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GENETIC POLYMORPHISM OF OROSOMUCOID (ORM) IN POPULATIONS OF THE UNITED KINGDOM, INDIAN SUBCONTINENT, AND CAMBODIA

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Summary The genetic variation of the human serum orosomucoid (ORM) was investigated by isoelectric focusing (IEF) followed by immunofixation in 15 different populations from East Midlands (United Kingdom), India, Sri Lanka, and Cambodia. Statistically significant differences were observed between various Asiatic and British populations, however differences within Asiatic and European populations were minor. The distribution of *ORM1* alleles in populations investigated to date suggests an interesting east-west geographical cline. There is a suggestion that present day wide polymorphism at the *ORM1* locus may be influenced by selection.

Key Words polymorphism, orosomucoid, ethnic groups, United Kingdom, India, Cambodia, Sri Lanka

INTRODUCTION

Orosomucoid (ORM), or alpha-1-acid glycoprotein, is an acute-phase reactant protein (mol wt 40,000) present in the human serum at levels between 0.5 and 1 mg/ml. Its serum concentration increases in the inflammatory response in number of diseases and in pregnancy (Schmid, 1976). Though its biological function is still obscure, it seems to play a role in inhibiting erythrocyte invasion by the malarial parasite and therefore preventing parasite increase and reducing their survival

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(Freidman, 1983). The genetic polymorphism of ORM was first reported by Tokita and Schmid (1963) using starch gel electrophoresis. Subsequently, Johnson *et al.* (1969) described three different phenotypes using immunofixation which are the expression of two codominant alleles *ORM*F* and *ORM*S* at a single locus. Recent studies using isoelectric focusing have shown further heterogeneity in ORM. It has been established that ORM is coded by two loci, *ORM1* and *ORM2* (Yuasa *et al.*, 1986; Weidinger *et al.*, 1987) which are closely linked on chromosome 9 near ABO and AK1 systems (Eiberg *et al.*, 1982).

The *ORM1* locus is polymorphic in most populations investigated with three common alleles (*ORM1*F1* or *ORM1*1*, *ORM1*F2* or *ORM1*3*, and *ORM1*S* or *ORM1*2*) (Escallon *et al.*, 1987; Thymann and Weidinger, 1988; Yuasa *et al.*, 1987). Several rare alleles have also been found a few being common in certain geographical regions. *ORM2* is polymorphic in the U.S. blacks (Escallon *et al.*, 1987) and the Mongoloid populations (Yuasa *et al.*, 1987), while in the Europe it is practically monomorphic (Yuasa *et al.*, 1986). The ORM polymorphism has also been proven useful in the forensic characterisation of human and animal blood and semen stains (Harada *et al.*, 1989; Yuasa *et al.*, 1990a). So far the ORM genetic polymorphism has not been investigated extensively in several different regions of the world. The aim of this study was to increase our understanding of the ORM distribution by analysing population samples from four geographical regions of the East Midlands (Britain), ten endogamous and ethnic populations from Western India, and Sri Lanka and the population of Khmer from Cambodia.

MATERIALS AND METHODS

Sera from a total of 1,581 healthy and unrelated individuals were collected as part of various genetic surveys. The samples belonged to the native residents of the four geographical regions of Britain, northwest Derbyshire (105), northeast Derbyshire (105), south Derbyshire (242), and Leicestershire (103); five endogamous groups of India, Brahmins (119), Marathas (140), Gujarati Hindu (84), Parsee (53), and Andhra Pradesh Hindus (115); five ethnic groups of Sri Lanka Sinhalese (88), Tamils (100), Burghers (100), Moors (98) and Malays (99), and Khmers of Cambodia (31). Serum samples were stored at $-20°C$ before use and tested within one year. Desialyzation of serum samples was performed by mixing 20 μl neuraminidase (Sigma) (1 U/ml, pH 5.5) to 5 μl of serum followed by incubation of the mixture overnight at 37°C. ORM typing of neuraminidase treated plasma samples was carried out according to Yuasa *et al.* (1986, 1987) using 5% Ampholine 4.5–5.4 (Pharmacia-LKB, Bromma, Sweden).

RESULTS AND DISCUSSION

The distribution of observed and expected phenotype numbers of the *ORM1* system in 15 populations studied is given in Table 1. All the populations investi-

Table 1. Observed and expected phenotype numbers of *ORM1*.

	Phenotypes						
Population	**F1-F1**	**F1-F2**	**F1-S**	**F2-F2**	**F2-S**	**S-S**	**No.**
Britain, South Derbyshire							
Observed	99	-	109	-	-	34	242
Expected	97.36		112.27			32.36	
χ^2 0.21							
Northwest Derbyshire							
Observed	36	-	49	-	-	20	105
Expected	34.86		51.28			18.86	
χ^2 0.21							
Northeast Derbyshire							
Observed	35	-	53	-	-	17	105
Expected	36.02		50.96			18.02	
χ^2 0.17							
Leicestershire							
Observed	26		57	-	-	20	103
Expected	28.84		51.33			22.84	
χ^2 1.26							
India, Bomaby							
Brahmin							
Observed	57	1	42	0	0	15	116
Expected	53.8	1.4	49	0	0.6	11.2	
χ^2 3.43							
Maratha							
Observed	74	3	55	0	0	12	144
Expected	73.6	2.2	56.5	0	0.8	10.8	
χ^2 1.34							
G. Hindu							
Observed	57		21	-	-	6	84
Expected	54.2		26.5			3.2	
χ^2 2.16							
Parsee							
Observed	26	-	19	-	-	6	51
Expected	24.7		21.6			4.7	
χ^2 0.73							

	Phenotypes						
Population	**F1-F1**	**F1-F2**	**F1-S**	**F2-F2**	**F2-S**	**S-S**	**No.**
India, Hyderabad							
Hindu							
Observed	59	2	40	0	4	9	115
Expected	59.17	4.3	44.48	0.07	1.62	8.35	
χ^2 0.57							
Sri Lanka							
Sinhalese							
Observed	51	2	25	0	1	9	88
Expected	47.3	2.2	32.2	0	0.8	5.5	
χ^2 4.28							
Tamils							
Observed	51	2	42	0	0	5	100
Expected	53.3	1.5	37.9	0	0.5	6.8	
χ^2 1.72							
Burghers							
Observed	45	2	48	0	0	5	100
Expected	49	1.4	40.6	0	0.6	8.4	
χ^2 3.91							
Moors							
Observed	54	2	41	0	0	6	98
Expected	55.3	1.5	38.9	0	0.5	6.8	
χ^2 0.96							
Malays							
Observed	53	-	41	-	-	5	99
Expected	54.6	-	37.9	-	-	6.5	
χ^2 0.96							
Cambodia							
Khmer							
Observed	16	-	15	-	-	0	31
Expected	17.8	-	11.4	-	-	1.8	
χ^2 0.42							

Table 2. *ORM1* allele frequencies in different populations.

Population		No.	ORM1*F1	ORM1*F2	ORM1*S	ORM1*V	Reference
Americas							
	US Whites	228	0.559	-	0.386	-	Escallon et al.1987
	US Blacks	181	0.619	-	0.384	-	Escallon et al.1987
	Candian Indians	169	0.547	-	0.453	-	Escallon et al.1987
Paraguay	Paraguayan	200	0.645	0.023	0.307	0.025	Umetsu et al. (1989)
United Kingdom							
	South Derbyshire	242	0.634	-	0.366	-	This study
	Northwest Derbyshire	105	0.576	-	0.424	-	This study
	Northeast Derbyshire	105	0.586	-	0.414	-	This study
	Leicestershire	103	0.529	-	0.471	-	This study
Danes		215	0.581	0.033	0.386	-	Thymann and Eiberg, (1986)
Germans							
	West Germany	670	0.627	-	0.373	-	Metzner and Schiel (1988)
	Munchen	272	0.610	0.040	0.348	0.002	Weidinger et al. (1987)
	South Germany	696	0.613	0.034	0.353	0.001	Thymann and Eiberg (1986)
Swiss		329	0.593	0.001	0.404	0.002	Eap et al. (1988)
Switzerland		220	0.607	-	0.393	-	Metzner and Scheil (1988)
French		112	0.563	0.049	0.388	-	Yuasa et al. (1986)
Spanish							
	Basque	150	0.573	0.033	0.393	-	Montiel et al. (1990)
	Galicia	880	0.557	0.033	0.406	0.003	Montiel et al. (1990)
	Madrid	315	0.621	0.005	0.375	-	Alonso et al. (1990)
Portuguese		260	0.552	0.031	0.415	0.002	Montiel et al. (1990)
Italy							
	Mainland Italy	567	0.621	-	0.379	-	Scacchi et al. (1992)
	Sardinia	244	0.564	-	0.436	-	Scacchi et al. (1992)
	Lombardy	600	0.599	0.015	0.386	-	Cerri and De Ferrari (1992)
Libya	Libyans	105	0.650	0.009	0.309	0.032	Sebetan and Sagisaka (1988)
India							
	Brahmin	116	0.681	0.009	0.310		This study
	Maratha	144	0.715	0.011	0.274		This study
	G.Hindu	84	0.804	-	0.196	-	This study
	Parsee	51	0.696	-	0.304	-	This study
	Hyderabad Hindus	115	0.717	0.026	0.270	-	This study
	Parsees	180	0.636	0.008	0.356	-	Saha et al. (1992)
Sri Lanka							
	Sinhalese	88	0.733	0.017	0.250	-	This study
	Tamils	100	0.730	0.010	0.260	-	This study
	Burghers	100	0.700	0.010	0.290	-	This study
	Moors	98	0.733	-	0.257	-	This study
	Malays	99	0.742	-	0.258	-	This study
	Sri Lankans	140	0.700	-	0.268	0.033	Umetsu et al. (1989)
Nepalese		141	0.674	0.014	0.312	-	Yuasa et al. (1986)
China							
	Chinese	163	0.756	-	0.141	0.104	Yuasa et al. (1990a)
	Han Chinese	286	0.703	0.021	0.276		Yiping et al. (1992)
Taiwanese		200	0.726	-	0.181	0.094	Umetsu et al.(1988a)
Cambodia	Khmer	31	0.758	-	0.242		This study
Filipinos		115	0.790	-	0.169	0.041	Umetsu et al. (1988b)
Japanese							
	Yamagata	500	0.779	-	0.221	-	Umetsu et al. (1985)
	Yamaguchi	200	0.680	0.022	0.163	0.135	Yuasa et al. (1990b)
	Okinawa	364	0.688	-	0.166	0.146	Yuasa et al. (1990a)
	Myagi	232	0.668	0.006	0.170	0.156	Sebetan and Sagisaka (1989)
Thailand	Thais	369	0.814	-	0.161	0.025	Umetsu et al. (1989)
Cook Islanders		318	0.789	-	0.211	-	Abe et al. (1988)
New Guinea		110	0.841	-	0.159	-	Escallon et al. (1987)

gated were in Hardy-Weinberg equilibrium. The gene frequencies in populations investigated along with the *ORM1* frequencies collected from different studies are listed in Table 2.

The *ORM2* locus was monomorphic in British population from East Midlands, Indians from Western India and Khmer from Cambodia, however the locus exhibited polymorphic variation in populations of Sri Lanka. The allele *ORM2*4* (*ORM2*L7*) was observed in all populations of Sri Lanka with frequency ranging from 1–2.5%, however the allele *ORM2*5* (*ORM2*H6*) was observed in three populations (Sinhalese, Tamils, and Malays) with frequencies between 0.5–2.2%. The number of populations studied for this locus are very few; it is therefore difficult to comment if these alleles are the result of gene flow and admixture in the present day inhabitants of Sri Lanka.

The polymorphism on the *ORM1* is more extensively studied. In the four regional samples of Britain the frequency of *ORM*F1* allele was within the European range and there was no significant genetic heterogeneity among the four regional subpopulations of East Midlands ($\chi^2=9.57$, df=6, $p>0.05$), but the phenotypic distribution in the two subpopulations, Leicestershire and south Derbyshire, was statistically significant ($\chi^2=7.82$, df=2, $p<0.05$).

In the Indian subcontinent this is the first extensive study on *ORM1* polymorphism. In different ethnic and endogamous groups of India and Sri Lanka, the lowest frequency of *ORM1*F1* was found in Indian Brahmins (68%) while the highest was found in Gujarati Hindus (80%). The five ethnic groups of Sri Lanka show a very close range of *ORM1*F1* allele (70–74%). The overall pattern of allele frequency variation in Sri Lanka is compatible with another study on Sri Lankans (Umetsu *et al.*, 1989). The present Parsee *ORM1*F1* (70%) frequency is higher than observed in another sample (64%) (Saha *et al.*, 1992). This difference was statistically not significant. Overall the populations of Western Indian and Sri Lankan seems to be relatively homogenous except the Gujarati Hindus which differed significantly from Brahmins and Marathas of India ($\chi^2=7.06$, df=2, $p<0.05$ and $\chi^2=6.17$, df=2, $p<0.05$, respectively) and from Tamils and Burghers ($\chi^2=6.39$, df=2, $p<0.05$ and $\chi^2=11.06$, df=2, $p<0.05$) of Sri Lanka.

The *ORM1*F1* frequency in the Khmer population of Cambodia is 76%, which fits well in the range observed for Indian subcontinent and other Mongoloid populations of the southeast Asia (Table 2).

Within the subpopulations from Britain, India, and Sri Lanka, there was no significant genetic diversity but the variation between the total British sample against each of total Indian and Sri Lankan samples was statistically highly significant (British *vs.* India $\chi^2=36.26$, df=2, $p<0.0001$ and British *vs.* Sri Lanka $\chi^2=43.46$, df=2, $p<0.0001$).

It has been observed from the Table 2 that in all the European populations the *ORM1*F1* gene is low and restricted around 60%, and the *ORM1*1S* gene may be as high as 47% averaging around 40%. However the allele *ORM1*1F*

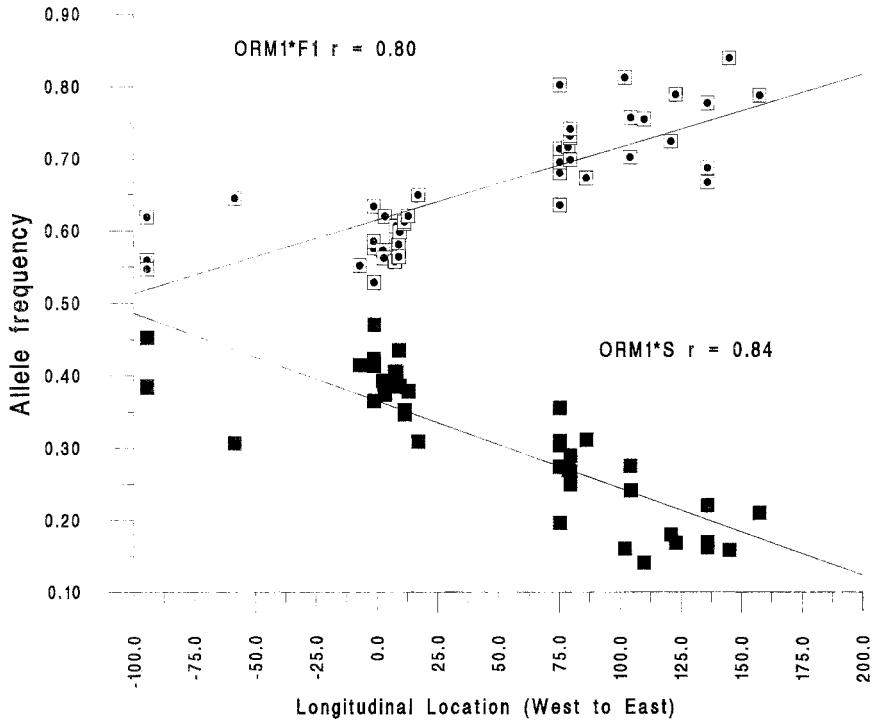

Fig. 1. Geographical cline of *ORM1* allele frequencies.

increases in central Asia and this increase continues towards the Southeast Asia, where the populations from Japan, Cambodia, Thailand, and Philippines give high value of *ORM1*F1* allele ranging between 69–81%. The highest value of *ORM-1*F1* so far reported is from New Guinea (84%).

For 48 populations reported in the literature including this study, there is significant clinal increase of *ORM1*F1* allele frequency from west to east ($r=0.8$, $p<0.01$). The *ORM1*S* allele frequency shows the opposite longitudinal trend ($r=84$, $p<0.01$) (Fig. 1).

In conclusion, the allele frequencies at the *ORM1* locus show an interesting geographical distribution. At present, it is difficult to explain the observed clinal increase of *ORM1*F1* allele. More physiological data is needed to understand biological differences between the *ORM1*F1* and *ORM1*S* alleles but it is possible that in addition to the gene flow and random drift the present day polymorphism of the *ORM1* locus may significantly influenced by yet an unknown selection.

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