Orosomucoid (ORM) Subtyping: Application to Paternity Testing

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INTRODUCTION

An extended genetically determined polymorphism of human alpha-1-acid glycoprotein or orosomucoid (ORM) can be recognized using isoelectric focusing (IEF) in polyacrylamide gel followed by immunofixation or immunoblotting. After neuraminidase treatment of plasma or sera five of the six common ORM phenotypes were observed in population studies indicating the existence of three autosomal codominant alleles (Thymann and Eiberg 1986; Yuasa et al. 1986). According to the nomenclature of Johnson et al. (1969) the three allelic genes were designated ORM*F1, ORM*F2 and ORM*S. The ORM*F gene product can be separated into F1 and F2 only with the high resolving power of IEF. ORM subtyping has been carried out recently in populations from Japan, Denmark and Germany (Umetsu et al. 1985; Yuasa et al. 1986; Thymann and Eiberg 1986; Weidinger et al. 1987). There is evidence for the existence of a second structural locus in the ORM system. ORM has been mapped to chromosome 9 by linkage to ABO, adenylate kinase 1 and delta-aminolevulinate dehydrase (Eiberg et al. 1983).

In this study the ORM polymorphism was investigated in a sample from Southern Germany using a combination of IEF and print immunofixation. The distribution of ORM1 subtypes and alleles is given along with family data. The usefulness of this marker system for paternity testing will be discussed.

MATERIALS and METHODS

The population examined in this study comprised 484 unrelated individuals from Southern Germany. Families were obtained from the paternity testing laboratory. Desialylation of serum samples was carried out prior to analysis by IEF. To 20 µl of serum 10 µl neuraminidase (Clostridium perfringens 1U/mg) were added. Digestion for at least 20 h at 37 °C was required for essentially complete removal of the sialyl residues.

Isoelectric focusing was carried out with the LKB Multiphor chamber. A 0.5 mm thin polyacrylamide gel (dimension 250x115 mm) consisted of 7.5 ml acrylamide (9.7 g/dl)- and bis-acrylamide (0.3 g/dl) solution, 2 ml glycerol (87%), 0.6 ml Pharmalyte pH 4.2-4.9, 0.4 ml Pharmalyte pH 4.5-5.4, 50 mg sulfonic acid (ACES), and 5 ml destilled water.

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Polymerization was performed within 30 min. after addition of 6 µl TEMED and 180 µl ammonium persulfate (36 mg/ml). 8 µl of serum samples were applied to the gel with Whatman No.3 filter paper pieces at a distance of 2 cm from the cathodal strip. As electrode solutions a mixture of 0.025 M aspartic acid and 0.025 M glutamic acid was used for the anode and 0.1 M NaOH was used for the cathode. Separation was performed at 8°C for 3 h at a maximum of 2000 V, 16 mA, and 20 W.

Print immunofixation was carried out using a cellulose acetate strip (micro-solid, Biotec-Fischer GmbH) soaked with a 1:3 diluted monospecific ORM-antiserum (Behringwerke AG). The strip was placed on the surface of the gel for 150 s. After washing for 20 min. it was stained with Coomassie Brillant Blue R-250.

RESULTS and DISCUSSION

Figure 1 shows the banding patterns of the ORM phenotypes as observed by IEF with subsequent print immunofixation. After neuraminidase treatment of sera the ORM bands focused between pH 4.7 and 4.9. With a narrow pH gradient the common ORM1 F can be differentiated into two subtypes. The most acidic major band is ORM1 F1, the other ORM1 F2. The difference between these two genetic types corresponds to only 0.02 pH units. In the phenotype ORM1 F1F2 a single band is present in the cathodal region which is due to ORM2. In the presence of the allele ORM1*S two major bands can be observed. The anodal band is genetically determined by ORM1*S, the cathodal band belongs to the monomorphic ORM2. In addition three uncommon ORM variants are presented. Two of these have a single band cathodal to ORM2 A. They were classified as ORM2 B1 and ORM2 B2 by family studies. The asymmetrical double band pattern (lane 7) cathodal to ORM2 A is most likely a rare ORM1 variant. This variant phenotype has been tentatively named ORM1 F1S1.

Figure 2 gives a diagrammatic representation of the observed ORM phenotypes, including the hypothetical ORM1 F2 subtype.

Table 1 shows the distribution of ORM1 phenotypes observed in 484 unrelated individuals from Southern Germany. Five common ORM1 subtypes and one variant phenotype were found in this study. The distribution of phenotypes is in good agreement with the Hardy-Weinberg equilibrium. The allele frequencies calculated from the data are: ORM1*F1 = 0.6146, ORM1*F2 = 0.0352, ORM1*S = 0.3492, and ORM1*S1 = 0.001. The population data are very similar to those found in Denmark (Thymann and Eiberg 1986). Asian populations have different frequencies for the ORM1 alleles (Umetsu et al. 1985; Yuasa et al. 1986).

Results from a study of 108 families with a total of 112 children are given in Table 2. In 13 different matings the segregation of genotypes in the children is in accordance with the assumed codominant mode of inheritance.

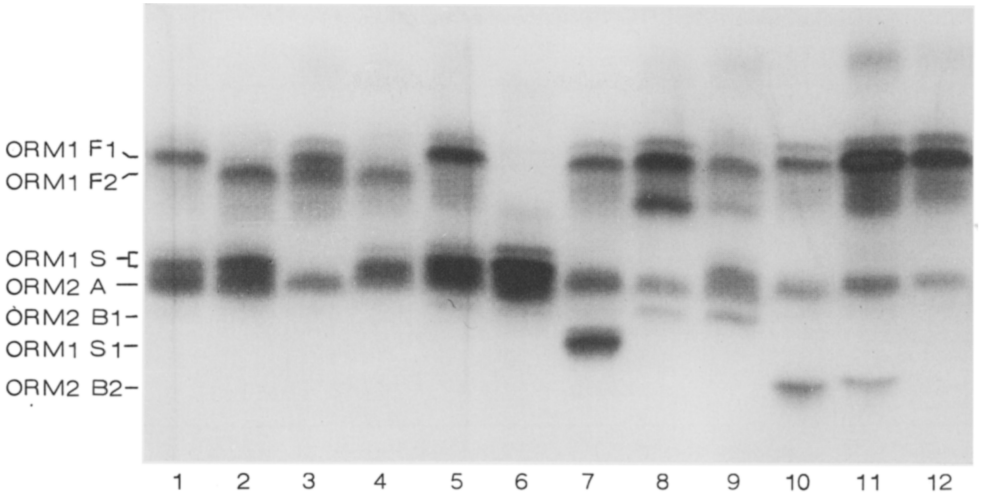

Fig. 1. ORM phenotypes observed by isoelectric focusing on polyacrylamide gel with print immunofixation. Anode is at top. ORM1 phenotypes: lane (1) F1S, (2) F2S, (3) F1F2, (4) F2S, (5) F1S, (6) S, (7) F1S1, (8) F1, (9) F1S, (10) F1, (11) F1 and (12) F1. ORM2 variant phenotypes: lanes (8) and (9) AB1 and lanes (10) and (11) AB2. The other ORM2 banding patterns are monomorphic

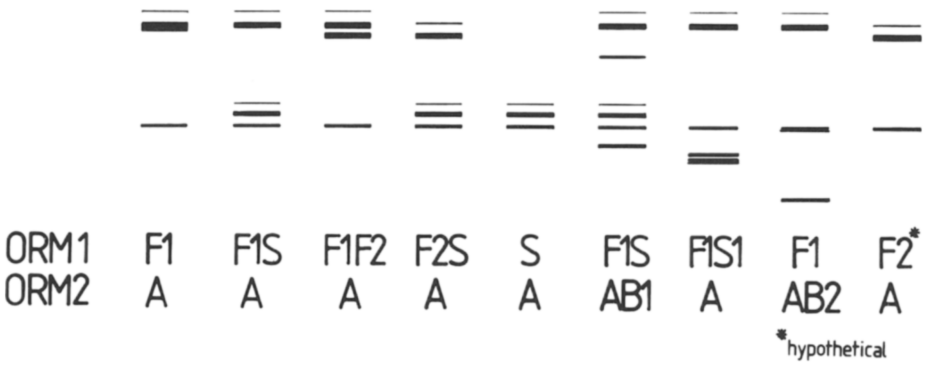

Fig. 2. Diagrammatic representation of the observed ORM1 and ORM2 phenotypes, including the hypothetical ORM1 F2 subtype

Table 1. Distribution of ORM1 phenotypes and ORM1 alleles in a sample from Southern Germany

Phenotype	Observed		Expected		Allele frequencies
	n	%	n	%	
F1	185	38.22	182.82	37.80	ORM1*F1 = 0.6146
F1F2	22	4.55	20.94	4.33	ORM1*F2 = 0.0352
F2	0	0	0.60	0.12	ORM1*S = 0.3492
F1S	202	41.74	207.75	42.96	ORM1*S1 = 0.0010
F2S	12	2.48	11.90	2.46	
S	62	12.81	59.02	12.21	
F1S1	1	0.20	0.60	0.12	
Total	484	100.00	483.63	100.00	

$\sum \chi^2$[a] = 0.4234; df = 3; $P > 0.20$. [a] Phenotypes with n (exp) below 10 were combined for χ^2 calculation

Table 2. Segregation of ORM1 in 108 families with a total of 112 children

Mating genotypes	Families n	Children n	Genotypes of the children					
			F1	F1F2	F1S	F2S	S	F1S1
F1 x F1	15	16	16	-	-	-	-	-
F1 x F1S	50	53	23	-	30	-	-	-
F1 x F1F2	2	2	2	0	-	-	-	-
F1 x F2S	1	1	-	1	0	-	-	-
F1 x S	2	2	-	-	2	-	-	-
F1S x F1S	14	14	6	-	6	-	2	-
F1S x F1F2	4	4	1	1	1	1	-	-
F1S x S	15	15	-	-	6	-	9	-
F1S x F2S	1	1	-	0	0	0	1	-
F2S x F2S	1	1	-	-	-	1	0	-
F2S x S	1	1	-	-	-	0	1	-
S x S	1	1	-	-	-	-	1	-
F1S x F1S1	1	1	0	-	0	-	-	1
Total	108	112	48	2	45	2	14	1

In 165 cases of disputed paternity, we have thus far observed 16 exclusions in the ORM1 system. This exclusions were confirmed in all cases by further exclusions in other blood group systems. The theoretical chance of exclusion was calculated to be 20.8%. The ORM1 system, therefore, appears to be a useful genetic marker, not only for population studies, but also for paternity testing.

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