Human Heredity
Editor: J. Ott, New York, N.Y.

Reprint
Publisher: S. Karger AG, Basel
Printed in Switzerland

Original Paper

Hum Hered 1995;45:181–185

Orosomucoid Phenotyping with Monoclonal Antibodies: Polymorphic Occurrence of *ORM1*Q0* in Aboriginal Taiwanese Populations

Kazuo Umetsu[a]
Isao Yuasa[b]
Akira Harada[a]
Tsuneo Suzuki[a]
I-Hung Pan[c]
Takafumi Ishida[d]
Naruya Saitou[e]
Satoshi Horai[e]

[a] Department of Forensic Medicine, Yamagata University School of Medicine, Yamagata;
[b] Department of Legal Medicine, Tottori University School of Medicine, Yonago, Japan;
[c] National Taiwan University School, Taipei, Republic of China;
[d] Department of Anthropology, Graduate School of Science, University of Tokyo, and
[e] National Institute of Genetics, Mishima, Japan

Key Words
Orosomucoid
Monoclonal antibodies
Aboriginal Taiwanese
Silent allele

Abstract
Three monoclonal antibodies (OR35, OR40 and OR48) against orosomucoid (ORM) were prepared for the phenotyping of the human ORM system. The OR35 and OR48 antibodies recognized ORM1 and ORM2 products, respectively. OR40 reacted strongly to the products of ORM1 but poorly to those of ORM2. With the help of these monoclonal antibodies, ORM phenotyping was performed on 658 individuals from nine subpopulations of aboriginal Taiwanese, with close attention to two individuals with an ORM1 Q0 homozygous phenotype. The *ORM1*Q0* allele was found to be at a polymorphic frequency in eight of the nine subpopulations.

Introduction

Human orosomucoid (ORM) or α_1-acid glycoprotein is a major acute-phase plasma protein with a molecular mass of approximately 49 kD, of which 45% is carbohydrate. ORM is predominantly produced in the liver and suppresses blastogenesis of lymphocytes and neutrophil activation [1]. ORM shows extensive genetic variation, and about 30 alleles have been described at the two loci ORM1 and ORM2 [2]. The ORM1 and ORM2 alloproteins are encoded by two closely linked genes on the long arm of chromosome 9 [3–5] and are distinguishable from each other in band intensity [6].

In the course of ORM phenotyping, we have occasionally encountered some bands

Received:
June 31, 1994
Accepted:
November 3, 1994

Kazuo Umetsu
Department of Forensic Medicine
Yamagata University School of Medicine
Yamagata 990–23 (Japan)

0001–5652/95/
0454–0181$8.00/0

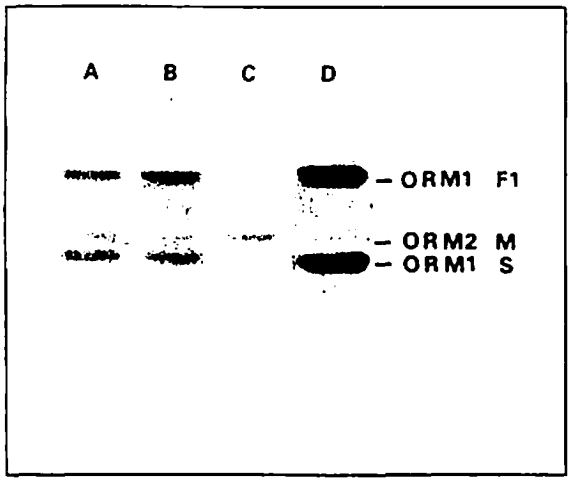

Fig. 1. IEF patterns of ORM1 F1-S/ORM2 M. Immunoprint using polyclonal antibody to ORM (**A**) and immunoblot using monoclonal antibodies to ORM (**B–D**): OR35, OR48 and OR40, respectively. The immunoblot was performed with monoclonal antibodies as the first antibodies, and alkaline-phosphate-labelled goat anti-mouse immunoglobulin as the second antibody. The anode is at the top.

for which it was difficult to determine whether they belonged to the ORM1 or ORM2 product. To overcome such confusions, we attempted to prepare locus-specific monoclonal antibodies. Using polyclonal and monoclonal antibodies, we performed ORM phenotyping on native Taiwanese populations and carried out ORM1 Q0 typing.

Materials and Methods

Monoclonal Antibodies

Female BALB/c mice were immunized twice at 2-week intervals by intraperitoneal injection of purified desialylated ORM (about 100 μg protein, Cosmo Bio, Tokyo, Japan) emulsified in 100 μl Hunter's TiterMax adjuvant (CytRx, Norcross, Ga., USA). A booster injection was given 10 days after the second injection. The spleen was removed 3 days after the booster injection and used for hybridization. The spleen cells were hybridized with P3-X-63-Ag8.653 myeloma cells [7] according to Oi and Herzenberg [8]. Three monoclonal antibodies, OR35 (IgG1), OR40 (IgM) and OR48 (IgG1) were selected. The supernatants of the ascites from BALB/c mice injected with the hybridoma cells were used for immunological detection of ORM bands without further purification.

Subjects

Plasma samples from 658 aboriginal Taiwanese were collected from the following subpopulations: 100 Atayal, 64 Saisiat, 87 Bunun, 80 Tsou, 72 Ami, 63 Puyma, 54 Rukai, 60 Paiwan and 78 Yami. Preliminary genetic studies in these groups have been reported by Umetsu et al. [9]. The samples were treated overnight with a fourfold volume of sialidase (0.5 U/ml, 50 m*M* sodium acetate buffer, pH 5.0, Type V, Sigma, St. Louis, Mo., USA) at room temperature.

ORM Phenotyping

Polyacrylamide gel isoelectric focusing (IEF) was carried out as described elsewhere [10], except that the concentration of Triton X-100 was decreased from 0.2 to 0.05%. The ORM patterns were developed by immunoprinting with polyclonal antibody (anti-ORM antiserum from rabbit, Dako, Glostrup, Denmark) and by immunoblotting with monoclonal antibodies. The immunoblotting was performed using alkaline-phosphate-labelled goat anti-mouse immunoglobulin (Dako). The band patterns were visualized by a BCIP/NBT reaction. The symbols of these alleles were based on the nomenclature proposed by Yuasa et al. [2].

Results and Discussion

Figure 1 shows the band patterns of an ORM1 F1-S/ORM2 M type analyzed by IEF with the pH gradient 4.5–5.4, using polyclonal and monoclonal antibodies. All three monoclonal antibodies, OR35, OR40 and OR48, were found to be specific to ORM products. The OR35 and the OR48 antibodies recognized ORM1 and ORM2 bands, respectively. The OR40 antibody reacted strongly to the products of ORM1 but poorly to those of ORM2. The results of further experiments concerning the reaction profiles of some ORM phenotypes indicated that OR35 recognized the common ORM1 types, while OR40 recog-

nized the common ORM1 types but reacted poorly with the ORM2 types including ORM2 M and ORM2 H19 bands. OR48 did not recognize ORM2 H19 but did label ORM2 M. The three monoclonal antibodies reacted to both native and desialylated forms of ORM (data not shown), although Fraeyman et al. [11] have shown that some of the monoclonal antibodies react to both the native and the desialylated ORM, whereas others react only to the desialylated form of ORM.

ORM phenotyping was performed on 658 individuals from nine subpopulations of aboriginal Taiwanese by IEF and immunoprinting using the polyclonal anti-ORM antiserum from Dako. Because some samples were difficult to analyze due to unusual patterns, as shown in lanes 2–4 of figure 2A, we thought that these samples might represent a new ORM1 variant with the same mobility as the ORM2 M band. If this is the case, such a variant band should be more intense than ORM1 F1 and ORM1 S bands because of their overlapping with the ORM2 M band. To clarify this point, we employed the monoclonal antibodies for further ORM phenotyping. Figure 2 B–D show immunoblotted patterns after IEF of desialylated samples using monoclonal antibodies. Judging from the reactivity of each monoclonal antibody, the band corresponding to the ORM2 region must be the ORM2 M origin. Since there is considerable variation in the ORM concentrations in the sera, a reduced level in comparison with controls does not necessarily indicate the presence of an inheritable silent allele. Because the relative concentrations of the ORM1 and ORM2 products in a sample could be estimated by comparing the intensities of the two bands, *ORM1*Q0* carriers were distinguishable from the common ORM1 types. Thus, the samples in lanes 2–4 of figure 2 were identified as ORM1 F1-Q0/ORM2 M, Q0/M, and S-Q0/M phenotypes, respectively.

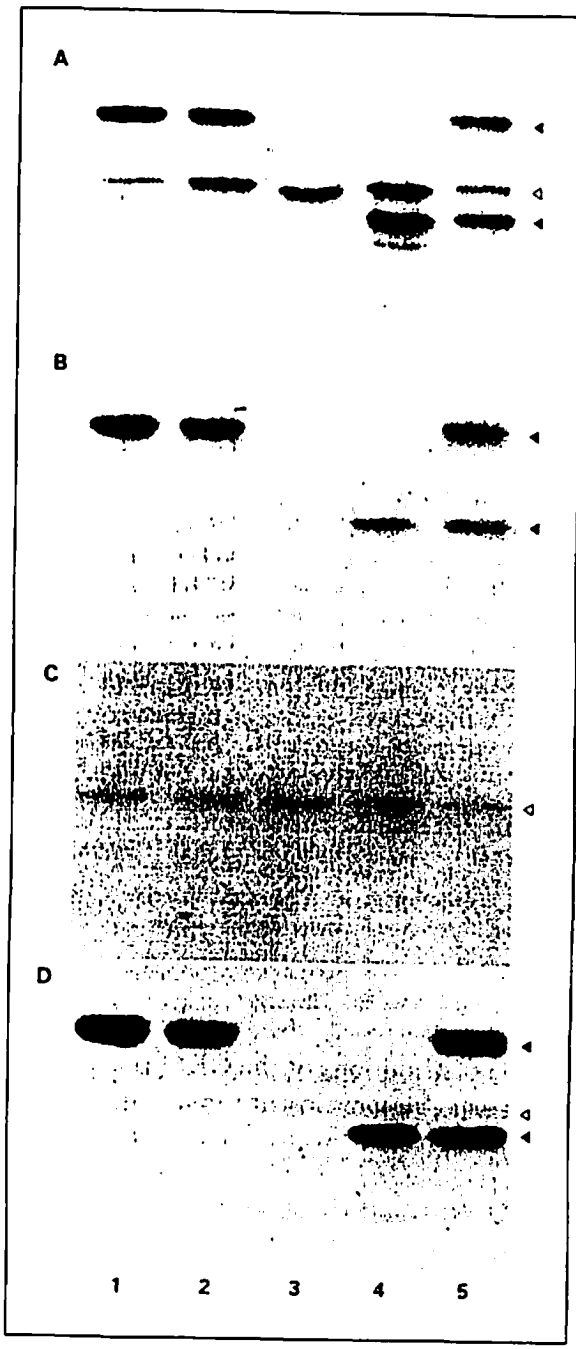

Fig. 2. Immunoprint using polyclonal antibody to ORM (**A**) and immunoblots using monoclonal antibodies to ORM (**B–D**): OR35, OR48 and OR40, respectively. Lane 1 = ORM1 F1/ORM2 M; 2 = F1-Q0/M; 3 = Q0/M; 4 = S-Q0/M; 5 = F1-S/M. Filled and open triangles indicate ORM1 and ORM2 bands, respectively. The anode is at the top.

Table 1. Distribution of ORM phenotypes and allele frequencies in nine subpopulations of aboriginal Taiwanese

Phenotypes	Atayal	Saisiat	Bunun	Tsou	Ami	Puyuma	Rukai	Paiwan	Yami
ORM1 F1	49	49	37	56	48	38	47	42	75
F1-S	23	11	34	19	13	18	4	14	3
S	0	0	6	3	0	1	0	1	0
F1-dF1S	0	2	4	0	0	2	0	1	0
F1-B9	0	0	0	0	3	0	0	0	0
S-B9	0	0	0	0	0	1	0	0	0
F1-Q0	25	1	4	2	8	3	3	2	0
S-Q0	2	1	1	0	0	0	0	0	0
Q0	1	0	1	0	0	0	0	0	0
Total	100	64	87	80	72	63	54	60	78
Allele frequencies									
*ORM1*F1*	0.730	0.875	0.667	0.831	0.833	0.786	0.935	0.842	0.981
*ORM1*S*	0.125	0.094	0.270	0.156	0.090	0.167	0.037	0.133	0.019
*ORM1*dF1S*	–	0.016	0.023	–	–	0.016	–	0.008	–
*ORM1*B9*	–	–	–	–	0.021	0.008	–	–	–
*ORM1*Q0*	0.145	0.016	0.040	0.013	0.056	0.024	0.028	0.017	–
ORM2 M	99	64	84	79	62	56	53	54	78
M-H19	1	0	2	1	10	7	1	6	0
M-H2	0	0	1	0	0	0	0	0	0
Total	100	64	87	80	72	63	54	60	78
Allele frequencies									
*ORM2*M*	0.995	1.000	0.983	0.994	0.931	0.944	0.991	0.950	1.000
*ORM2*H19*	0.005	–	0.012	0.006	0.069	0.056	0.009	0.050	–
*ORM2*H2*	–	–	0.006	–	–	–	–	–	–

The distributions of the ORM phenotypes and allele frequencies are given in table 1. In the present study, five ORM1 alleles (*ORM1*F1*, *ORM1*S*, *ORM1*dF1S*, *ORM1*B9* and *ORM1*Q0*) and three ORM2 alleles (*ORM2*M*, *ORM2*H2* and *ORM2*H19*) were identified by IEF in combination with immunodetection using polyclonal and monoclonal antibodies. All of these alleles have been reported in Asians and American Indians [10, 12, 13]. At the ORM1 locus, *ORM1*F1* was the most common allele in all nine aboriginal Taiwanese populations, with its frequency ranging from 0.667 to 0.981. At the ORM2 locus, *ORM2*M* is highly prevalent with a frequency between 0.931 and 1.0. Although *ORM1*Q0* has been reported to be very rare worldwide [14, 15], it is distributed throughout the aboriginal Taiwanese populations, with the exception of the Yami. It is to be noted that the present paper is the first documentation of the presence of the homozygote for *ORM1*Q0* in human populations. Its highest allele frequency was observed in the Atayal (0.145), with the frequency decreasing toward peripheral regions. In the present study, the *ORM2*H19* allele is fairly common in seven aboriginal Taiwanese populations, ranging from 0.5 to 6.9%. Since *ORM2*H19* is very common in Han Chinese

[12] but not high in Southeast Asians [16], it is suggested that the *ORM2*H19* allele in subpopulations of aboriginal Taiwanese probably originated from neighboring Chinese.

In conclusion, we found monoclonal antibodies to be of great value in ORM phenotyping of the *ORM1*Q0* carriers. Further studies will be required to characterize the silent allele at both the DNA and protein level.

Acknowledgements

This work was supported by grants-in-aid for the international program (human genetical studies on native minority groups in Taiwan) and scientific research on the priority area (prehistoric Mongoloid dispersals) from the Ministry of Science, Education and Culture of Japan.

References

1 Bennet M, Schmid K: Immunosuppression by human plasma alpha-1 acid glycoprotein: Importance of the carbohydrate moiety. Proc Natl Acad Sci USA 1980;77:6109–6113.

2 Yuasa I, Weidinger S, Umetsu K, Suenaga K, Ishimoto G, Eap CB, Duche JC, Baumann P: Orosomucoid system: 17 additional orosomucoid variants and proposal for a new nomenclature. Vox Sang 1993;64: 47–55.

3 Dente L, Pizza MG, Metspalu A, Cortese R: Structure and expression of the genes coding for human α_1-acid glycoprotein. EMBO J 1987;6: 2289–2296.

4 Merritt CM, Board PG: Structure and characterization of a duplicated human α_1-acid glycoprotein gene. Gene 1988;66:97–106.

5 Smith M, Simpson NE: Report of the committee on the genetic constitution of chromosomes 9 and 10. Cytogenet Cell Genet 1989;51:202–225.

6 Yuasa I, Umetsu K, Suenaga K: Orosomucoid (ORM) typing by isoelectric focusing: Evidence for two structural loci ORM1 and ORM2. Hum Genet 1986;74:160–161.

7 Kearney JF, Radbruch A, Liesegang B, Rajewsky K: A new mouse myeloma cell line that has lost immunoglobulin expression but permits the constitution of antibody-secreting hybrid cell lines. J Immunol 1979; 123:1548–1550.

8 Oi VT, Herzenberg JA: Immunoglobulin-producing hybrid cell lines; in Mishell BB, Shigieds SM (eds): Selected Methods in Cellular Immunology. San Francisco, Freeman, 1980, pp 351–372.

9 Umetsu K, Yuasa I, Suzuki T, Cheih-Shan S, I-Hung P, Ishida T, Saitou N, Horai S: Polymorphisms of complement component I and C1R subcomponent of C1 in nine aboriginal Taiwanese populations. Hum Biol 1994;66:339–348.

10 Yuasa I, Suenaga K, Umetsu K, Ito K, Robinet-Levy M: Orosomucoid (ORM) typing by isoelectric focusing: Evidence for gene duplication of ORM1 and genetic polymorphism of ORM2. Hum Genet 1987; 77:255–258.

11 Fraeyman NH, Smet FH, van de Velde EJ: A study of the heterogeneity of human alpha$_1$-acid glycoprotein with monoclonal antibodies. Hybridoma 1987;6:565–574.

12 Umetsu K, Yuasa I, Chen ER, Suzuki T: Orosomucoid 1 and orosomucoid 2 types in the Taiwanese and Japanese: Evidence for five new orosomucoid variants. Electrophoresis 1988;9:224–226.

13 Salzano FM, Umetsu K, Yuasa I, Black FL, Suzuki T: Isoelectric focusing studies in Brazilian Indians – Uncovering variation of ORM, AHSG and IF–. Jpn J Hum Genet 1990;35:283–290.

14 Yuasa I, Umetsu K, Suenaga K, Ikebuchi J, Suzuki T: Orosomucoid (ORM) typing by isoelectric focusing: Evidence for several new variants including ORM1 and ORM2 silent alleles. Vox Sang 1990;58: 129–134.

15 Kasulke DH, Weidinger S: A silent allele in the orosomucoid (ORM) system; in Polesky HF, Mayr WR (eds): Advances in Forensic Haemogenetics. Berlin, Springer, 1990, vol 3, pp 313–315.

16 Umetsu K, Yuasa I, Yamashita T, Saito S, Yamaguchi T, Ellepola SB, Isida T, Suzuki T: Genetic polymorphisms of orosomucoid and alpha-2-HS-glycoprotein in Thai, Sri Lankan and Paraguayan populations. Jpn J Hum Genet 1989;34:195–202.