 | 31.983 | $PLG^2$: 0.1999 |
| 2 | 29 | 4.13 | 28.08 | 3.994 | |
| Total | 703 | 100.00 | 703 | 100.000 | |

$\sum \chi^2 = 0.04708$; $df = 1$; $0.90 > P > 0.75$

**Table 4.** Frequencies of Hp phenotypes and Hp alleles in the Galicia population

| Phenotypes | Observed | | Expected | | Allele frequencies |
|---|---|---|---|---|---|
| | *n* | (%) | *n* | (%) | |
| Hp 1 | 145 | 18.01 | 146.15 | 18.155 | $Hp^1$: 0.4261 |
| 2-1 | 396 | 49.19 | 393.70 | 48.907 | $Hp^2$: 0.5739 |
| 2 | 264 | 32.80 | 265.15 | 32.938 | |
| Total | 805 | 100.00 | 805 | 100.000 | |

$\sum \chi^2 = 0.027473$; $df = 1$; $0.90 > P > 0.75$

European populations. Nevertheless, the results are in agreement with previously reported results from the Spain Basque country and Northern Portugal [5].

The distribution of Hp phenotypes and their allele frequencies are shown in Table 4. Good agreement was noted for the Hardy-Weinberg distribution. The observed frequencies for common Hp alleles are not significantly different from the results of a previous study of the Galician population [6], and they are in the range expected for European populations.

To evaluate the precise value of a particular marker in paternity testing in a human population, a genetic population analysis should be carried out a priori. Such an analysis is much more important in paternity probability calculations, in which knowledge of the markers gene frequency of the involved population is necessary.

All markers generally used in paternity investigation in our Department have been studied at the local level in the Galician population to investigate any possible variations in frequency in different areas.

Except for a few small endogamic areas, such variations so far have not been sufficiently important to be taken into account.

The frequencies of protein and enzyme markers studied in the Galician population by now are shown in Tables 5 and 6.

Table 7 shows the a priori exclusion rate of all systems which were used in our laboratory, classified in descending order of exclusion efficiency. This order

**Table 5.** Frequencies of protein electrophoretic markers in Galicia population

| Marker | Number | Allele frequencies | | Reference |
|---|---|---|---|---|
| 1. Haptoglobin (Hp) | 805 | $Hp^1$ | = 0.4261 | This paper |
| | | $Hp^2$ | = 0.5739 | |
| 2. Gc (Gc) | 480 | $Gc^{1S}$ | = 0.5719 | A. Carracedo and L. Concheiro [9] |
| | | $Gc^{1F}$ | = 0.1198 | |
| | | $Gc^2$ | = 0.3083 | |
| 3. Alpha-1-antitrypsin (Pi) | 480 | $Pi^{M1}$ | = 0.6604 | A. Carracedo and L. Concheiro [9] |
| | | $Pi^{M2}$ | = 0.1146 | |
| | | $Pi^{M3}$ | = 0.0604 | |
| | | $Pi^S$ | = 0.1490 | |
| | | $Pi^Z$ | = 0.0094 | |
| | | $Pi^F$ | = 0.0052 | |
| | | $Pi^I$ | = 0.0010 | |
| 4. Transferrin (Tf) | 484 | $TFC^1$ | = 0.7749 | A. Carracedo and L. Concheiro [9] |
| | | $TFC^2$ | = 0.1795 | |
| | | $TFC^3$ | = 0.0404 | |
| | | $TFC^6$ | = 0.0010 | |
| | | TFB | = 0.0041 | |
| 5. $C_3$ | 252 | $C_3^F$ | = 0.2083 | Goedde et al. [10] |
| | | $C_3^S$ | = 0.7838 | |
| | | $C_3^{S0.5}$ | = 0.0079 | |
| 6. Properdin factor B (Bf) | 339 | $Bf^S$ | = 0.8142 | M. S. Rodriguez-Calvo [11] |
| | | $Bf^F$ | = 0.1799 | |
| | | Others | = 0.0059 | |
| 7. Plasminogen (PLG) | 703 | $PLG^1$ | = 0.8001 | This paper |
| | | $PLG^2$ | = 0.1999 | |
| 8. Cholinesterase ($C_5$) | 500 | $E_2^+$ | = 0.04816 | J. L. Blazquez [12] |
| | | $E_2^-$ | = 0.95184 | |
| 9. Cholinesterase (Locus $E_1$) | 483 | $E_1^u$ | = 0.9731 | J. L. Blazquez [12] |
| | | $E_1^a$ | = 0.0269 | |
| 10. Amylase ($AMY_2$) | 497 | $AMY_2^1$ | = 0.9708 | J. L. Blazquez (unpublished results) |
| | | $AMY_2^2$ | = 0.0292 | |
| 11. Orosmucoid (ORM) | 650 | $ORM^1$ | = 0.460 | A. Carracedo et al. [13] |
| | | $ORM^2$ | = 0.540 | |
| 12. Ceruloplasmin (Cp) | 517 | $Cp^A$ | = 0.00193 | J. L. Blazquez (unpublished results) |
| | | $Cp^B$ | = 0.99807 | |

does not completely coincide with the biostatistical efficiency of each marker [7].

Recently, Hummel and Clausen [8] have demonstrated that using the frequencies of the German population (Hummel's tables) there is a 100% coincidence of "verbal predicates" with the East German, French, English, West German, Swedish, Turkish, and Yugoslavian populations.

**Table 6.** Frequencies of enzyme electrophoretic markers in Galicia population

| Marker | Number | Allele frequencies | | Reference |
|---|---|---|---|---|
| 1. Phosphoglucomutase-Locus 1 ($PGM_1$) | 1086 | $PGM_1^{1+}$ | = 0.621 | A. Carracedo and L. Concheiro [14] |
| | | $PGM_1^{1-}$ | = 0.114 | |
| | | $PGM_1^{2+}$ | = 0.211 | |
| | | $PGM_1^{2-}$ | = 0.054 | |
| 2. Acid phosphatase (AcP) | 1086 | $AcP^b$ | = 0.686 | A. Carracedo and L. Concheiro [15] |
| | | $AcP^a$ | = 0.278 | |
| | | $AcP^c$ | = 0.036 | |
| 3. Glutamic-pyruvic transaminase (GPT) | 302 | $GPT^1$ | = 0.5099 | A. Carracedo and J. L. Blazquez [16] |
| | | $GPT^2$ | = 0.4901 | |
| 4. Esterase D (EsD) | 394 | $EsD^1$ | = 0.8744 | This paper |
| | | $EsD^2$ | = 0.1040 | |
| | | $EsD^5$ | = 0.0216 | |
| 5. Adenylate kinase (AK) | 1086 | $AK^1$ | = 0.9613 | A. Carracedo and L. Concheiro [14] |
| | | $AK^2$ | = 0.0387 | |
| 6. Adenosine deaminase (ADA) | 1086 | $ADA^1$ | = 0.9503 | A. Carracedo and L. Concheiro [15] |
| | | $ADA^2$ | = 0.0497 | |
| 7. Phosphogluconate dehydrogenase (6-PGD) | 1086 | $6\text{-}PGD^A$ | = 0.9779 | A. Carracedo and L. Concheiro [14] |
| | | $6\text{-}PGD^C$ | = 0.0221 | |
| 8. Glyoxalase (GLO) | 500 | $GLO^1$ | = 0.493 | A. Carracedo and J. L. Blazquez [16] |
| | | $GLO^2$ | = 0.507 | |
| 9. Galactose-1-phosphate uridyl transferase (GALT) | 179 | $GALT^N$ | = 0.9302 | This paper |
| | | $GALT^{D1}$ | = 0.0447 | |
| | | $GALT^{D2}$ | = 0.0251 | |
| 10. Uridine monophosphate kinase (UMPK) | 489 | $UMPK^1$ | = 0.95429 | J. L. Blazquez (unpublished results) |
| | | $UMPK^2$ | = 0.04571 | |
| 11. Phosphoglycollate phosphatase (PGP) | 485 | $PGP^1$ | = 0.92784 | M. D. Rey [17] |
| | | $PGP^2$ | = 0.04639 | |
| | | $PGP^3$ | = 0.02577 | |
| 12. Aminolevulinate dehydrase (ALADH) | 500 | $ALADH^1$ | = 0.9170 | J. L. Blazquez (unpublished results) |
| | | $ALADH^2$ | = 0.0830 | |
| 13. Malic enzyme ($ME_2$) | 434 | $ME_2^1$ | = 0.5922 | C. Llano [18] |
| | | $ME_2^2$ | = 0.4078 | |
| 14. Phosphoglucomutase-Locus 3 ($PGM_3$) | 417 | $PGM_3^1$ | = 0.6667 | C. Llano [18] |
| | | $PGM_3^2$ | = 0.3333 | |
| 15. Glutamic oxaloacetic transaminase ($GOT_2$) | 432 | $GOT_2^1$ | = 0.9745 | C. Llano [18] |
| | | $GOT_2^2$ | = 0.0255 | |

In addition, accepting $\pm 1$ difference in verbal predicates, the above-mentioned tables can be used with most European populations, although the agreement for the Galician population is not perfect (99%).

Therefore, even though Hummel's tables can be used for Iberian populations, especially when a $\pm 1$ variation of the "verbal predicate" cannot be significant for judgement because of the distinctive traits of the Galician population

**Table 7.** Exclusion rate

| Marker | % | Marker | % |
|---|---|---|---|
| 1. $PGM_1$ | 31.90 | 21. EsD | 10.87 |
| 2. MNSs | 31.63 | 22. ALADH | 7.03 |
| 3. Pi | 31.34 | 23. PGP | 6.88 |
| 4. Rh | 29.90 | 24. Xg | 6.74 |
| 5. Gc | 29.01 | 25. GALT | 6.67 |
| 6. Gm (1,2,4) | 23.62 | 26. ADA | 4.50 |
| 7. AcP | 20.43 | 27. Cholinesterase ($C_5$) | 4.37 |
| 8. ABO | 19.11 | 28. UMPK | 4.17 |
| 9. GPT | 18.75 | 29. K | 3.69 |
| 10. GLO | 18.75 | 30. AK | 3.58 |
| 11. Jk | 18.69 | 31. $AMY_2$ | 2.75 |
| 12. ORM | 18.67 | 32. Cholinesterase (Locus $E_1$) | 2.55 |
| 13. Hp | 18.47 | 33. $GOT_2$ | 2.42 |
| 14. $ME_2$ | 18.32 | 34. 6-PGD | 2.11 |
| 15. Tf | 17.87 | 35. Lu | 1.92 |
| 16. Fy | 17.80 | 36. Pep A | 1.11 |
| 17. $PGM_3$ | 17.28 | 37. Gd | 1.01 |
| 18. $C_3$ | 14.76 | 38. $P_1$ | 0.93 |
| 19. PLG | 13.44 | 39. Cp | 0.19 |
| 20. Bf | 13.31 | | |

(e.g., Pi, Tf, EsD), greater accuracy could be obtained by using the frequency reported in the present paper.

## References

1. Vaccaro AM, Muscillo M, Mandara I, Salvioli R (1982) Improved isoelectric focusing of normal and variant forms of erythrocyte galactose-1-phosphate uridyl transferase. Electrophoresis 3:58–61
2. Hopkinson DA, Mestriner MA, Cortner J, Harris H (1973) Esterase D: a new human polymorphism. Ann Hum Genet 37:119–137
3. Pascali V (1985) Isoelectric focusing techniques and their application to serum protein genetic marker typing. In: Lee HC, Gaensslen RE (eds) Advances in forensic science, vol 1. Biomedical Publications, Foster City, California, pp 160–205
4. Baxter SJ, Rees B (1974) Simultaneous haptoglobin and haemoglobin typing of blood and bloodstains using gradient polyacrylamide gel electrophoresis. Med Sci Law 14:231–236
5. Riobo MI (1985) Estudio sobre el polimorismo del plasminogeno (PLG) y properdina factor B (Bf) en las poblaciones vasca, gallega y del Norte de Portugal. Graduate dissertation. University of Santiago de Compostela
6. Requena L (1979) Estudio sobre la distribución de los grupos haptoglobinicos en la provincia de La Coruña. Graduate dissertation. University of Santiago de Compostela
7. Concheiro L, Carracedo A (1985) Marcadores genéticos de la población gallega. Su utilidad en la investigación de la paternidad. In: Villanueva E (ed) Temas medico-legales. Libro homenaje al Prof JA Gisbert, Departamento de Medicina Legal de la Facultad de Medicina de Granada

8. Hummel K, Claussen M (1981) Exclusion efficiency and biostatistical value of conventional blood group systems in European and non-European populations. In: Hummel K, Gerchow J (eds) Biomathematical evidence of paternity. Springer, Berlin Heidelberg New York
9. Carracedo A, Concheiro L (1983) Distribution of the Pi, TfC, and Gc subtypes in Galicia (North West Spain). Z Rechtsmed 90:153–158
10. Goedde HW, Hirth L, Benkmann HG, Pellicer A, Pellicer T, Stahn M, Singh S (1973) Population genetic studies of serum protein polymorphisms in four Spanish populations. Hum Hered 23:131–145
11. Rodriguez-Calvo MS (1986) Marcadores genéticos del cromosoma 6p: Análisis de desequilibrio de ligamiento y su aplicación en la investigación biológica de la paternidad. Graduate dissertation, University of Santiago de Compostela
12. Blazquez JL (1982) Estudio del polimorfismo genético de proteinas plasmáticas en una muestra de población gallega. Doctoral thesis, University of Santiago de Compostela
13. Carracedo A, Concheiro L, Estefania J, Suarez C (1986) Polymorphism of serum orosomucoid. Family and population studies in Galicia. In: Brinkmann B, Henningsen K (eds) Advances in forensic haemogenetics, vol 1. Springer, Berlin Heidelberg New York Tokyo
14. Carracedo A, Concheiro L (1982) $PGM_1$ subtypes in Galicia (NW Spain). Hum Hered 32:133–135
15. Carracedo A, Concheiro L (1982) Polymorphism of erythrocyte acid phosphatase and adenosine deaminase in Galicia (NW Spain) by AGIF and PAGIF. Z Rechtsmed 88: 143–145
16. Carracedo A, Blazquez JL (1984) Determinación das isoenzimas GPT por focaxe isoelectrica en xeles de poliacrilamida. Acta Cientifica Compostelana 21:231–237
17. Rey MD (1981) Análisis del polimorfismo de la fosfoglicolato fosfatasa y de la esterasa D en una muestra de la poblácion gallega. Graduate disseration, University of Santiago de Compostela
18. Llano C (1986) Heterogeneidad alozímica de sistemas ($GOT_2$, $ME_2$, $PGM_3$) en Galicia. Graduate dissertation, University of Santiago de Compostela

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