

ORM1 Subtypes in Hanover and Lower Saxony, FRG,  
by PAG SIEF and Immunoblotting (N=1934); 11 Variants

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## INTRODUCTION

ORM1 subtypes have first been described by Thymann and Eiberg (1986). Since then, their existence has been confirmed by several working groups, e.g. Yuasa et al. (1986), Weidinger et al. (1987), Eap et al. (1988), Umetsu et al. (1989), and Luckenbach et al. (1989). The technique of IEF with different modifications (HIEF, IPG, SIEF) and following immunoprinting or immunoblotting was applied by all these investigators.

The aim of our study using the advantages of SIEF and immunoblotting was an improvement of protein separation and visualization. Furthermore, the exploration of population and family data for a Northern German population sample was intended.

## MATERIALS AND METHODS

Sera of 1934 unrelated healthy individuals from Hanover and Lower Saxony (plus 29 variant carriers) and of 115 families with 126 children were processed.

### Sample preparation

45  $\mu$ l serum, 5  $\mu$ l CPN (Sigma, type V; 10 U/0.4 ml 1 M KH<sub>2</sub>PO<sub>4</sub> buffer, pH 7.0), incubated 24 h at RT.

### Separator isoelectric focusing (SIEF)

Gel: 230x105x0.5 mm; T=4.8%, C=3.0%. Separator: 0.0175 M ACES. Ampholytes: Pharmalytes; 3.8% pH 4.2-4.9, 2.6% pH 4.5-5.4. Additive: 13% glycerol. Electrolytes: anolyte 1 M H<sub>3</sub>PO<sub>4</sub>, catholyte 0.2 M NaOH. Running conditions: prerun 30'; 5 W, 2000 V, mA unlimited; run 150'; 20 W, 2000 V, 16 mA; cooling 8 °C. Sample application: 3  $\mu$ l CPN-treated serum on 4x4 mm Whatman 1; 2 cm from cathode for 30'. Apparature: LKB; Multiphor II, Macrodrive 5, Multitemp II.

## Immunoblotting

Blotting: passive blot (0.45  $\mu$ m NC, Schleicher & Schüll); 20'. Blocking: 2% liquid gelatin (NORLAND) in washing buffer; 1 h. 1st antibody: rabbit anti-human ORM (Dakopatts); 1/500 diluted in blocking solution; 1 h. Washing 1: 5x5' in washing buffer (0.01 M Tris/HCl, 0.9% NaCl, 1% Triton X-100; pH 7.4). 2nd antibody: swine anti-rabbit IgG, AP-conjugated (Dakopatts); 1/1000 diluted in blocking solution; 45'. Washing 2: 2x5' in washing buffer. Color development: BCIP: 15 mg/1 ml dimethylformamid (DMF). NBT: 30 mg/1 ml 70% DMF solution. 1 ml BCIP and 1 ml NBT diluted in 100 ml carbonate buffer (0.1 M NaHCO<sub>3</sub>, 1.0 mM MgCl<sub>2</sub>; pH 9.8); 30'. Stopping: distilled water.

## RESULTS

An original blot of common ORM1 phenotypes including the rare F2 homozygote is shown in figure 1.

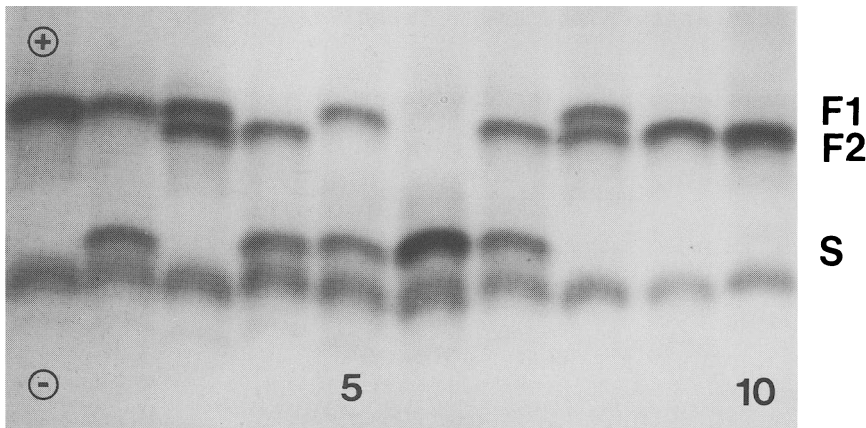


Fig. 1. ORM1 subtypes by SIEF and immunoblotting. From left to right, 1: F1, 2: F1S, 3: F1F2, 4: F2S, 5: F1S, 6: S, 7: F2S, 8: F1F2, 9: F2 (hypothetical until recently), 10: F2

The homozygous F2 carriers are siblings (sister lane 9, brother lane 10), the father's type (lane 8) is F1F2. The type of the deceased mother was deduced from those of her living siblings (lane 4 - 7) to be F1F2 or F2S.

Figure 2 represents another blot including 2 variant ORM1 phenotypes.

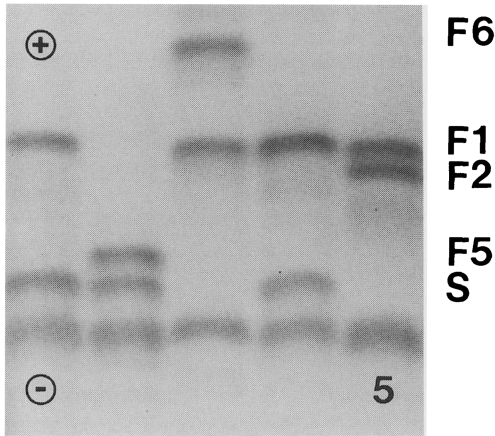


Fig. 2. Two ORM1 variants by SIEF and immunoblotting. From left to right, 1: F1S, 2: F5S, 3: F1F6, 4: F1S, 5: F1F2; F5 and F6 being provisional designations

Nine further variants apparently caused by gene duplication found in this study are not shown.

The distribution of ORM1 subtypes observed in 1934 unrelated individuals from Hanover and Lower Saxony is listed in Table 1.

Table 1. Population data (with exclusion of variant phenotypes)

phenotypes	observed		expected	allele frequencies	
	n	o/o	n		
ORM1					
F1	645	33.35	645.18		
F1F2	55	2.84	57.63		
F2	1	0.05	1.35		
F1S	889	45.97	886.16	ORM1*F1	0.5776
F2S	43	2.22	39.65	*F2	0.0258
S	301	15.56	304.22	*S	0.3966
total	1934	99.99	1934.19		1.0000
chi-square: 0.5392; p: 0.95 > p > 0.9; df: 3; SECNF 0.2137					

Table 2 presents the results of family studies with 115 matings and 126 children.

Table 2. Family data

parents		children								
ORM1	n	n	F1	F2	S	F1F2	F1S	F2S	F1-F2.S	
F1 x F1	12	16	16	-	-	-	-	-	-	
S x S	2	2	-	-	2	-	-	-	-	
F1S x F1S	19	20	3	-	2	-	15	-	-	
F1 x S	8	8	-	-	-	-	8	-	-	
F1 x F1S	43	46	22	-	-	-	24	-	-	
F1 x F2S	5	6	-	-	-	4	2	-	-	
S x F1S	15	16	-	-	6	-	10	-	-	
F1F2 x F1S	6	6	2	-	-	1	2	1	-	
F1F2 x F2S	1	1	-	-	-	-	1	-	-	
F1S x F2S	3	3	-	-	-	2	-	1	-	
F1S x F1.F4-F2.S	1	2	-	-	-	-	-	-	2	
total	115	126	43	-	10	7	62	2	2	
other matings not observed										

The allele frequencies of subtyped ORM1 in various European and non-European populations correlated to our own data are given in Table 3.

Table 3. Allele frequencies of ORM1 subtypes in various populations

population		allele frequencies				references
	n	ORM1*F1	ORM1*F2	ORM1*S	others	
Danes	215	0.5810	0.0330	0.3860	0.0000	THYMANN and EIBERG (1986)
French	112	0.5625	0.0491	0.3884	0.0000	YUASA et al. (1986)
Germans						
Hanover	1934	0.5776	0.0258	0.3966	*)	this study (1989)
Münster	167	0.5625	0.0298	0.3929	0.0149	UMETSU et al. (1989)
Munich	272	0.6103	0.0404	0.3475	0.0018	WEIDINGER et al. (1987)
Swiss	329	0.5927	0.0015	0.4043	0.0015	EAP et al. (1988)
Filipinos	115	0.7904	0.0000	0.1687	0.0409	UMETSU et al. (1988b)
Japanese	200	0.6800	0.0000	0.1525	0.1675	YUASA et al. (1988)
Taiwanese	200	0.7255	0.0000	0.1805	0.0941	UMETSU et al. (1988a)

\*) not taken into account

## DISCUSSION

SIEF combined with immunoblotting achieved a considerable technical improvement of ORM1 subtyping. The distribution of ORM1 subtypes found by this technique for an extended population sample from North Germany was in good agreement with the Hardy-Weinberg equilibrium. 11 different variants were observed, 9 apparently caused by gene duplication. Family data were available to elucidate inheritance of the homozygous ORM1 F2 subtype hypothetical until recently. The segregation of ORM1 subtypes in 115 Northern German families with 126 children was in accordance with an assumed autosomal codominant mode of inheritance. The single exclusion chance for non-fathers (SECNF) being 0.2137, subtyped ORM1 can be considered as an informative marker for paternity testing.

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