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Genetic polymorphism of orosomucoid (ORM1 and ORM2) in Lombardy (Italy)

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Summary. Orosomucoid subtypes have been analysed in 600 unrelated individuals from the Brescia Area (Lombardy-Italy) by the isoelectric focusing technique and immunoprinting. Five ORM1 phenotypes were observed. The ORM2 pattern was found to be virtually monomorphic, but one variant with an additional single band was found. The estimated allele frequencies were: ORM1*F1 = 0.5992, ORM1*F2 = 0.0150, ORM1*S = 0.3858.

Key words: Blood groups – Orosomucoid typing – ORM1 and ORM2 polymorphism

Zusammenfassung. Bei 600 nicht verwandten Individuen aus Brescia (Italien) wurden die Orosomucoid-Subtypen mittels isoelektrischer Fokussierung und anschließender PrintImmunofixation bestimmt. Die beobachteten Allelefrequenzen waren: ORM1*F1 = 0.5992, ORM1*F2 = 0.0150, ORM1*S = 0.3858.

Schlüsselwörter: Blutgruppen – Orosomucoid Typen – ORM1 und ORM2 Polymorphismus

Introduction

Orosomucoid (ORM), also named alpha-1-acid glycoprotein, is a plasma protein (mol wt 40000) present in serum at levels between 0,5 and 1 mg/ml.

The genetic polymorphism was first reported by Tokita and Schmid (1963) using a starch gel electrophoresis technique. Subsequently Johnson et al. (1969) described 3 different phenotypes using immunofixation which are the expression of 2 codominant alleles ORM*F and ORM*S at a single autosomal locus.

A microheterogeneity of the ORM band pattern has been demonstrated by Berger et al. (1980) by means of isoelectric focusing. Umetsu et al. (1985) carried out ORM-subtyping by IEF and print-lectinofixation, Thymann and Eiberg (1986) by IEF and immunofixation and further studies have confirmed that ORM represents the products of 2 different loci ORM1 and ORM2 (Yuasa et al. 1986; Weidinger et al. 1987) which are mapped to chromosome 9 (Cox and Francke 1985; Webb et al. 1988) and linked to the ABO and AK1 systems (Eiberg et al. 1982, 1983).

The ORM1 locus is polymorphic with 3 common alleles (ORM1*F1 = ORM1*1, ORM1*F2 = ORM1*3 and ORM1*S = ORM1*2 (Escallon et al. 1987; Thymann and Weidinger 1988; Yuasa et al. 1987) and several rare variants which are polymorphic in some geographical areas (Sebetan and Sagisaka 1989; Umetsu et al. 1989a; Yuasa et al. 1990a). The ORM2 locus is polymorphic in U.S. blacks (Escallon et al. 1987) and many populations of Asia, while in Europe only one type is normally present (Yuasa et al. 1986) but several variants with very low frequencies have been reported (Weidinger et al. 1987, 1988; Umetsu et al. 1989b).

Using IEF in the presence of Triton X-100 three duplicated ORM1 locus products (Yuasa et al. 1987, 1988) and new haplotypes containing the silent alleles ORM1*QO and ORM2*QO have been identified (Yuasa et al. 1990b, c). The presence of a null allele, showing a difference in band intensity, has also been confirmed by Kasulke and Weidinger (1990).

In the last few years, the genetic variation of ORM has proven useful in the forensic analysis of blood and semen stains (Harada et al. 1989; Umetsu et al. 1989b 1990) and isoelectric focusing has also been employed to study the differences between human and animal ORM patterns (Yuasa et al. 1990a).

So far, the ORM genetic polymorphism has not been extensively investigated in Italy, and is not routinely used for forensic purposes. The aim of this study was to increase the knowledge of the ORM distribution through a population sample from the Brescia area (Northern Italy).

Materials and methods

Sera from 600 healthy and unrelated blood donors from the Transfusion Centre of the "Spedali Civili" of Brescia (Lombardy, Italy)

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Table 1. ORM1 phenotype distribution and allele frequencies in Brescia (Lombardy, Italy)

Phenotype	Observed		Expected		Allele frequencies
	n	%	*n*	%	
F1	216	36.00	215.42	35.90	ORM1*F1 = 0.5992
F1–F2	12	2.00	10.79	1.80	ORM1*F2 = 0.0150
F2	0	0.00	0.14	0.02	ORM1*S = 0.3858
F1–S	275	45.83	277.41	46.24	
F2–S	6	1.00	6.94	1.16	
S	91	15.17	89.30	14.88	
Total	600	100.00	600.00	100.00	

$\chi^2 = 0.4579$; 3 *d.f.*; $0.90 < P < 0.95$

have been studied. Samples were stored at −20°C before use and tested within 6 months. Neuraminidase treatment (Clostridium perfrigens 1 U/mg, Boehringer) was carried out by addition of 10 µl enzyme to 20 µl serum and the mixture was incubated at 37°C for 24 h.

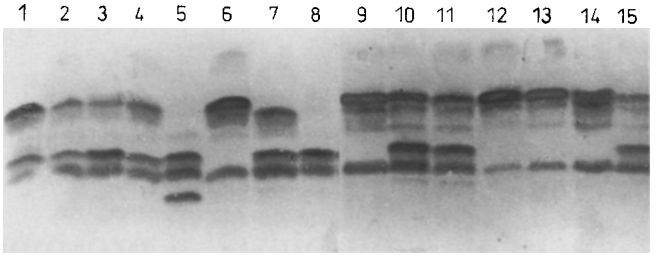

Fig. 1. Electrofocusing and immunoprinting pattern of ORM1 phenotypes. From left to right: (1) F1-S, (2) F1-S, (3) F1-S, (4) F1-S, (5) S, (6) F1, (7) F2-S, (8) S, (9) F1, (10) F1-S, (11) F1-S, (12) F1, (13) F1, (14) F1-F2, (15) F1-S. Sample (5) is an ORM2 B1 Var, whereas all others are ORM2 A. Anode is at the top

Orosomucoid typing was performed by thin layer isoelectric focusing in polyacrylamide gels (250 × 120 × 0.5 mm), containing Pharmalyte carrier according to Weidinger et al. (1987). IEF was performed using an LKB Ultrophor chamber connected to the LKB 2197 power supply for a total of 3 h at a maximum of 2000 V, 16 mA and 20 W.

Table 2. ORM1 allele frequencies in different populations

Population	*N*	ORM1*F1	ORM1*F2	ORM1*S	ORM1*Others	References
Danes	215	0.5810	0.0330	0.3860	–	Thymann and Eiberg (1986)
Germans						
München	272	0.6103	0.0404	0.3475	0.0018	Weidinger et al. (1987)
Tübingen	336	0.6090	–	0.3880	0.0030	Wimmer et al. (1988)
Münster	167	0.5625	0.0298	0.3929	0.0149	Umetsu et al. (1989b)
Hanover	1934	0.5776	0.0258	0.3966	–	Krüger et al. (1990)
S. Germany	696	0.6127	0.0345	0.3521	0.0007	Thymann and Weidinger (1988)
S. Bavaria	468	0.6463	0.0145	0.3387	–	Gathof et al. (1990)
Swiss	329	0.5927	0.0015	0.4043	0.0015	Eap et al. (1988)
French	112	0.5626	0.0491	0.3884	–	Yuasa et al. (1986)
Spanish						
Basque C.	150	0.5733	0.0330	0.3933	–	Montiel et al. (1990)
Galicia	650	0.460 (F)		0.5400 (S)	–	Carracedo et al. (1986)
Galicia	880	0.5574	0.0330	0.4063	0.0028	Montiel et al. (1990)
Madrid	315	0.6206	0.0047	0.3746	–	Alonso et al. (1990)
Portuguese	260	0.5520	0.0308	0.4153	0.0019	Montiel et al. (1990)
Italians						
Veneto	96	0.4219 (F)		0.5781 (S)	–	Caenazzo et al. (1988)
Veneto	735	0.3496 (F)		0.6531 (S)	–	Caenazzo et al. (1989)
Lombardy	600	0.5992	0.0150	0.3858	–	This study
U.S. whites	228	0.5590	–	0.4410	–	Escallon et al. (1987)
U.S. blacks	181	0.6190	–	0.3840	–	Escallon et al. (1987)
Canadian Ind.	169	0.5470	–	0.4530	–	Escallon et al. (1987)
Japanese						
Yamagata	500	0.7790	–	0.2210	–	Umetsu et al. (1985)
Yamaguchi	200	0.6800	0.0225	0.1625	0.1350	Yuasa et al. (1987)
Myagi	232	0.6680	0.0060	0.1700	0.1560	Sebetan and Sagisaka (1989)
Goto	168	0.7052	–	0.1671	0.1277	Fukuma et al. (1990)
Okinawa	364	0.6882	–	0.1662	0.1456	Yuasa et al. (1990b)
Chinese	163	0.7564	–	0.1411	0.1043	Yuasa et al. (1990a)
Taiwanese	200	0.7255	–	0.1805	0.0940	Umetsu et al. (1988a)
Nepalese	141	0.6738	0.0142	0.3121	–	Yuasa et al. (1986)
Filipinos	115	0.7904	–	0.1687	0.0409	Umetsu et al. (1988b)
New Guinea	110	0.8410	–	0.1590	–	Escallon et al. (1987)

After separation, ORM phenotypes were identified by immunoprinting. A cellulose acetate strip was soaked in anti-human α1-acid glycoprotein (ATAB) diluted 1:5 in saline (0.9%) and placed on the gel surface at room temperature for 8 min. The strip was removed and washed in saline for 30 min, stained with nigrosine (1%) and destained in acetic acid (5%).

Results and discussion

The distribution of ORM1 phenotypes and allele frequencies in the population sample of Brescia area are given in Table 1. Good correlation was found between the observed and expected phenotype distribution, assuming Hardy-Weinberg equilibrium conditions.

The electrophoretic pattern (Fig. 1) according to Weidinger et al. (1987) shows 5 phenotypes (F1, F1F2, F1S, F2S and S) but the homozygote F2 was not observed in this study. In the anodal region, 2 major bands were observed: the most anodal major band being ORM1 F1, the other, ORM1 F2. In the cathodal region, one single band was present which is the product of the ORM2 locus (ORM2A) and in the ORM1 S phenotype an additional double band was found.

Figure 1 (lane 5) also shows a rare ORM variant. This phenotype, observed in 2 different individuals, is characterized by an additional band located cathodally to the ORM2 band and could be tentatively classified as an ORM2 variant.

Table 2 shows the distribution of the ORM1 gene locus in different populations and ethnic groups. No significant differences have been found between the allele frequencies calculated for our sample and other samples from Europe. The differences between the distributions of the Spanish population of Galicia (Carracedo et al. 1986) and the Italian population of Veneto were, in our opinion, probably the result of a different interpretation of the data. A comprehensive comparison between the published data is difficult because many alleles are only polymorphic in some geographical areas and not in others. An analysis of the most common phenotypes shows that the data of the American and European populations are similar but with a lower frequency for ORM*F1 than Asian populations, whereas the highest frequency for ORM*F1 is found in Filipinos and in a population of New Guinea (Oceania).

This is additional confirmation of obvious variations in allelic frequencies in ethnic groups and demonstrates the usefulness of the ORM system for genetic population studies.

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