

Orosomuroid (ORM) Phenotyping by Isoelectric Focusing in Immobilized pH-Gradient Followed by Immunoblotting

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INTRODUCTION

Orosomuroid or α 1-acid glycoprotein is an acute phase reactant. It is coded by chromosome 9 (12) and its concentration increases in response to non-specific stimuli. The protein has a binding capacity for several drugs (2, 5, 9, 13, 17, 24, 25, 29). Clinical investigations did not lead to significant association with diseases (7,10,23). Numerous population data have been published (6, 15, 16, 22, 33, 34, 36, 37). Rare variants (11, 14, 19, 20, 21, 22, 31, 32, 36) have been reported using different improved techniques (4, 8, 14, 16, 18, 27, 28, 29, 30, 33, 38).

MATERIALS and METHODS

Serum samples had been stored at -50° C until examined.

Treatment of samples: desialylation by neuraminidase (*C. perfringens* ; serum/enzyme 2:1) at 37° C overnight (20-24 hours). Adding of 30 μ l reductive clearance solution 1: 0.12 g boric acid, 4.81 g urea, 0.015 g DDT ad 10 ml aqua dest. (adjusted to pH 9, NaOH). Components mixed by Vortex and incubated for 20 min. at room temperature.

Adding of 30 μ l reductive clearance solution 2 : 0.12 g boric acid and 0.36 g jodacetamide ad 10 ml aqua dest. (adjusted to pH 9, NaOH). Components mixed by Vortex and incubation for 20 min. at room temperature. Isoelectric focusing (Immobiline dry plate; LKB, pH 4.5-5.4): 3000 V, 6 mA, 12 W, 4 hours.

Immunoblotting (20 min.) by passive transfer to nitrocellulose membrane, stopped by incubating the membrane in 2% TWEEN 20 (PBS-buffer, pH 10.2) for 15 min..

Primary antibody: rabbit-antihuman ORM (DAKOPATTS), 1:300 in PBS buffer (1% bovine albumine 30%), incubation overnight.

Washing the membrane three times for 5 minutes in the same buffer.

Second antibody: peroxidase-conjugated swine-rabbit immunoglobine (DAKOPATTS) 1:1000 solution (PBS buffer, see above) for 2 hours.

Visualization: Washing the membrane in 0.05 M acetate buffer pH 5;

incubation in the staining solution: 20 mg 3-amino-9-ethylcarbazol, 2,5 ml acetone, 50 ml acetate buffer 0.05 M (pH 5), 25 μ l hydrogen peroxidase(30%); staining time about 5 min.

The reaction is stopped by washing the membrane in aqua dest.

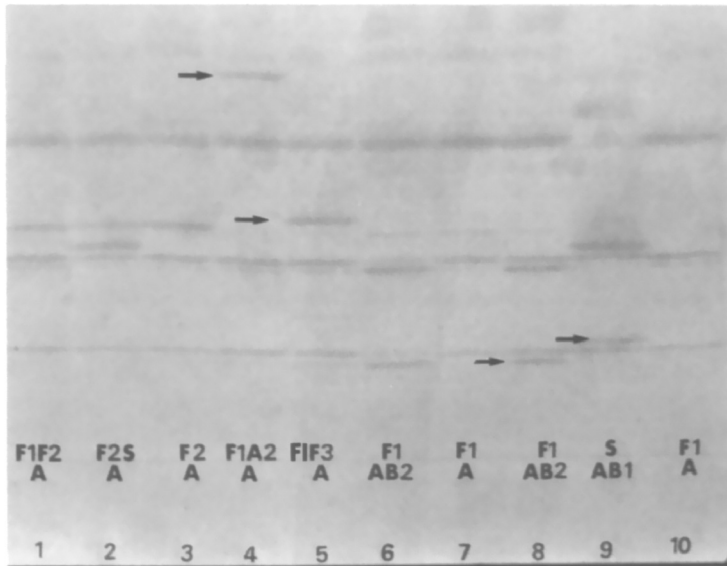
RESULTS and DISCUSSION

Figure 1 shows the banding pattern of the ORM phenotypes observed by IEF with the method described above. Five of the six common phenotypes are presented in high resolution and can be identified definitely. In case of the heterozygous ORM1*F1F2 and the homozygous ORM1*F2 the examiner has to observe different intensities. Interpreting the banding pattern of a fresh visualized blot this means no problem. In lane 3 and lane 4 two rare ORM1 variants are presented : ORM1 F1A2 and ORM1 F1F3. The additional bands anodally and cathodally from the

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F1 band are marked. The cathodal bands may be assigned to the ORM2 locus. The common phenotype is ORM2 A. Nevertheless the examiner has to reckon on rare variants : on this blot ORM2*AB1 and ORM2*AB2 (lane 6,8,9). The B2 variant was found in a family with putative father from Africa.

Fig.1 ORM PHENOTYPING, IEF FOLLOWED BY IMMUNOBLOTTING



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