Jpn. J. Human Genet. **25**, 1–8, 1980

GENETIC POLYMORPHISM OF SERUM PROTEINS AND LEVELS OF IMMUNOGLOBULIN AND COMPLEMENT COMPONENTS IN HIGH CASTE COMMUNITY (BRAHMINS) OF MADHYA PRADESH, INDIA

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Summary Quantitation of C_3 and C_3 proactivator in a healthy Indian population shows lower levels than in European populations, whereas the levels of all immunoglobulins except IgA are elevated. It is suggested that for the Indian subcontinent the normal IgE level should be taken as 400–500 iu/ml, a figure considerably higher than that in Europe. To assess the possibility of a genetic element in the regional serum protein variation, the association of the immunoglobulin and complement levels with several serum polymorphic characters is examined.

INTRODUCTION

While the last few decades have brought considerable increase in knowledge of the genetic variation that occurs among populations in India, particularly in relation to the blood groups and isoenzyme types, the distribution of genetic variants of the serum proteins is less well known. There is also very little evidence on the extent of quantitative variation in these proteins, so that the normal levels say of complement and its components or of immunoglobulins are not yet established; yet such data are important on account of the role of variation in these variables in the diagnosis of a number of disorders.

MATERIAL AND METHODS

Blood specimens were obtained from 100 Chattisghari Brahmin male students and staff members at various colleges in Raipur, Madhya Pradesh. The ages of all subjects lay within the range 16–32 years. From each, 2 ml of blood was obtained by venipuncture into vials containing EDTA. After centrifugation the plasma was separated, and the specimens were transported personally in dry ice to the Department of Human Genetics, Newcastle upon Tyne.

Received December 13, 1979

On arrival, the specimens were stored at −80°C. They were first analysed for quantitative and phenotype variation of the C_3 component of complement. The C_3 phenotypes were characterised electrophoretically by the method of Alper and Propp (1968) with minor laboratory modification. Alpha-1-antitrypsin(Pi) was examined by starch gel electrophoresis, using the conditions described by Cook (1975). Group specific components (Gc) were examined by agarose electrophoresis followed by immunofixation by specific human anti-Gc as described by Alper and Johnson (1969). Haptoglobins were studied in horizontal starch gel electrophoresis, using the discontinuous buffer system of Poulik (1957). Pseudocholinesterase (E_1 locus) was studied by standard techniques of dibucaine and RO_2–2038 inhibition by the method given by Morrow and Motulsky (1965).

For the continuous variables, quantitation of the C_3 component of complement and the C_3 proactivator was performed by radialimmunodiffusion techniques, with standard M partigen plates provided by Behringwerke. C_3 was estimated as C_3^c (β_1A globulin) and C_3-PA was determined in plates with antisera against the C_3 activator. Immunoglobulins G and A were measured by immunodiffusion on LC partigen plates, and immunoglobulin M on tri-partigen plates supplied by Behringwerke; stabilised standard human serum already prediluted was used to form the respective calibration curves. Serum immunoglobulin E was measured by the Phadebas radioimmunosorbent test standard in this laboratory as described by Al-Agidi *et al.* (1977).

RESULTS

Polymorphisms

The distribution of the Hp,Gc,C_3,E_1 and Pi phenotypes and their gene frequencies in the Chattisghari Brahmins are shown in Table 1. In each system observed phenotype frequencies are very similar to those expected from the gene frequency in a population, in Hardy Weinberg equilibrium. In 3 of the 100 subjects no haptoglobin could be detected, and were omitted in the gene frequency calculations. Haptoglobin gene frequencies in India show a south-north gradient, Hp^1 increasing with latitude. In the present sample the Hp^1 gene frequency is very similar to that expected for the latitude, though at 17% it is rather lower than in the urban Hindu sample from Madhya Pradesh (Roberts *et al.*, 1974), and significantly higher than in the Bhil tribal population from the same state (Papiha *et al.*, 1978). Though in India there is a considerable range of gene frequencies of the Gc polymorphism, particularly in some tribal populations, in most the gene frequency falls between 20 and 35% and the frequency in the present sample of 27% falls at the same general level (Chopra, 1970; Goedde *et al.*, 1972; Walter *et al.*, 1972).

There is only a single study of C_3 polymorphism from the subcontinent which showed a very low incidence of $C'3^F$ gene in North Indians. In the present sample the fast allele $C'3^F$ has a frequency of 7%, similar to that observed in other Indian

Table 1. Distribution of serum group phenotypes and gene frequencies in Chattisghari Brahmins.

		Observed number	Observed frequency	Expected number	Gene frequencies
Hp	1	3	0.031	2.9	
	2-1	28	0.289	28.1	$Hp^1=0.175$
	2	66	0.680	66.0	$Hp^2=0.825$
		97		97.0	
Gc	1-1	40	0.533	40.3	
	2-1	30	0.400	29.4	$Gc^1=0.733$
	2-2	5	0.067	5.3	$Gc^2=0.267$
C_3	FF	0	0.000	0.5	
	FS	13	0.143	12.0	$C_3{}^F=0.071$
	SS	78	0.857	78.5	$C_3{}^S=0.929$
		91		91.0	
E_1	UU	90	0.989	90.1	
	UA	1	0.011	0.9	$E_1{}^u=0.995$
	AA	0	0.000	0.0	$E_1{}^A=0.005$
		91		91.0	
Pi	MM	96	0.960	98.0	$Pi^M=0.990$
	M_WK	2	0.020		
	MS	1	0.010	1.0	$Pi^S=0.005$
	MX	1	0.010	1.0	$Pi^X=0.005$
		100			

urban population samples studied in this laboratory but much higher than the nearby tribal and the North Indian populations (Papiha *et al.*, 1979; Sahai *et al.*, 1978). This is considerably lower than the frequeny of 17–23% usual in European populations (MacDonald, 1975; Hobart and Lachmann, 1976) and in south-west Asia among Iranians and Afghans (Farhud and Walter, 1973; Agarwal *et al.*, 1976). For α^1 antitrypsin the preponderance of the PiM gene (99%) is as expected from the earlier studies in India of Kellerman and Walter (1970). Altogether there are reported in the literature results on 1,583 individuals from regional populations of identified locality in India and 383 from unspecified Indians. In these, totals of 15 MX, 6 MS, 3 FM, 1 LM, and 18 M-weak and MZ variants are found (Cook, 1975; Mukherjee *et al.*, 1974). All of these variants except 3 MX were found in the northeastern state of Bengal, while the remaining 3 MX variants were found in Indians in England for whom the area of origin in India was unknown. The present sample establishes for the first time the existence of the Pi^X and Pi^S genes in a region of India other than Bengal. For pseudocholinesterase types, there is only one

Table 2. Concentration of complement components C_3 and C_3-PA (mg%) in various populations.

	Brahmin	Lambada tribe	Afghan	Ethiopian	Mari	German	Spanish	English
C_3No. tested	89	55	644	145	136	286	310	50
Mean	45.4	51.6	72.6	110.2	110.2	75.9	94.3	92.8
SD	17.0	12.7	23.9	26.0	26.0	22.7	33.1	
C_3-PA (raw data)								
No. tested	88	55	355	—	—	—	—	—
Mean	15.0	19.7	26.2	—	—	—	—	—
SD	5.7	4.8	2.7	—	—	—	—	—
C_3-PA (log)								
No. tested	88	55						
Mean	1.141	1.294						
SD	0.181	0.086						

individual showing heterozygosity, giving a gene frequency of $E_1^A = 0.5\%$, rather lower than the frequency reported for Punjabis (Singh *et al.*, 1971), but similar to that in West Pakistan reported by Neuman and Walter (1968).

Quantitative

In the analysis of the quantitative results, the form of the distribution of each continuous variable was first examined. Skewness and the Kolmogarov-Smirnov test showed significant departures from a normal distribution of IgE and C_3-PA and to these variables logarithmic transformations were applied for statistical testing. For the remaining variables (IgG, IgA, IgM and C_3^C) the form of the distribution could be regarded as normal.

The serum level of the C_3 component of complement in the Chattisghari Brahmin is compared in Table 2 with that in samples of other populations. A major problem of C_3 quantitation is its rapidity of breakdown with ageing of the specimen, and results from a number of existing studies are difficult to accept on account of the methods employed and internal inconsistencies. In the present sample from a healthy Brahmin community the mean C_3 level at 45.4 ± 2.4 mg/100 ml is significantly lower than in the tribal population of Lambada from Hyderabad ($t = 2.42$, $p < 0.02$; Papiha *et al.*, 1979) studied in the same laboratory using the same single radioimmunodiffusion technique in commercially-available plates from the same source. It is also significantly lower than that reported in West Asian tribes of Afghanistan (Agarwal *et al.*, 1976) in whom the mean occupies an intermediate level between the present Indian samples and those of Western Europe as exemplified by a sample of Germans (Agarwal *et al.*, 1972). The same authors using electroimmunoassay

Table 3. Immunoglobulin profile of Chattisghari Brahmins.

Immunoglobulin	Number tested	Range (mg%)	Mean	SD	SE
IgG	93	730–2,290	1,405	271	28
IgM	92	44–490	186	86	9
IgA	92	36–406	166	70	7
IgE	92	10–4,700 (iu/ml)	614	731	76
Log IgE	92	1–3.672	2.467	0.657	0.068

reported still higher values in healthy Spanish, Ethiopian and Mari populations (Agarwal *et al.*, 1974). The present low means in the Indian samples are not due to technique, because a sample of 50 English individuals studied at the same time, on specimens of the same age, showed mean values of 92.8 mg/100 ml, which agree well with reported European figures.

The concentrations of C_3 proactivator in the Brahmins is within the range reported for populations of healthy adults, though the amount of comparative material is much more limited. The Brahmin mean log concentration is significantly lower ($t=5.83$, $p<0.001$) than in the Lambada tribe, and appears to be lower than the Afghan sample (though direct testing is not possible against the latter).

The results relating to serum immunoglobulin level in Chattisghari Brahmins are summarised in Table 3. The mean IgG level at $1{,}405 \pm .271$ mg% falls in the range of means reported for other Indian populations (1,200–1,675 mg%, Sehgal *et al.*, 1970; Samuel *et al.*, 1970; Gupta *et al.*, 1972; Gupta *et al.*, 1975) but appears slightly elevated over those in south west Asis, in populations of Afghanistan and Iraq (Agarwal *et al.*, 1970; Al-Agidi *et al.*, 1977) and in western Europe (Hitzig, 1957; Stiehm and Fudenberg, 1966; Hobbs, 1972). The Brahmin mean level shows some similarity to samples from Tanzania, New Guinea and Venezuela (Bennett *et al.*, 1970; Roberts *et al.*, 1979; Crane *et al.*, 1971; Wells, 1968; Arends and Gallango, 1966). The IgM mean value in Brahmins is similar to other Indian and West Asian samples, and appreciably higher than in European samples (Jarnum *et al.*, 1968; Hobbs, 1972) but certainly does not attain the high values that are features of Tanzanian and New Guinea populations. The IgA mean is lower than in the majority of Indian and other populations reported, but resembles Iraq levels (Al-Agidi *et al.*, 1977). No matter whether the raw (mean 614) or logarithmically transformed (mean 2.467) data are considered, IgE levels are conspicuously elevated. They depart significantly from those in European populations and in Iraq, but are similar to those of a Tanzanian (Sukuma) sample (649 iu/ml) (Roberts *et al.*, 1979). Similar, indeed enhanced, elevation to a mean of 1,050 was also observed in 35 normal individuals used as a control in a study of bronchial asthmatic patients in India (Kulpati *et al.*, 1976). However, these high means are partly attributable

to the presence of three individuals in Kulpati's sample and four in the present sample with IgE levels greater than 2,000 iu/ml. There was no asthma, or indeed any other obvious clinical condition in these individuals which might be responsible. If they are excluded, however, then the means and SDs for the Kulpati's and Brahmin series become 445 ± 443 and 491 ± 410, respectively, and for the latter on a log scale 2.420 SD 0.631. From this it appears that a mean IgE level lying between 400 and 500 iu/ml should be taken as a normal standard for Indian populations, a figure still considerably higher than any European population.

Quantitative and qualitative associations

Possible associations between variation in the quantitative variables and the phenotypes of the haptoglobin and C'3 component of complement were sought. Analysis of variance was carried out between and within phenotypes for the levels of the immunoglobulins and the complement components. No significant association was observed with the C'3 phenotype. However, in the haptoglobin phenotype there was a suggested association with the log C'3 proactivator. The means in phenotypes Hp 1-1, 2-1 and 2-2 were respectively 0.931 ± 0.125, 1.073 ± 0.045, and 1.175 ± 0.020 ($F=4.25$, $p=0.018$). No significant variation with haptoglobin emerged in any of the immunoglobulins or in the C'3 component. To find one significant association out of 20 tested may be a random occurrence, and certainly the biological significance of the association is not clear.

DISCUSSION

The Brahmin sample shows two curious features. First, in this apparently normal Indian population the C_3 complement level is lower than in European healthy populations, and this does not seem to be due to technical error. Secondly, the mean levels of all immunoglobulins except A are elevated by comparison with western populations. Both these features are directly related to the pathogenesis of many diseases and the immune response to them. The tropical environment exposes the subjects to a variety of infectious diseases and to infestations by different types of parasite. It is reasonable to attribute the high immunoglobulin levels to frequently repeated or continual exposure to such stimulation, for the antigen persistence would maintain synthesis, and hence circulating titres, of antibodies at an elevated level. From this combination of antibody-antigen, soluble complexes may result; these may be present in some individuals without any ill effects. But such soluble complexes may lead to hypocomplementaemia of the initial components of the complement system (C_1, C_4, C_3) due to their constant involvement in the neutralisation of these complexes. The quantitative study of other components of complement in these populations would be informative in this regard.

Acknowledgments For the generous help in the collection and transport of blood specimens, we are greatly indebted to Professor S.G. Tandon, University of Raipur, and also to Professor D.F. Roberts, Head of the Department of Human Genetics, University of Newcastle upon Tyne, for his continual support and critical reading of this manuscript.

Our thanks also to Roche Products Limited, Hertfordshire, for providing gift sample of RO2-2038 Inhibitor, and to Mrs. N. Khetan for her technical assistance.

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