# POLYMORPHISM OF SERUM OROSOMUCOID. FAMILY AND POPULATION STUDIES IN GALICIA.

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## Introduction

Alpha-1-acid glycoprotein or orosomucoid (Or) is one of the most extremely studied plasma proteins and it is characterized by an exceptionally high carbohydrate content and a large number of syalic residues (12%) together with an extremely acidic isoelectric point (around 2.5-3).

The current state of research into this protein has been reviewed by Schmid (1) and Arnaud and Gianazza (2).

At present this protein is not habitually used in paternity testing and bloodstain analysis due to the technical difficulties involved in the methods used until recently for the study of this polymorphism.

In this paper we present a simple method for Or typing by PAGIF followed silver staining together with the results of population and family studies in 650 individuals and 115 families from Galicia (NW Spain).

## Material and Methods

Serum from freshly collected blood samples from 650 healthy donors and 115 families was used.

Samples were stored at -30°C and pretreated with fresnly Clelland's reagent (0.05 M dithiothreitol) before typing.

PAGIF was carried out in 0.4 mm thin-layer polyacrylamide gels at a gel concentration of T=5.5% and cross-linking of C=3%. Ampholine (pH 2.5-4) concentration was 5%.

Sucrose was added as a stabilizing agent at a final concentration of 12% (w/v).

Polymerization was carried out with ultraviolet light and a 0.02% riboflavin solution (20 mg in 100 ml of distilled water).

The electrode solutions were 0.7% ethanolamine for the cathode and 1M phosphoric acid for the anode.

Samples were applied 2 cm from the cathode using Whatman



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3 MM filter papers (1 x 1 cm). Focusing was carried out at 15 W constant power, current unlimited. A maximum voltage of 2000 V and a cooling temperature of 10°C were used.

A focusing time of 3.5 hours was needed.

After isoelectric focusing the gels were stained with the silver staining method of Carracedo et al. (3) with some modifications:

First they were prefixed in 12% trichloroacetic acid for 15 min and washed three times for 20 min in 200 ml of 50% ethanol at 50°C in a shaking water bath to remove ampholytes, and then fixed in 100 ml of 10% glutaraldehyde for 20 min. The gels were then washed four times for 15 min in 200 ml of distilled water. They were then soaked in 100 ml of 0.01% dithiothreitol and the treated for 30 min in 100 ml of 0.1% silver nitrate. The gels were then given two rinses. First in 100 ml of distilled water and then with a small amount of developer (75 l of 37% formaldehyde in 150 ml of 3% sodium carbonate) until enough contrast in the bands was obtained.

The staining was stopped by adding 10 ml of 2M citric acid and shaken for 5 min. The gels can also be wrapped in protective celophane sheets, being stable the colours for several months.

## Results and discussion

Figures 1 and 2 demonstrate the Or phenotypes as observed with the method described. A clear distinction between phenotypes was found.

The first studies about the electrophoretic pattern of serum orosomucoid were carried out by Schmid and Binette (4) in 1961 using starch gel electrophoresis.

Later (5) it was found that when the sialic acid residues had been removed from the protein, one of three alternative patterns (designated I, II and III) were observed. Once the Or polymorphism had been further elucidated by general population and family studies, the phenotypes were designated FF, SS and FF.

At present, orosomucoid is not habitually used in paternity testing, due to technical difficulties in the methods used

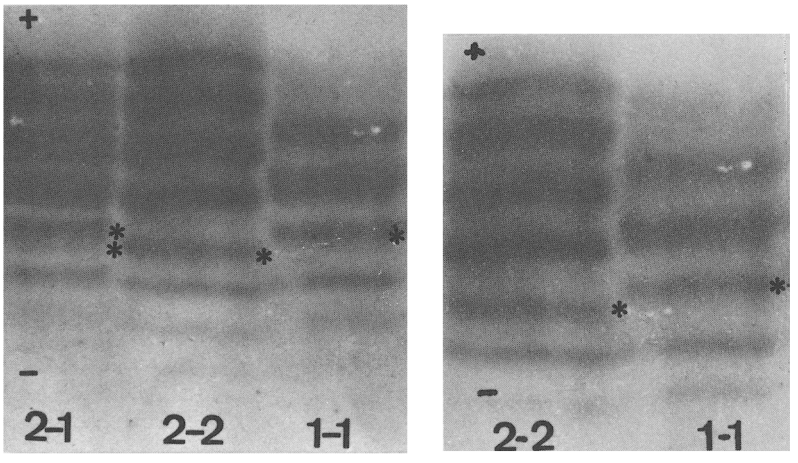


Fig. 1-2 Orosomucoid variants after PAGIF pH 2.5-4 and silver staining.

Table 1. Frequencies of Or phenotypes and Or alleles in Galicia

| Phenotype | Observed | Expected | |
|---|---|---|---|
| 1-1 | 134 | 137.5 | |
| 2-1 | 330 | 322.9 | $Or^1$:0.460 |
| 2-2 | 186 | 189.5 | $Or^2$:0.540 |
| | 650 | 649.9 | $\chi^2$=0.292 |
| | | | p>0.50 |

Table 2. Segregation of Or phenotypes in 115 families with 203 children

| | nF | 1-1 | 2-1 | 2-2 |
|---|---|---|---|---|
| 1 X 1 | 4 | 7 | | |
| 1 X 2-1 | 29 | 20(22) | 24(22) | |
| 2-1 X 2-1 | 30 | 13(14) | 29(28) | 14(14) |
| 2 X 2-1 | 34 | | 35(33) | 31(33) |
| 2 X 2 | 6 | | | 10 |
| 1 X 2 | 12 | | 20 | |

until recently for the study of this polymorphism. These methods include starch gel electrophoresis after protein purification or specific immunofixation and cross immunoelectrophoresis.

Using PAGIF followed by immunofixation, Arnaud and Gianazza(2) found that orosomucoid shows both microheterogeneity and polymorphism, the latter being under genetic control.

With the method described above we have observed that silver staining render the use of immunotechniques unnecessary.

The results of population and family studies are given in Tables 1 and 2.

The observed frequencies were 0.460 for the allele $Or^1$ and 0.540 for the allele $Or^2$. Good agreement was noted for the Hardy-Weinberg distribution, which is particularly important for the use of this marker in paternity testing since the Hardy-Weinberg equilibrium of the orosomucoid seems to be greatly disturbed in the Japanese group. Furthermore, investigation of 115 families with 203 children support the assumed autosomal codominant way of inheritance (Table 2).

With a theoretical chance of exclusion of non-fathers 18.7% in our population, Or polymorphism typed by PAGIF and silver staining becomes a useful marker in paternity testing.

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