Polymorphism of Orosomucoid (ORM).Formal genetics and population data

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INTRODUCTION

Acid α1-Glycoprotein or Orosomucoid (ORM) represents a plasma sialoglycoprotein with unusually high carbohydrate content. It consists of a single 40 kD polypeptide chain with 181 aminoacids and five heteropolysaccharide side chains (Schmid et al.1973). Its biological function is described as acute phase reactant (Kushner 1982) with protective responses in inflammatory and infectious states (Friedman 1983).

ORM exhibits a genetically determined polymorphism (Tokita & Schmid 1963) with two common alleles at an autosomal locus (Johnson et al.1969). The inherited structural variability of ORM can be demonstrated from desialyzed plasma samples (Tokita & Schmid) using PAGIEF (Berger et al.1980) with addition of urea in the gels (Yuasa et al.1986), followed by immunofixation (Johnson et al.1969), lectinofixation (Umetsu et al.1985) or by enzyme-immuno-assay after Western-blotting.

From the investigation of 159 families with 479 children and of 336 unrelated individuals from southwestern Germany we present data on formal and population genetics of ORM.

MATERIAL AND METHODS

Sample preparation: Serum- or EDTA plasma samples stored up to 6 years at -30°C were treated with Neuraminidase; 3µl plasma plus 20 µl Neuraminidase (Boehringer, 1 mg/ml Aq.bid.) for 18 hrs at 37°C in a moist chamber.

Polyacrylamide gels (245 x 115 x 0,5 mm) : 2,7 ml Acrylamide (LKB, 28% w/v); 1,2 ml N, N'-Methylenbisacrylamide (LKB, 2% w/v); 0,8 ml Pharmalyte pH 4.5 - 5.4; 10 ml Aq.bid. (alternatively 7 ml Aq. bid. plus 4,68 g urea, Merck); 0,1 ml TMED (Serva, 30 µl/ml Aq. bid.); 0,5 ml Persulfate (Serva, 10 mg/ml Aq.bid.). Polymerization was overnight at room temp. or for 60' at 50°C.

Focusing: Ultraphor with 10°C temp. of cooling water; Anolyte: 0,04 M Glutamic acid; Catholyte: 0,1 M NaOH; Maximum settings: 15 mA, 2000 V; 45' prefocusing with 5W; 6 hrs focusing with 12W; sample application in 3 x 4 mm filter papers (LKB) 2 cm from the cathodal end for 30'.

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Detection: Immunofixation with anti-human α1-Glycoprotein (Sigma, 1 : 4 in 0,9% saline) in CAF (Schleicher & Schüll) with 10' print contact. Foils were washed overnight in 0,9% saline and stained with 0,115% w/v Coomassie Brilliant Blue G 250 (Serva) in destaining solution (25 % abs. ethanol, 8% glacial acetic acid in Aq. dest.). EIA was carried out after Western blotting onto nitrocellulose filters (NC, Schleicher & Schüll, 0,45 μm) for 40' at room temp. Membranes were blocked with 5% Albumin (Sigma) in PBS, washing steps were done with 0,05% Tween 20 (Merck) in PBS. I. antibody: anti-human α1 acid Glycoprotein (Sigma, 1:200 in 1% Albumin/PBS), II. antibody: anti-goat Immunglobuline conjugated POD (ATAB, 1:1000 in 1% Albumin/PBS) Reaction time was 1 h at room temp. Visualization: 100 μl 4 Chloro-1-Naphthal 2% in Diethyleneglycol; 500 μl saturated O-Toluidine in 7% acetic acid; 500 μl 3% H202 in 40 ml PBS for 10' at room temp.

RESULTS AND DISCUSSION

Pherogram description

Figure 1 shows common (1) and rare (2) ORM-phenotypes after PAGIEF in the pH-range 4.5 - 5.4 detected by immunofixation. For comparison the effect of 5 M urea in the gel is demonstrated.

Without urea homozygotes ORM1 F and ORM1 S show single bands with different IEPs, respectively, whereas hterozygotes ORM1 FS show a double banded pattern. Independently from these phenotype-specific patterns monomorphic gene products appear anodally from the orm1*S band, which seem to be coded for by the locus ORM2.

Unsatisfactory separation of the gene products orm2 and orm1*S however will cause problems with the differentiation of the phenotypes ORM1 F and ORM1 FS.

After separation in gels containing 5 M urea each of the common phenotypes is represented by a characteristic pattern of maior and minor bands. Gene products orm2 appear as faint bands cathodally from the orm1*S band.

Two different rare heterozygous phenotypes were observed, which were designated as ORM1 FS1 and ORM1 SS2. In gels without urea the orm1*S1 product takes a position cathodally from orm1*S. In presence of 5 M urea however the phenotype ORM1 FS1 will be characterized by a band anodally to the maior fraction of orm1*F. Gene products orm1*S2 are clearly separated, independently from the use of urea. In order to characterize variants it seems therefore to be necessary to phenotype ORM also in gels without urea.

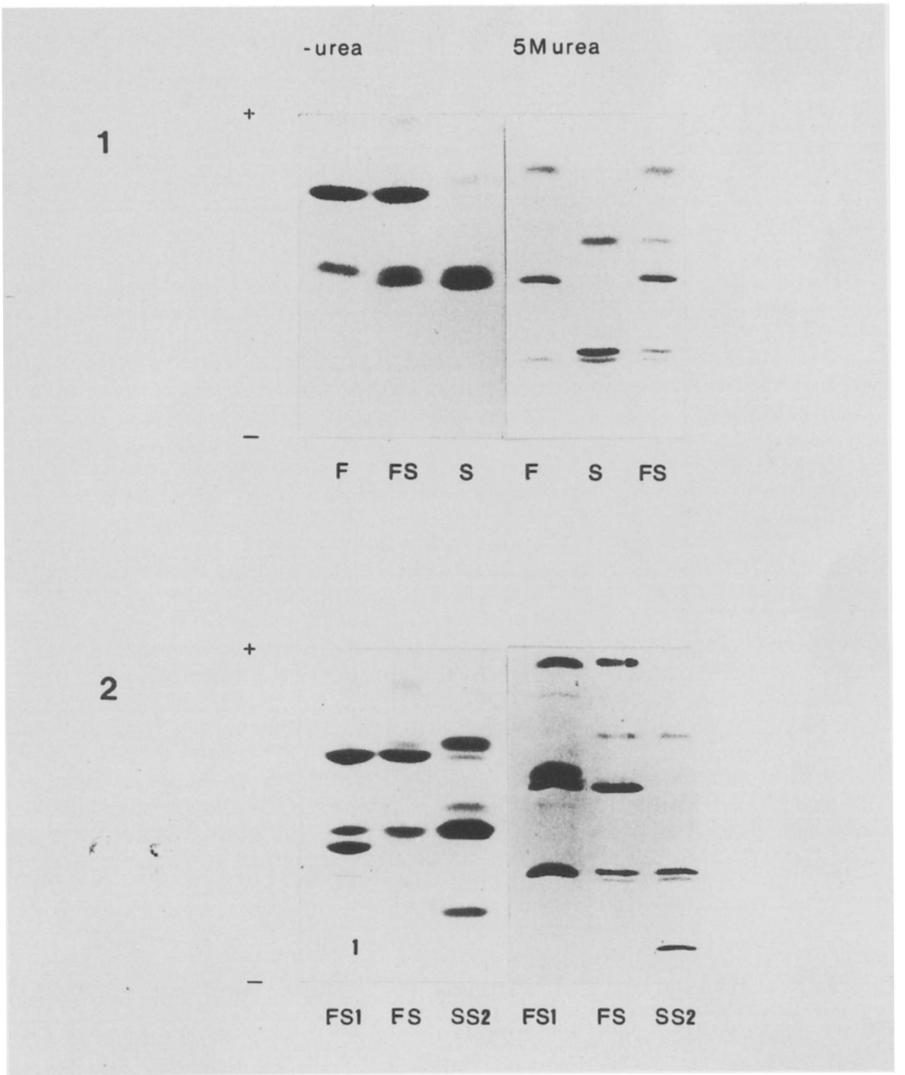

Fig. 1. Common (1) and rare (2) ORM-phenotypes after PAGIEF in the pH-range 4.5 - 5.4 in presence or absence of 5 M urea. Detection by immunofixation.

Formal genetics

Table 1 presents the segregation of the children's phenotypes in 159 families with 479 children from southwestern Germany.

The results of the segregation analysis are in agreement with the formal hypothesis "2 common alleles ORM1*F and ORM1*S at an autosomal locus ORM1". Observed and expected values of the children's phenotypes differ only by chance (X^2=1.447,df=4, 0.80-P-0.85). Since investigations at the DNA-level (Dente et al.1985) revealed the existence of two ORM loci, we preferred to designate the alleles ORM1*F and ORM1*S respectively.

Table 1. Segregation of the children's phenotypes in 159 families with 479 children.

mating type	number of fam.	ch.	phenotypes of children F	FS	S
F x F	21	62	62	-	-
F x FS	54	159	81	78	-
			79,5	79,5	
F x S	18	54	-	54	-
FS x FS	44	133	38	66	29
			33,25	66,5	33,25
FS x S	18	55	-	29	26
				27,5	27,5
S x S	4	16	-	-	16
Total	159	479	181	227	71

Population genetics

The population sample comprises of 336 unrelated individuals from southwestern Germany. Table 2 summarizes the observed distribution of the phenotypes and the calculated allele frequencies.

Table 2. Distribution of ORM1 phenotypes and ORM1 allele frequencies in a population sample from southwestern Germany.

Phenotypes	N	Alleles	frequency
ORM1 F	120	ORM1*F	0.609
FS	168	ORM1*S	0.388
S	46	ORM1*V	0.003
FS1	1		
SS2	1	(ORM1*V = ORM1*S1 + ORM1*S2)	
Total	336		

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