TECHNICAL NOTE

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Human Orosomucoid (ORM_1) 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 orosomucoid (ORM_1) was investigated in 1072 unrelated German Caucasians using isoelectric focusing followed by Western blotting and EIA. The estimated allele frequencies were ORM_1 *F1 = 0,5690, ORM_1 *S = 0,3927, ORM_1 *F2 = 0,0368, ORM_1 *F2S = 0,0009 and ORM_1 *F5 = 0,0005. The method was successfully applied to determine ORM_1 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, orosomucoid, ORM_1, serum protein, polymorphism, population genetics, bloodstains, aged blood samples

Orosomucoid (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). ORM_1 gene is located on chromosome 9 at position 9q31-q34.1 in close neighborhood to loci *ABO, adenylate kinase 1* and *delta-aminolevulinate-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, ORM_1 and ORM_2. At locus ORM_1 two alleles ORM_1 *F and ORM_1 *S could be identified (6–8). Thereafter, Thymann and Eiberg (9) found that ORM_1 *F product can be subdivided into two proteins (ORM_1 *F1 and ORM_1 *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 eluated in 200 μL distilled water by gentle shaking for approximately 3 hours. Aliquots of 20 μL and 40 μL were treated for desialysation 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.

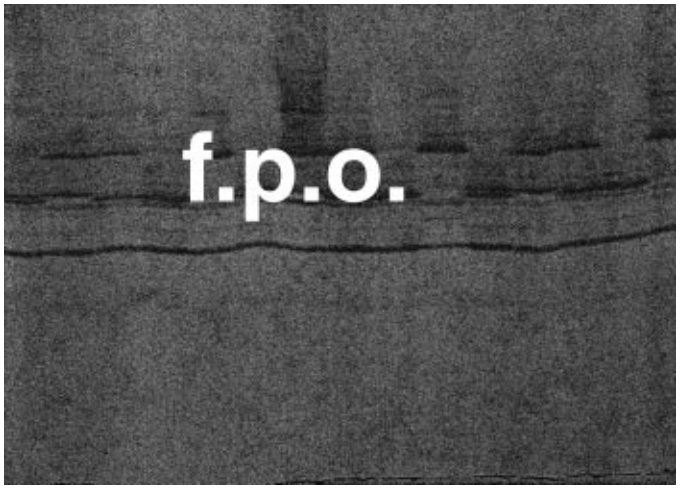

FIG. 3—*ORM_1 patterns in stored sera. From left to right: F2S, F1F2, F1S, S, F1, F2S, F1F2, F1S, S, F1, F2S, F1F2, F1S, S, F1 (anode on top).*

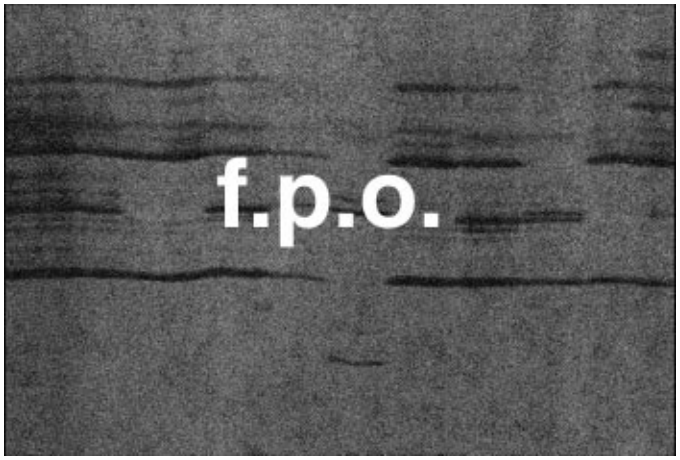

FIG. 4—*Patterns of ORM-phenotypes in aged and fresh blood samples. From left to right F1S, F1S, F1S, F1, F1, F1S, F1F2, aged blood samples. Fresh reference samples F1, F1S, F2S, F1, F1 (anode on top).*

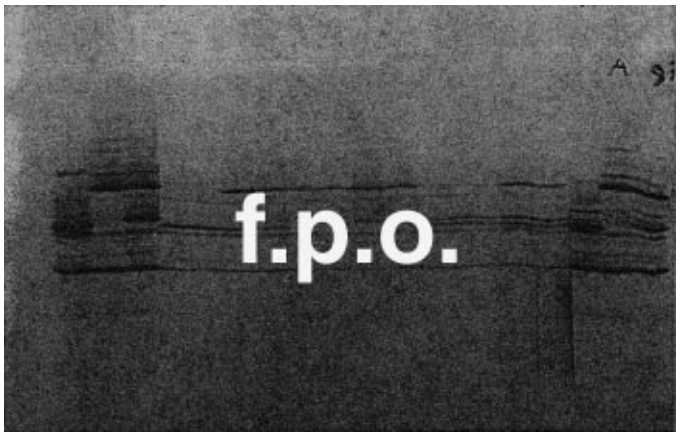

FIG. 5—*ORM_1 patterns in bloodstains. From left to right S, F1, F1F2 fresh reference samples; bloodstains of types S, S, F1S, F1S, F1, F1, F1F2, F1F2, F2S, F2S, F1F2S, F1F2S, followed by fresh reference samples of types S, F1, F1S (anode on top).*

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 ORM_1 *Q O allele, what may indicate that this "silent" allele is very rare (19).

ORM_1 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 ORM_1 (F1) phenotype "became" an ORM_1(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 ORM_1 protein in blood stains. The earlier finding of Umetsu et al., that ORM_1 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.

ORM_1 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).

References

1. Schmid K. α_1-acid glycoprotein. In: Putnum FW, editor. The plasma proteins. New York: Academic Press, 1975(1);183–228.
2. Schmid K, Binette JP. Polymorphism of α_1-acid glycoprotein. Nature 1961;190:630–1.
3. Eiberg H, Mohr J, Nielsen LS. Linkage of orosomucoid (ORM) to ABO and AK1. Cytogenet Cell Genet 1982;32:272.
4. Eiberg H, Mohr J, Nielsen LS. δ-Aminolevulinate dehydrase: synteny with ABO, AK1, ORM and assignment to chromosome 9. Clin Genet 1983;23:150–4.
5. Webb DC, Earle ME, Merritt C, Board PG. Localization of human alpha-1-acid-glycoprotein genes to 9q31-q34.1. Cytogenet Cell Genet 1988;47:18–21.
6. Dente L, Ciliberto G, Cortese R. Structure of the human alpha-1-acid glycoprotein gene: Sequence homology with other human acute phase protein genes. Nucleic Acids Res 1985;13:3941–52.
7. Johnson AM, Schmid K, Alpen CA. Inheritance of human alpha-1-acid glycoprotein (orosomucoid) variants. J Clin Invest 1969;48: 2293–9.
8. Yuasa I, Umetsu K, Suenaga K, Robinet-Levy M. Orosomucoid (ORM) typing by isoelectric focusing: Evidence for two structural loci ORM_1 and ORM_2. Hum Genet 1986;74:160–1.
9. Thymann M, Eiberg H. Orosomucoid polymorphism determination by separatory isoelectric focusing and determination of ORM_1 *F subtypes. Adv Forensic Haemogenet 1986;1:162–6.
10. Yuasa I, Suenaga K, Umetsu K, Ito K, Robinet-Levy M. Orosomucoid (ORM) typing by isoelectric focusing: Evidence for gene duplication of ORM_1 and genetic polymorphism of ORM_2. Hum Genet 1987;77:235–58.
11. Rocha J, Amorim A, Luckenbach C, Kömpf J, Ritter H. Subtyping of alkylated human orosomucoid: Evidence for a duplicated gene, ORM_1 *F2S. Electrophoresis 1993;14:235–7.
12. Luckenbach C, Kömpf J, Ritter H. Improved subtyping of orosomucoid 1 (ORM, alpha-1 acid glycoprotein). Ärztl Lab 1989;35: 192–3.
13. Sebetan JM, Sagisaka K. Genetic polymorphism of orosomucoid ORM_1 and ORM_2 in a Japanese population: occurrence of new ORM_1 alleles. Z Rechtsmed 1989;102:5–9.
14. Umetsu K, Yuasa J, Nishi K, Brinkmann B, Suzuki T. Orosomucoid (ORM) typing by isoelectric focusing: Description of two new alleles in a German population and thermostability in bloodstains. Z Rechtsmed 1989;102:171–7.

15. Weidinger S. Untersuchungen zum Polymorphismus des sauren Alpha-1-Glycoproteins mit der isoelektrischen Fokussierung. Elektrophorese-Forum 1987:217–24.
16. Weidinger S, Mϋller T, Schwarzfischer F, Cleve H. Three new orosomucoid (ORM) variants revealed by isoelectric focusing and print immunofixation. Hum Genet 1987;77:286–8.
17. Luckenbach C, Kϋmpf J, Rocha J, Amorim A. Formal genetic data on ORM_1 subtypes. Advances in forensic haemogenetics 4 Ch. Ritter, P.M. Schneider. Heidelberg 1992:403–6.
18. Luckenbach C, Kϋmpf J, Ritter H. Orosomucoid (ORM_1) subtyping and formal genetics. Hum Genet 1991;87:429–32.
19. Kasulke DH, Weidinger S. A silent allele in the Orosomucoid (ORM) system. Advances in forensic haemogenetics 3 H.F. Polesky, W.R. Mayr, Heidelberg 1990:313–5.
20. Sebetan IM, Oshida S, Tie J. An improved high resolution single method for orosomucoid ORM_1 and ORM_2 phenotyping. J Forensic Sci 1997;42(1):115–7.

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