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## Polymorphism of the Plasminogen (PLG) System in Cádiz Province, Southern Spain

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**ABSTRACT:** In this work, the authors report a plasminogen (PLG) system genetic-population study in a sample of 378 healthy subjects, of both sexes and unrelated, all resident in the province of Cádiz in Southern Spain. In this study, the PLG types were determined by isoelectric focusing in polyacrylamide gels (PAGIF), followed by staining with Coomassie blue.

The genic frequencies obtained were the following: PLG A = 0.833 333 3; PLG B = 0.166 666 7.

**KEYWORDS:** pathology and biology, genetic typing, plasminogen (PLG), genetic polymorphism, paternity testing, isoelectric focusing

Plasminogen (PLG) is a normal plasma protein which, when activated, is converted into plasmin. Plasmin is capable of adhering to fibrin mesh.

In spite of the fact that the PLG system was initially studied by authors such as Heberlein and Barnhart [1] and Wallen and Wiman [2], who used electrophoresis and zymographic methods, the polymorphism of this protein was not really demonstrated until concurrent investigations by Hobart [3] and by Raum, Marcus, and Alper [4] identified, by means of similar electrophoretical techniques, three common variants—which were called, in line with the different nomenclature used by the above-mentioned authors, PLG 1, PLG 2, and PLG 2-1 or PLG A, PLG B, and PLG A-B. Subsequently, a considerable number of new variants were discovered, whose common characteristic was their low rate of incidence in the population.

The present work discloses the results obtained from a study carried out on the PLG system in a representative sample of the population in Cádiz Province in Southern Spain (see Fig. 1).

### Materials and Methods

In this study, we have used 378 blood samples extracted from the same number of healthy individuals, resident in the province of Cádiz and randomly chosen from among

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FIG. 1—Cádiz Province, Andalusia, Spain.

outpatients at different hospitals who were treated for trauma. Their number was established in proportion to the population size of each municipality.

Sera were obtained by centrifugation and were stored in Eppendorf's capsules at  $-20^{\circ}\text{C}$ . The samples were processed electrophoretically within 26 months of extraction. Before PLG typing, the serum samples were pretreated with a neuraminidase (Sigma, Type V) solution (1 unit/mL) for at least 12 h at  $-4^{\circ}\text{C}$ .

The PLG types were determined by isoelectric focusing in polyacrylamide gels (PAGIF), following the technique described by Carracedo et al. [5].

PAGIF was carried out in 0.4-mm thin-layer polyacrylamide gels at a gel concentration of  $T = 5.5\%$  and a cross-linking of  $C = 3\%$ . The ampholine (LKB, Bromma, Sweden) concentration was 5%. Polymerization was carried out using 0.5% (v/v) riboflavin and ultraviolet light.

The pH range used for PLG typing was pH 4 through 8. The electrode solutions used were 11% ethanolamine for the cathode and 0.04M glutamic acid for the anode. The sera samples, after treatment with neuraminidase, were applied on Whatmann 3MM paper (0.5 by 0.5 cm), at 4 cm from the anode.

Electric focusing was carried out at 5 W for 270 min, with a voltage limited to 1,500 V. After focusing, the marker under study was stained with Coomassie blue R-250, according to the method of Pascali [6].

## Results and Discussion

The results obtained for the phenotype and genic frequencies of the plasminogen (PLG) system in the province of Cádiz are given in Table 1, which shows that the studied population is in Hardy-Weinberg equilibrium for this marker.

TABLE 1—PLG phenotypes and gene distribution in a population sample in Cádiz Province, Southern Spain.<sup>a</sup>

| Phenotype | Observed |          | Expected |         | Allele Frequencies  |
|-----------|----------|----------|----------|---------|---------------------|
|           | N        | %        | N        | %       |                     |
| A/A       | 266      | 70.3704  | 262.50   | 69.4444 |                     |
| A/B       | 98       | 25.9259  | 105.00   | 27.7778 | PLG A = 0.833 333 3 |
| B/B       | 14       | 3.7037   | 10.50    | 2.7778  | PLG B = 0.166 666 7 |
| Total     | 378      | 100.0000 | 378.00   | 100.000 |                     |

$$^a\chi^2 = 1.679\ 999; \text{df} = 1; 0.25 > P > 0.10.$$

TABLE 2—Geographical distribution and genic frequencies of PLG system.

| Population                         | N    | PLG A | PLG B | PLG V | Reference  |
|------------------------------------|------|-------|-------|-------|------------|
| Italy                              |      |       |       |       |            |
| Arezzo                             |      | 0.830 | 0.160 | 0.010 | [7]        |
| Rome                               | 2116 | 0.810 | 0.180 | 0.010 | [7]        |
| Benevento                          |      | 0.810 | 0.180 | 0.010 | [7]        |
| Veneto                             | 1325 | 0.840 | 0.158 | 0.001 | [8]        |
| Udine                              | 716  | 0.858 | 0.140 | 0.001 | [9]        |
| Spain                              |      |       |       |       |            |
| Barcelona                          | 752  | 0.784 | 0.215 | ...   | [10]       |
| Galicia                            | 703  | 0.800 | 0.199 | ...   | [5]        |
| Cádiz                              | 378  | 0.833 | 0.166 | ...   | this study |
| Mexico                             |      |       |       |       |            |
| Low social strata                  | 197  | 0.882 | 0.111 | 0.007 | [11]       |
| High social strata                 | 123  | 0.858 | 0.110 | 0.032 | [11]       |
| United States                      |      |       |       |       |            |
| Latins                             | 295  | 0.844 | 0.143 | 0.003 | [12]       |
| Whites                             | 3240 | 0.676 | 0.294 | 0.030 | [12]       |
| Blacks                             | 368  | 0.727 | 0.227 | 0.001 | [12]       |
| Denmark                            | 1664 | 0.639 | 0.303 | 0.058 | [13]       |
| Switzerland                        | 1392 | 0.662 | 0.302 | 0.036 | [14]       |
| North Germany                      | 604  | 0.712 | 0.269 | 0.017 | [15]       |
| South Africa<br>(Black population) | 1252 | 0.698 | 0.274 | 0.029 | [16]       |
| Japan                              | 795  | 0.944 | 0.019 | 0.014 | [17]       |

From the PLG study carried out, we would underline the similarities observed between the distribution of gene frequencies found in our province and those found in the majority of populations of Latin origin, as well as the existing differences between the populations of Northern and Central Europe and the Mongolian and Black African races. This situation is reflected in Table 2 [7-17].

The exclusion probability *a priori* obtained for the PLG system in this study undertaken in our province is 11.95%. This value is somewhat lower than those determined for the same marker in other national populations [5,10].

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