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GENETIC POLYMORPHISMS OF OROSOMUCOID ORM1 AND ORM2 IN LIBYANS: OCCURRENCE OF *ORM1*2.1* AND THREE NEW ORM2 ALLELES

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Summary Genetic polymorphisms of human serum orosomucoid ORM1 and ORM2 gene loci were investigated in a Libyan population sample from Tripoli, n=110, using isoelectric focusing in ultrathin layer polyacrylamide gel containing either Triton X-100 or *N*-(2-acetamido)-2-aminoethanesulfonic acid (ACES) with subsequent immunofixation. In ORM1 locus, six common phenotypes ORM1 1-1, ORM1 2-1, ORM1 2-2, ORM1 2.1-1, ORM1 2.1-2 and ORM1 3-1 were identified, with allele frequencies as follows: *ORM1*1*=0.6500, *ORM1*2*=0.3091, *ORM1*2.1*=0.0318 and *ORM1*3*=0.0091. In ORM2 locus, one common phenotype ORM2 1 and four new heterozygote types represent products of three new variant alleles tentatively designated *ORM2* Tripoli 1, ORM2* Tripoli 2* and *ORM2* Tripoli 3* were observed. In addition, one subject in which no ORM2 was detectable, the phenotype ORM2 0-0 is suggested. The ORM2 allele frequencies were *ORM2*1*=0.9816, *ORM2* Tripoli 1*=0.0092, *ORM2* Tripoli 2*=0.0046 and *ORM2* Tripoli 3*=0.0046.

INTRODUCTION

Recently, the human serum or plasma orosomucoid (ORM) pattern observed after using isoelectric focusing for the analysis of this protein is interpreted in terms of two gene loci ORM1 and ORM2 (Yuasa *et al.*, 1986). The ORM1 is coded by an autosomal gene with five common and a number of rare alleles (Carracedo *et al.*, 1986; Thymann and Eiberg, 1986; Yuasa *et al.*, 1986; Escallon *et al.*, 1987; Weidinger *et al.*, 1987; Yuasa *et al.*, 1987; Sebetan and Sagisaka, submitted for publication). The ORM2 gene locus was first described as a monomorphic (Yuasa *et al.*, 1986),

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but later several variants have been detected (Escallon *et al.*, 1987; Weidinger *et al.*, 1987; Yuasa *et al.*, 1987). Absence of the second locus components was found by Escallon *et al.* (1987), and is explained as a possible artifact of sample storage or due to the presence of a null allele.

In this study results of analysis of ORM1 and ORM2 genetic variants in a Libyan population sample using two electrofocusing methods are presented. Comparison of the different reported methods for the separation of ORM phenotypes is also given.

MATERIALS AND METHODS

Samples. Serum samples were collected from 110 healthy unrelated mixed urban Libyans of diverse origins residing Tripoli and transported in ice bag by air, then kept frozen at $-70°C$ until tested for ORM1 and ORM2 polymorphisms. Neuraminidase treatment was performed by adding 15 μl of 1 U/ml enzyme to 5 μl serum and the mixture was incubated at 37°C for about 24 hr prior to analysis.

Isoelectric focusing. Electrofocusing run was carried out using two different procedures. In the first one, ultrathin layer polyacrylamide gels of pH range 4.5–5.4 and $160 \times 110 \times 0.2$ mm dimensions 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 few minutes, then 150 μl 0.01% riboflavin was added for the polymerization. The electrode solutions were 1 M phosphoric acid for the anode and 0.2 M sodium hydroxide for the cathode. The maximum voltage was 1,600 V with free mA. The gel was prefocused for 40 min, then 5 μl of neuraminidase treated sera were applied near the cathode using 5×7 mm paper strips (Toyo No. 2). The sample strips were removed after 30 min and the total run time was 5 hr at 2°C. In the second electrofocusing condition, the above mentioned procedure was followed except that 20 mg ACES and 300 μl Pharmalyte pH range 4.2–4.9 were used instead of Triton X-100 and Pharmalyte pH range 4.5–5.4. All samples were typed at least once by both methods and chosen samples representing the different phenotypes were also subjected to the analysis in the presence of urea and using gels without additives.

Immunofixation. Print immunofixation was done using a cellulose acetate membrane soaked in five times diluted monospecific ORM antiserum (DAKO). The membrane was placed in contact with the surface of the gel for 5–6 min at room temperature, then removed and washed for overnight with saline, and stained with Acid Violet 49.

RESULTS AND DISCUSSION

Figures 1 and 2 demonstrate the band pattern of the various ORM1 and ORM2 phenotypes as detected by isoelectric focusing in ultrathin layer polyacrylamide gels

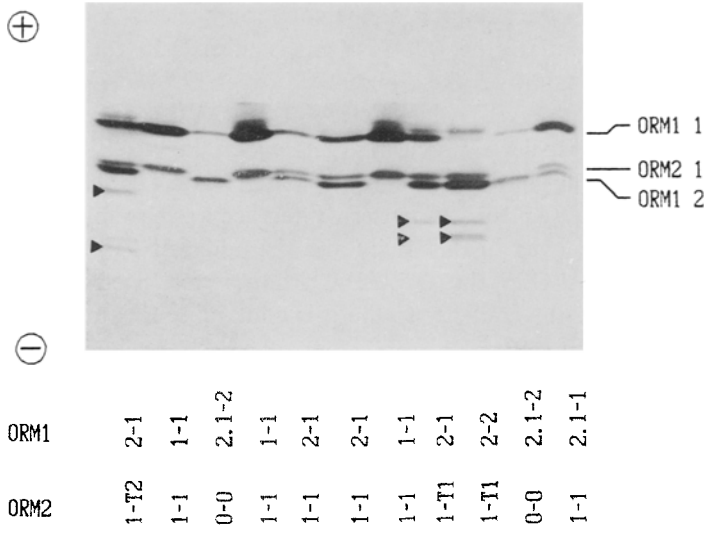

Fig. 1. ORM1 and ORM2 phenotypes after electrofocusing in polyacrylamide gel containing Triton X-100 followed by immunofixation of neuraminidase treated sear. Filled triangles indicate variant ORM2 alleles.

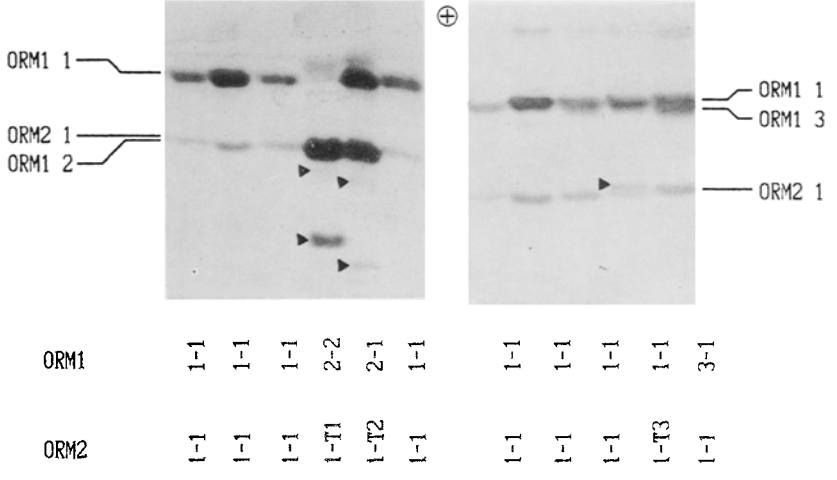

Fig. 2. ORM1 and ORM2 phenotypes after electrofocusing in polyacrylamide gel containing ACES followed by immunofixation of neuraminidase treated sera. Filled triangles indicate variant ORM2 alleles.

containing either Triton X-100 (Fig. 1) or ACES (Fig. 2) with subsequent immunofixation. Adding Triton X-100 to the gel produces sharp and reproducible pattern, and this method has the capability of detecting the duplicated *ORM1*2.1* allele which is difficult to identify by all other methods. But this method fails to resolve

the difference between the products of the common alleles *ORM1*1* and *ORM1*3*, and could not differentiate the *ORM2*Tripoli3* allele. In the presence of ACES or using gels without additives the difference between *ORM1*1* and *ORM1*3* products could be easily resolved, while detection of *ORM2*Tripoli3* allele was only reliable by separator isoelectric focusing (gels containing ACES). Gels containing urea showed inferior resolution for many of ORM1 and ORM2 phenotypes. This method is better to be avoided. A pattern difference was also found between the two main procedures employed in this study and the additional ones. In the presence of Triton X-100 or ACES the *ORM2*1* product was electrofocused anodal to *ORM1*2* product, on the contrary to the gels containing urea or without additives, the *ORM2*1* was electrofocused cathodal to *ORM1*2* product. Two facts seem noteworthy: (1) It is necessary to apply more than one analytical system, (2) The homozygote form of the duplicated allele *ORM1*2.1* which is characterized by the occurrence of double-banded pattern with isoelectric points identical to those of *ORM1*1* and *ORM1*2* alleles, indistinguishable of the ordinary ORM1 2-1 phenotype, as both phenotypes attain double-banded pattern with equal staining intensity.

Table 1. ORM1 and ORM2 phenotypes and allele frequencie in Libyans.

ORM1	ORM2					Combined	%	No. expected
	1-1	1-T1	1-T2	1-T3	0-0			
1-1	45			1		46	41.81	46.48
2-1	42	1	1			44	40.00	44.20
2-2	10	1				11	10.00	10.51
2.1-1	5					5	4.55	4.55
2.1-2	1				1	2	1.82	2.16
2.1-2.1	0					0	0.00	0.11
3-1	2					2	1.82	1.30
3-2	0					0	0.00	0.62
3-2.1	0					0	0.00	0.06
3-3	0					0	0.00	0.01
Total	105	2	1	1	1	110	100.00	100

Allele frequencies

ORM1	ORM2
*ORM1*1*=0.6500	*ORM2*1*=0.9816
*ORM1*2*=0.3091	*ORM2*T1*=0.0092
*ORM1*2.1*=0.0318	*ORM2*T2*=0.0046
*ORM1*3*=0.0091	*ORM2*T3*=0.0046

ORM1 : $\Sigma\chi^2 = 1.26$, $df = 6$, $0.95 < p < 0.99$. Note: ORM2 0-0 phenotype is excluded from frequency calculation and the observed and expected ORM2 phenotypes are identical. T, Tripoli.

The heterozygote form of *ORM1*2.1* with *ORM1*1* or *ORM1*2* shows double-banded pattern with two bands of different staining intensity. After the two main techniques, the studied population sample showed six ORM1 phenotypes as a products of the three common alleles; *ORM1*1*, *ORM1*2*, *ORM1*3* reported in Caucasians (Thymann and Eiberg, 1986; Yuasa *et al.*, 1986; Weidinger *et al.*, 1987), and *ORM1*2.1* allele which is common in Mongoloids (Yuasa *et al.*, 1987). At the second locus, beside the most common allele detected in all the studied populations, three variant ones were also identified. Family study was not available, but the pattern of these variants was constant and reproducible after repeated typing using different stored sera. The banding profiles of these alleles are clearly different from those reported in previous publications (Escallon *et al.*, 1987; Weidinger *et al.*, 1987; Yuasa *et al.*, 1987). The variant ORM2 alleles are tentatively named *ORM2* Tripoli 1*, *ORM2*Tripoli 2* and *ORM2*Tripoli 3*.

Distribution of ORM1 and ORM2 phenotypes and allele frequencies are shown in Table 1. The observed and expected phenotypes at both loci provide a good fit to the Hardy-Weinberg equilibrium. The obtained ORM frequencies from our population sample are markedly different from all other reported populations (Carracedo *et al.*, 1986; Thymann and Eiberg, 1986; Yuasa *et al.*, 1986; Escallon *et al.*, 1987; Weidinger *et al.*, 1987; Yuasa *et al.*, 1987). However, appropriate comparison is not possible now because of the technical difficulties and differences discussed above play an important role in the previously published data.

The ORM systems will become valuable markers for forensic medicine, anthropology and genetics if proper methods will be applied.

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