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Orosomucoid (ORM) typing by isoelectric focusing: description of two new alleles in a German population and thermostability in bloodstains

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Summary. The genetic polymorphism of serum orosomucoid (ORM) was studied in 168 unrelated German individuals using isoelectric focusing followed by immunoprinting. Two new alleles, tentatively designated *ORM1*14* and *ORM2*13,* were identified. The method was successfully applied to demonstrate ORM1 types in dried bloodstains. Each type of ORM1 was also correctly determined in bloodstains heated at 130°C for 30 min. The results indicated that ORM1 is a new powerful genetic marker system for the grouping of bloodstains.

Key words: Serum groups, ORM-phenotyping – Bloodstains, orosomucoid (ORM) typing – Orosomucoid typing

Zusammenfassung. Bei 168 nicht verwandten Deutschen wurden die Orosomucoid(ORM)-Typen mittels isoelektrischer Fokussierung und Printimmunfixation untersucht. Zwei neue Allele, vorläufig *ORM1*14* and *ORM2*13,* wurden beobachtet. Die Isoelektrofokussierungs-Methode wurde mit Erfolg zur ORM1-Typisierung an Blutspuren angewandt. Jeder Phänotyp konnte an bis 130°C, bis zu 30 min erhitzten Blutspuren sicher nachgewiesen werden. Die Resultate zeigen, daß der ORM1-Polymorphismus ein neues und aussagekräftiges genetisches Merkmalsystem für die Serumgruppen-Typisierung an Blutspuren ist.

Schlüsselwörter: Serumgruppen, ORM-Typisierung – Blutspuren, Orosomucoid(ORM)-Typen – Orosomucoid-Typen

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Introduction

The genetic polymorphism of orosomucoid (ORM, α_1-acid glycoprotein) was first recognized by Tokita and Schmid (1963) and Johnson et al. (1969). The application of isoelectric focusing (IEF) to ORM typing has been carried out in various populations (Umetsu et al. 1985; Thymann and Eiberg 1986; Yuasa et al. 1986; Escallon et al. 1987; Weidinger et al. 1987; Wimmer et al. 1988). Recently, IEF methods in the presence of Triton X-100 for typing of ORM polymorphism have increased the discrimination power of these systems (Umetsu et al. 1987) and many variant alleles *(ORMI*2 · 1, ORMI*5 · 2)* have been detected (Yuasa et al. 1987; Umetsu et al. 1988; Yuasa et al. 1988).

ORM is characterized by exceptionally high carbohydrate content and stability (Schmid 1975). These properties of ORM glycoproteins suggest a potential usefulness for the analysis of bloodstains.

In the present study, the polymorphism of ORM in a German population was investigated with the immunoprinting technique after IEF in gels containing 0.2% Triton X-100. Furthermore, the method was successfully applied to phenotyping ORM1 in bloodstains.

Materials and Methods

Serum Samples

Blood samples were obtained from 168 unrelated German individuals in Münster (FRG). Desialization was performed with the method described previously (Umetsu et al. 1987). The samples were applied onto the gel surface by using Whatman no. 3 filter papers (4 × 2 mm).

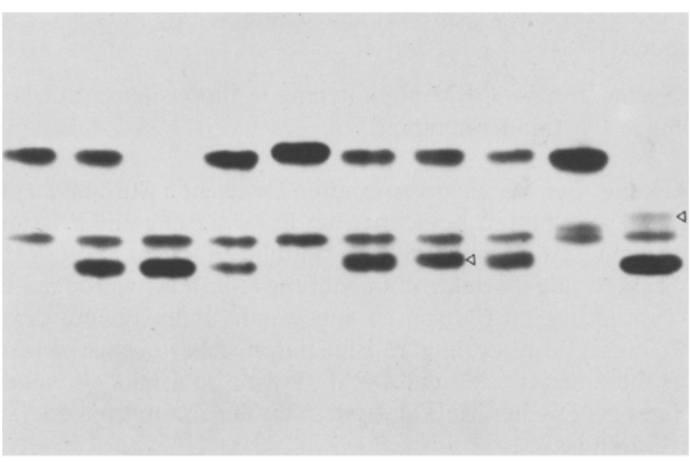

Fig. 1. Immunoprinted band patterns of several ORM phenotypes after IEF in the presence of glycerol. Anode at the *top*. *Open triangle* indicates the new variant band

Bloodstains

Blood samples of whole blood (2 µl) from known phenotypes of ORM1 1, ORM1 2–1 and ORM1 2 were dropped on Whatmann no. 3 filter paper (4 × 4 mm) and air-dried at room temperature. Blood stains were heated at every 10°C from 80°C up to 180°C for 30 min. The heated blood stains were treated with 10 µl of 0.2 *M* Na-acetate buffer, pH 5.0, containing 1 U/ml neuraminidase (type V, Sigma, St. Louis, MO) and 0.2% v/v Triton X-100 overnight at room temperature. The filter papers with bloodstains were applied directly onto the IEF gel surface.

Isoelectric Focusing Methods

IEF of the serum samples was performed on polyacrylamide gel in the presence of glycerol as described previously (Umetsu et al. 1987) and in the absence of glycerol as described by Yuasa

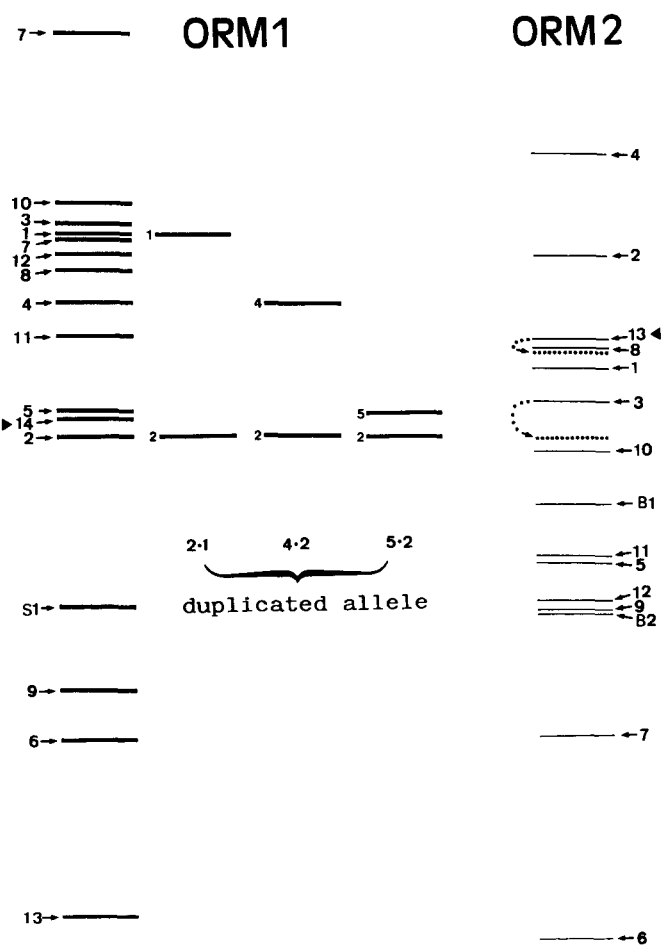

Fig. 2. Diagrammatic representation of ORM variant bands detectable on an IEF gel in the presence of Triton X-100 and glycerol. Anode at the *top*. *Dotted bands* show those which change in the absence of glycerol. ORM1 9-ORM1 13, ORM2 11 and ORM2 12; Yuasa et al. (in preparation) ▲; newly found allele in German population

et al. (1988). Because the ORM1 3 band was not always correctly defined by these methods, it was also confirmed by another previously described method (Yuasa et al. 1986).

For the typing of bloodstains, polyacrylamide gels (5%T, 3%C, 110 × 110 × 0.5 mm) were prepared containing 2.4% Pharmalyte (pH 4–6.5), 12% v/v glycerol and 0.2% v/v Triton X-100. The catholyte and anolyte were 0.2 *M* NaOH and 1 *M* H_3PO_4 respectively. IEF was performed at a maximum setting of 2000 V, 10 mA and 10 W at 10°C for 2.5 h.

Detection of ORM Bands

ORM bands were detected by immunoprinting and subsequent staining with Coomassie brilliant blue R-250 (Umetsu et al. 1988a). The time of immunoprinting for serum samples was 8 min and that for bloodstain samples was 15 min.

Results and Discussion

Population Study

Figure 1 presents several ORM band patterns as revealed by IEF on the polyacrylamide gel in the presence of glycerol and immunoprinting. The figure includes the apparently new ORM variant phenotypes. One variant phenotype is seen in the ORM1 locus. It has a more intense ORM1 band located between the ORM1 5 and ORM1 2 bands and it was tentatively designated ORM1 14. The other variant phenotype is observed in the ORM2 locus. It has a less intense ORM2 band on the anodal side of the ORM2 8 band and it was tentatively designated ORM2 13. Using IEF on polyacrylamide gel in the absence of glycerol, the ORM2 13 band came out slightly cathodal to the ORM2 8 band. These two new variants were apparently different from the variant phenotypes identified in a southern German population by Weidinger et al. (1987). In addition, the

Table 1. Distribution of ORM1 and ORM2 phenotypes in a German population

ORM1	ORM2		Combined	Number expected	χ^2
	1	13–1			
1	52	–	52	53.2	0.0271
2–1[a]	73	1	74	74.3	0.0012
2	28	–	28	25.9	0.1703
3–1	7	–	7		
3–2	1	–	1		
3	1	–	1		
2·1–1	3	–	3	14.6	0.0247
2·1–2	1	–	1		
14–1	1	–	1		
Others	–	–	0		
Total	167	1	168	168.0	0.2456

[a] Including two "ORM1 2/1 and 2·1/2·1" genotypes.
*ORM1*1* = 0.5625, *ORM1*2* = 0.3929, *ORM1*3* = 0.0298, *ORM1*2·1 = 0.0119, ORM1*14* = 0.0030; *ORM2*1* = 0.9970; *ORM2*13* = 0.0030
$\chi^2 = 0.2456$, $df = 1$, $0.70 < P < 0.50$

Table 2. ORM1 allele frequencies in various populations

Population (*n*)	Allele frequencies					References
	*ORM1*1*	*ORM1*2*	*ORM1*2 · 1*	*ORM1*3*	*Others*	
Germans						
München (272)	0.6103	0.3475	*	0.0404	0.0018	Weidinger et al. (1987)
Tübingen (336)	0.609	0.388	*	*	0.003	Wimmer et al. (1988)
Münster (167)	0.5625	0.3929	0.0119	0.0298	0.0030	This study
Danes (215)	0.5810	0.3860	*	0.0330	0.0000	Thymann and Eiberg (1986)
French (112)	0.5625	0.3884	*	0.0491	0.0000	Yuasa et al. (1986)
USA Blacks (181)	0.6160	0.3840	*	*	0.0000	Escallon et al. (1987)
Japanese (200)	0.6800	0.1525	0.1550	0.0000	0.0125	Yuasa et al. (1988)
Taiwanese (200)	0.7255	0.1805	0.0641	0.0000	0.0300	Umetsu et al. (1988a)
Filipinos (115)	0.7904	0.1687	0.0409	0.0000	0.0000	Umetsu et al. (1988b)

*This allele was not taken into consideration

present study revealed the homozygous ORM1 3 phenotype which had not been observed in all the various population studies. A diagrammatic representation of ORM variant bands known at present is shown in Fig. 2.

Table 1 presents the distribution of ORM1 and ORM2 phenotypes in 168 serum samples from a northern German population. The observed and expected numbers are in equilibrium according to the Hardy-Weinberg law. Table 2 summarizes the differences in the distribution of ORM1 allele frequencies among various populations. The *ORM1*1* frequency in the present sample is almost similar to that reported in Germans (Weidinger et al. 1987; Wimmer et al. 1988), French (Yuasa et al. 1986) and Danes (Thymann and Eiberg 1986). In three previous studies, the separation of the duplicated allele *(ORM1*2 · 1)* band was not performed. The duplicated allele, *ORM1*2 · 1,* however, has been shown to be widespread in Mongoloids (Umetsu et al. 1988; Yuasa et al. 1988). In the present study, the separation of the duplicated allele was made and a few cases with *ORM1*2 · 1* bands were observed. From these findings, it is revealed that the frequency of *ORM1*2 · 1* allele is much lower in Germans than in Mongoloids.

Phenotyping in Bloodstains

The IEF method was successfully applied to the phenotyping of ORM in bloodstains at room temperature up to 1 year after stain formation (data not shown). Therefore, in the present study the thermostability of ORM was investigated.

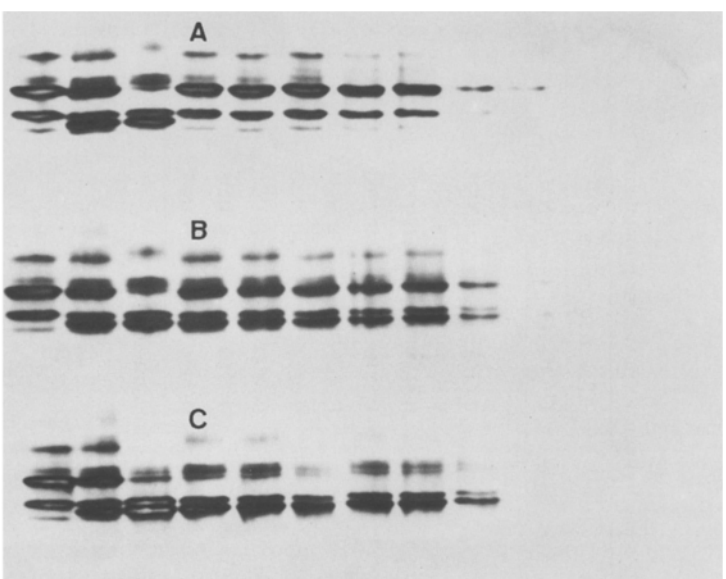

Fig. 3. Isoelectric focusing patterns of ORM in bloodstains heated at every 10°C from 80°C up to 160°C for 30 min. Anode at the *top*. *A* ORM1 1, *B* ORM1 2–1, *C* ORM1 2

Figure 3 shows the ORM1 patterns of bloodstains heated at every 10°C from 80°C up to 180°C for 30 min. All the bloodstains heated up to 130°C for 30 min. were successfully phenotyped. ORM is one of the most thermostable glycoproteins in the serum protein systems. The thermostability of ORM is considered to be due to its high carbohydrate content.

The present study demonstrates that ORM can be reliably phenotyped in bloodstains. The easiness and reliability of the typing method makes ORM a very recommendable genetic marker for the medicolegal analysis of bloodstains.

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