

TECHNICAL NOTE

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Human Orosomuroid (ORM₁) Subtyping: Further Population Genetic Data and Reports on the Feasibility to Type Aged Blood Samples and Stains

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ABSTRACT: Genetic polymorphism of serum orosomuroid (ORM₁) was investigated in 1072 unrelated German Caucasians using isoelectric focusing followed by Western blotting and EIA. The estimated allele frequencies were ORM₁*F1 = 0,5690, ORM₁*S = 0,3927, ORM₁*F2 = 0,0368, ORM₁*F2S = 0,0009 and ORM₁*F5 = 0,0005. The method was successfully applied to determine ORM₁ phenotypes in aged blood samples and blood stains. The results indicated that the ORM protein is a informative and remarkably robust blood group system.

KEYWORDS: forensic science, orosomuroid, ORM₁, serum protein, polymorphism, population genetics, bloodstains, aged blood samples

Orosomuroid (ORM) or Alpha-1 Acid Glycoprotein is an *acute phase reactant* which may be significantly increased during various diseases. The protein consists of a single polypeptide chain composed of 183 amino acids.

Together with five carbohydrate chains including a large number of sialic acid residues the molecular weight is about 40,000 dalton (1,2). ORM₁ gene is located on chromosome 9 at position 9q31-q34.1 in close neighborhood to loci *ABO*, *adenylate kinase 1* and *delta-aminolevulinatase-dehydratase* (3–5).

Genetic heterogeneity of ORM was first observed in 1961 by means of starchgel electrophoresis (2). Isoelectric focusing (IEF) in combination with immunofixation as well as molecular genetic studies revealed that the protein is controlled by two closely linked loci, *ORM₁* and *ORM₂*. At locus *ORM₁* two alleles ORM₁*F and ORM₁*S could be identified (6–8). Thereafter, Thymann and Eiberg (9) found that ORM₁*F product can be subdivided into two proteins (ORM₁*F1 and ORM₁*F2), which have different isoelectric points.

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Evidence for gene duplications was given by Yuasa and Luckenbach (10,11). Various population genetic studies were carried out and rare variants could be described (12–17).

This paper aims at reporting allele frequencies found in a larger group of Caucasian individuals (n = 1072) and focuses on the issue of stability of the protein in aged blood samples and blood stains.

Material and Methods

Samples—Serum samples from 1072 random German Caucasians were phenotyped.

Treatment of Samples—Ten µL serum was incubated with 30 µL neuraminidase (*Clostridium perfringens*; 2 U/mL; Sigma N-2876) at 37°C for at least 18 hours. According to the description by Luckenbach et al. (12,18) alkylation was carried out: samples were incubated with 5 µL 1.18 M iodacetamid in 0.2 M boric acid/NaOH (pH 9.0) containing 8 M urea for 1.5 hours at 37°C.

Preparation of Blood Stains—Fifteen blood stains were prepared by placing either 1 mL EDTA blood or whole blood on cotton sheets (2 × 2 cm). Blood stains were stored for approximately 4 weeks at room temperature. Without delay stains of 1 × 1 cm in size were eluted in 200 µL distilled water by gentle shaking for approximately 3 hours. Aliquots of 20 µL and 40 µL were treated for desialylation with neuraminidase. Alkylation, focusing, immunoblotting and detection was carried out as described below.

Preparation of Aged Samples—Aged blood samples were at least 6 months old. The appropriate serum samples were stored at –20°C. Those samples were analyzed exactly like the fresh ones.

Isoelectric Focusing (IEF)—Isoelectric focusing was carried out in horizontal polyacrylamide gels sized: 250 × 115 × 0.5 mm. The gel contents of 2.4 mL acrylamide stock solution (29.1% acrylamide and 0.9% N,N'-methylene bisacrylamide), 10.4 mL distilled water, 6.92 g urea, 80 µL pharmalyte pH 3.5–5 (LKB #1809-111), 600 µL pharmalyte pH 4.5–5.4 (Pharmacia #17-0563-01) and 80 µL servalyte pH 5–6 (Serva #42924). Gel was polymerized using 14.4 µL TEMED and 100 µL ammoniumpersulfate (91 mg/mL). Anodal solution consisted of 0.04 M glutamic acid while 0.1 M NaOH was used as cathodal electrolyte.

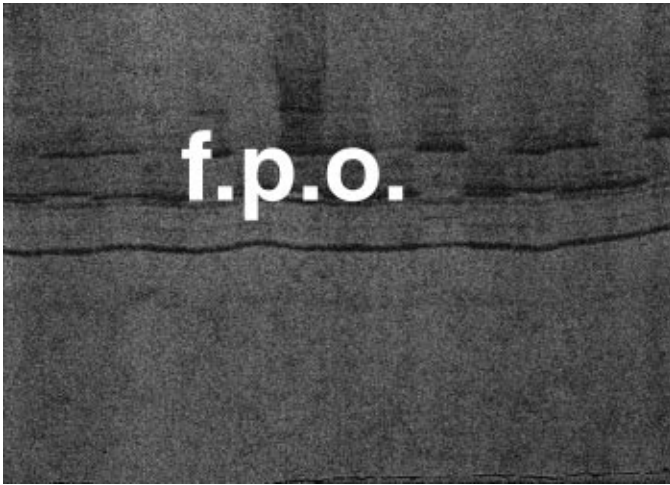


FIG. 3—ORM₁ patterns in stored sera. From left to right: F2S, F1F2, F1S, S, F1, F2S, F1F2, F1S, S, F1, F2S, F1F2, F1S, S, F1 (anode on top).

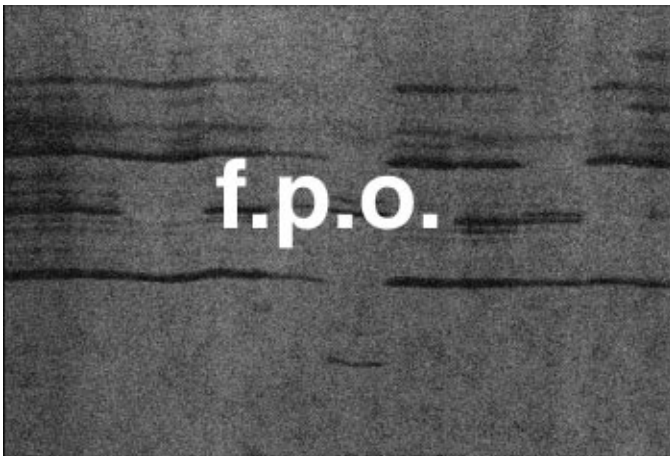


FIG. 4—Patterns of ORM-phenotypes in aged and fresh blood samples. From left to right F1S, F1S, F1S, F1, F1, F1S, F1F2, aged blood samples. Fresh reference samples F1, F1S, F2S, F1, F1 (anode on top).



FIG. 5—ORM₁ patterns in bloodstains. From left to right S, F1, F1F2 fresh reference samples; bloodstains of types S, S, F1S, F1S, F1, F1, F1F2, F1F2, F2S, F2S, F1F2S, F1F2S, followed by fresh reference samples of types S, F1, F1S (anode on top).

Discussion

Because legislations or court systems may differ significantly among countries, it is well justified to hold on the ability to type informative, and last but not least, robust blood group markers. In parentage testing the overall exclusion chance for Caucasian non-fathers is approximately 22%.

In this study, we did not find an ORM₁*Q O allele, what may indicate that this “silent” allele is very rare (19).

ORM₁ phenotypes could be reliably determined in serum samples being stored for 6 months at -20°C. Even highly hemolytic blood samples except one could be typed unambiguously. In two exceptional cases an ORM₁(F1) phenotype “became” an ORM₁(F2) type. Because the entire banding patterns were shifted cathodically, we were alerted and expected that the particular sample might not give reliable typing results. The reason for these exceptional events could not be resolved.

Beside the issue of phenotyping aged blood samples, we also studied the robustness of the ORM₁ protein in blood stains. The earlier finding of Umetsu et al., that ORM₁ is remarkably stable, could be confirmed although we employed a different typing technique (14). All blood stains analyzed could be correctly typed after a storage time of 4 weeks.

ORM₁ subtyping technique as it was introduced by Luckenbach et al. (12) is considered to be a sensible alternative in comparison to methods used by other groups (14,20).

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