GENETIC STUDY OF SERUM OROSOMUCOID (ORM) POLYMORPHISM BY ULTRATHIN LAYER ISOELECTRIC FOCUSING

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INTRODUCTION

Orosomucoid (ORM), or Alpha-1-acid glycoprotein, serum polymorphism, has not been extensively investigated until now,so that ,it is not routinely used in paternity testing. Genetic polymorphism of human serum orosomucoid can be demonstrated with various electrophoretic techniques such as starch gel electrophoresis after protein purification(1), or immunofixation of neuraminidase treated serum (2), cross immunoelectrophoresis (3), separator isoelectric focusing.
The genetic heterogeneity of serum orosomucoid is due to two codominant alleles at a single autosomal locus: ORM*F and ORM*S which results in the three phenotypes FF, FS and SS (5,6). However, Thymann et al. (4), using separator isoelectric focusing, have demonstrated that the electrophoretic F band was subdivided in two bands named F1 and F2, so that, five phenotypes were observed.
Recently Carracedo et al. (7,8) have found that the application of silver staining after PAGIF makes the use of immunotechnique unnecessary. Based on these results we have applied the Carracedo's tecnique typing a group of human serum samples. Here we shall outline our procedure, with some modifications respect to Carracedo's technique, and show the results obtained from a preliminary study.

MATERIAL AND METHODS

Sera were obtained from 96 apparently healthy and urelated blood donors of the Blood Bank of Treviso (Veneto, Italy). They were stored frozen in small aliquots at -25°C and tested within some days.
Isoelectric focusing was carried out on polyacrylamide gel (250x125x0.5 mm) on a LKB apparatus connected to a LKB Power Supply, at cool temperature. Each gel was made to a final concentration of acrylamyde solution T=5% and C=3%, sucrose 12% (w/v), carrier ampholytes 3% (w/v) in the 2.5-5.0 pH range. After 15 min of degasation polymerization was achieved with Ammonium persulphate 0.05% (w/v).

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The following electrodic solutions were used: 0.1 M Glutamic acid in 0.5 M H_3PO_4 (anolyte) and 0.1 M β-Alanine (catholyte).
Undiluted serum samples were applied at 2 cm from the cathodal end by means of small papers (W 1 MM 7x5 mm).
Focusing was carried out for 270 min (papers were removed after 60 min) with the following maximal conditions: 1500 V, 5W, unlimited mA.
After isoelectric focusing, the gel was stained with the silver staining method of Carracedo et al. (7, 8) using shorter steps.

RESULTS AND DISCUSSION

In table 1 are reported the results obtained with this preliminary study. There is a good agreement between observed and expected values assuming a Hardy-Weinberg equilibrium.

TABLE 1

PHENOTYPE	OBSERVED	EXPECTED
S-S	33	32.0832
F-S	45	46.8289
F-F	18	17.0879
	96	96.0000

ORM^S= 0.5781 ORM^f=0.4219

χ^2 =0.1462 .95 p .90 for 1df

In our study the common variants are designated ORM F and ORM S.
The slow banded pattern (we considered the two most cathodally bands of the pattern, with the anode on the top), corresponds to the phenotype ORM S, the more rapidly migrating banded pattern corresponds to ORM F and the double-banded pattern is ORM FS. The corresponding genes are, obviously, designated ORM*S nad ORM*F.
Using the tecnique as described, no difficulties were encountered in the determination of ORM allotypes. The multiple-banded patterns are similar to those described by other Authors.
In comparison to the other electrophoretic techniques, the procedure above described is more simple, cheaper and shorter, for this, we believe that orosomucoid may be routinely used as useful marker both population studies and paternity testing.

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