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Genetic polymorphisms of orosomucoid ORM1 and ORM2 in a Japanese population: occurrence of new ORM1 alleles

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Summary. Genetic polymorphisms of orosomucoid ORM1 and ORM2 in a Japanese population from northern Japan were investigated using isoelectric focusing (IEF) in ultrathin layer polyacrylamide gels containing Triton X-100 and immunofixation. Nine ORM1 phenotypes which are determined by four common and one rare alleles were observed. Two of the identified alleles at this locus were considered to be new. The ORM2 pattern was classified into 14 phenotypes as products of one common and two variant alleles. The estimated allele frequencies were ORM1*1 = 0.668, ORM1*2 = 0.170, ORM1*2.1 = 0.136, ORM1*5.2 = 0.022 and ORM1*7 = 0.004; ORM2*1 = 0.972, ORM2*3 = 0.006 and ORM2*6 = 0.022.

Key words: Blood groups, orosomucoid ORM1 and ORM2 – Orosomucoid, polymorphism

Zusammenfassung. Die genetischen Polymorphismen des Orosomucoids ORM1 and ORM2 wurden mit Hilfe der Isoelektrofokussierung und Immunfixation in einer Stichprobe von 232 nicht verwandten Personen aus der nördlichen Region Japans untersucht. Die folgende Allelfrequenzen wurden ermittelt: ORM1*1 = 0.668, ORM1*2 = 0.170, ORM1*2.1 = 0.136, ORM1*5.2 = 0.022 und ORM1*7 = 0.004; ORM2*1 = 0.972, ORM2*3 = 0.006 und ORM2*6 = 0.022.

Schlüsselwörter: Blutgruppen, Orosomucoid ORM1 und ORM 2 – Orosomucoid, Polymorphismus

Introduction

Orosomucoid (ORM) or alpha-1-acid glycoprotein is a plasma protein of 40,000 daltons molecular weight (MW), present in human plasma at levels between

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0.36 and 1.46 mg/ml. Desialyzed ORM shows three common electrophoretic patterns which are determined by two codominant alleles at a single autosomal gene locus [1, 2]. Recently, the pattern obtained after using IEF for the analysis of this plasma (serum) protein has been interpreted in terms of two gene loci ORM1 and ORM2 [3]. The ORM1 locus is polymorphic with four common alleles and two rare ones [3–8], while the ORM2 locus is characterized by one common allele and several rare variants [4–6].

In the present study, ORM1 and ORM2 polymorphisms were investigated in a population sample from northern Japan. Two new ORM1 alleles were observed.

Materials and methods

Plasma specimens were obtained from 232 unrelated Japanese from the Miyagi prefecture in northern Japan. Fresh samples were treated with neuraminidase from Clostridium perfringens by adding 15 µl of IU/ml enzyme to 5 µl plasma and the mixture was incubated at 37°C for about 24 h.

IEF was carried out using ultrathin layer polyacrylamide gels of pH range 4.5–5.4 and 160 × 110 × 0.2 mm dimensions. The gels were prepared by mixing the following solutions: 1.6 ml acrylamide stock solution (29.1% acrylamide and 0.9% N, N′ methylene-bisacrylamide), 2.8 ml distilled water in which 0.5 g sucrose was dissolved, 300 µl pharmalyte pH 4.5–5.4 and 7 µl Triton X-100. The mixture was degassed for a few minutes, then 150 µl riboflavin (0.01%) was added for the polymerization. The anolyte and catholyte used were 1 *M* phosphoric acid and 0.2 *M* sodium hydroxide, respectively. The power unit was adjusted to supply an initial voltage of 280 V and a maximum of 1,600 V. The gel was prerun for 40 min, then 5 µl neuraminidase-treated plasma was placed on the gel surface at a distance of 2 cm from the cathode using 5 × 7 mm paper strips (Toyo no. 2). The paper strips were removed after 30 min, and the total running time was 5 h at 2°C. For immunoprinting 5 times diluted monospecific anti-ORM antiserum (DAKO) was used.

Results and discussion

Figures 1 and 2 show the band pattern of the different ORM1 and ORM2 phenotypes as seen by IEF in ultrathin layer polyacrylamide gels containing Triton X-100 with subsequent immunofixation. Besides the previously reported ORM1 alleles, two new ones were also detected at this locus. These alleles are

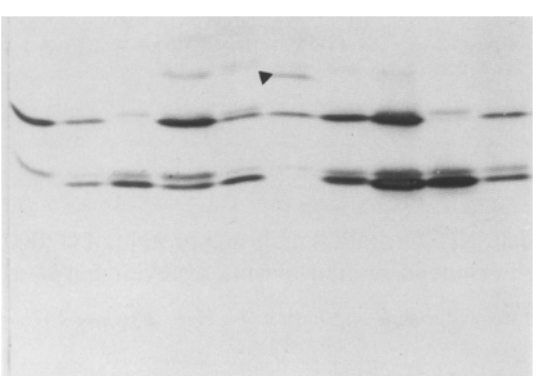

Fig. 1. Electrofocusing and immunofixation band patterns of various ORM1 phenotypes. Anode at the *top*. From *left* to *right:* 1, 2-1, 2, 2.1-1, 2.1-2, 7-1, 5.2-1, 5.2-2.1, 5.2-2 and 2-1

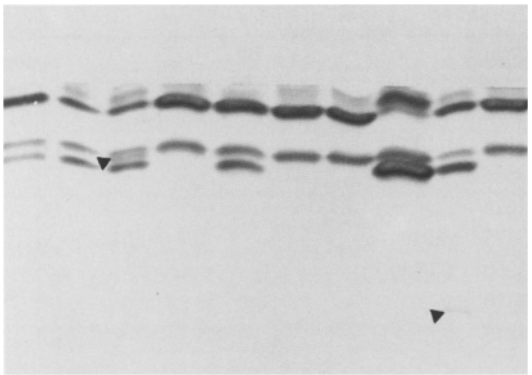

Fig. 2. Electrofocusing and immunofixation band patterns of various ORM2 phenotypes. Anode at the *top*. From *left* to *right:* 1, 1, 3-1, 1, 1, 1, 1, 1, 6-1 and 1

Table 1. ORM1 and ORM2 phenotypes and allele frequencies in northern Japanese

ORM1	ORM2			Combined	%	No. expected
	1–1	3–1	6–1			
1–1	96	2	2	100	43.1	103.52
2–1	46	1	6	53	22.8	52.69
2–2	6			6	2.6	6.70
2.1–1	48			48	20.7	42.15
2.1–2	12		1	13	5.6	10.73
5.2–1	6		1	7	3.0	6.82
5.2–2.1	2			2	0.9	1.39
5.2–2	1			1	0.4	1.74
7–1	2			2	0.9	1.24
Total	219	3	10	232	100.0	

Allele frequencies

ORM1	ORM2
ORM1*1 = 0.668	ORM2*1 = 0.972
ORM1*2 = 0.170	ORM2*3 = 0.006
ORM1*2.1 = 0.136	ORM2*6 = 0.022
ORM1*5.2 = 0.022	
ORM1*7 = 0.004	

ORM1: $\Sigma\chi^2 = 1.545$, $df = 2$, $0.30 < P < 0.50$
Note: Phenotypes of ORM1*5.2 and ORM1*7 were combined for χ^2 calculation. The observed and expected ORM2 phenotypes are identical

designated ORM1*5.2 and ORM1*7. The ORM1*5.2 allele is represented by a pattern consisting of two very close bands, the cathodal one has an isoelectric point almost identical to that of ORM1*2 allele. This allele gives a narrower corridor with the band of the ORM2*1 allele, if compared with the common ORM1*2 allele. The ORM1*5.2 is the fourth common ORM1 allele in Japanese. The ORM1*7 allele exhibits a single band migrating anodal to all other reported alleles. Similar alleles were also detected in Japanese and Taiwanese (K.

Table 2. ORM1 allele frequencies in different populations

Populations	*N*	Allele frequencies						References
		1	2	3	2.1	5.2	R	
Nepalese	141	0.6738	0.3121	0.0142	–	–	–	[3]
French	112	0.5625	0.3884	0.0491	–	–	–	[3]
Japanese	200	0.6800	0.1625	–	0.1575	–	–	[4]
Germans	272	0.6103	0.3475	0.0404	–	–	0.0018	[5]
Danes	215	0.5810	0.3860	0.0330	–	–	–	[7]
US whites	228	0.5590	0.4410	–	–	–	–	[6]
US blacks	181	0.6160	0.3840	–	–	–	–	[6]
Canadian Indians	169	0.5470	0.4530	–	–	–	–	[6]
Eskimos	220	0.5730	0.4270	–	–	–	–	[6]
Aleuts	237	0.5510	0.4490	–	–	–	–	[6]
South-American Indians	62	0.5560	0.4440	–	–	–	–	[6]
New Guinea Highlanders	110	0.8410	0.1590	–	–	–	–	[6]
Galicians	650	0.4600	0.5400	–	–	–	–	[8]
Japanese	232	0.6680	0.1700	–	0.1360	0.0220	0.0040	This study

Umetsu, personal communication). Addition of Triton X-100 to the gel gives a sharp and reproducible pattern, but the risk of missing the Caucasian allele ORM1*3 by this technique is very high. Applying this method for investigating Caucasians will require the use of an additional analytical system for detection of the ORM1*3 allele. At ORM2 locus, one common allele and two variants were encountered, of these variants the ORM2*6 seems more polymorphic in the northern Japanese as compared with the western Japanese [4].

Distribution of ORM1 and ORM2 phenotypes and allele frequencies are given in Table 1. The phenotype distribution at both loci fitted the Hardy-Weinberg equilibrium.

The published ORM1 allele frequencies with those obtained from our population are shown in Table 2. Comparison with the reported data is not possible at present, because of the apparent absence of one or more of the common alleles which is most probably due to the applied techniques. Recently, we have analyzed a Caucasian population sample and observed the occurrence of four common ORM1 alleles which included the duplicated ORM1*2.1. The ORM systems could become valuable markers for forensic medicine, anthropology, and genetics if the appropriate techniques are used.

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