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THE DISTRIBUTION OF TRANSFERRIN, GROUP-SPECIFIC COMPONENT AND PHOSPHOGLUCOMUTASE-1 SUBTYPES AMONG THE LEPCHAS OF DARJEELING, EASTERN INDIA

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Summary The distribution of serum transferrin (Tf), group-specific component (Gc), and red cell phosphoglucomutase-1 (PGM1) subtypes has been studied by polyacrylamide gel and starch-gel electrophoresis followed by isoelectric focusing in a group of 213 Lepchas in the Darjeeling district, West Bengal, India. The frequencies of Tf^{C1}, Tf^{C2}, Tf^{B} and Tf^{DChi} were found to be 0.804, 0.182, 0.014 and 0.00, respectively, among the Buddhists and 0.741, 0.244, 0.007 and 0.007, respectively, in the Christian Lepchas. The frequencies of Gc alleles were as follows: Gc^{1F} 0.587 and 0.539; Gc^{1S} 0.203 and 0.242; Gc^{2} 0.210 and 0.215 in these two groups, respectively. The allelic frequencies of PGM1 were found to be 0.706 and 0.714 for PGM^{1+}; 0.164 and 0.086 for PGM^{1-}; 0.116 and 0.175 for PGM^{2-} in Buddhists and Christians, respectively. No rare allele of Tf and PGM1 had been detected but one example of a variant from of Gc^{2} allele has been observed among the Christians.

INTRODUCTION

The introduction of isoelectric focusing (IEF) in the study of blood genetic markers enables a more refined and detailed investigation of the polymorphic system to be carried out than earlier studies by either starch-gel or polyacrylamide-gel electrophoresis. The most-studied blood genetic markers by IEF are serum transferrin (Tf), group-specific component (Gc) and red cell phosphoglucomutase-locus 1 (PGM1) (Kühnl and Spielman, 1978, 1979; Cleve *et al.*, 1978; Constans and Viau, 1977; Constans and Cleve, 1979; Constans *et al.*, 1979; Bark *et al.*, 1976). Very limited data

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are available on these three polymorphisms in the populations of Asia, especially in the Indian regions. Earlier, we had reported results of limited studies on Tf and PGM polymorphisms (IEF) in several population groups of the Indian subcontinent (Saha and Tan, 1983; Saha, 1983, 1985). We present here the results of a study on the distribution of Tf, Gc, and PGM1 subtypes among a group of Lepchas in the Darjeeling district of West Bengal, Eastern India. The Lepchas are the original inhabitants of the Sikkim State at the foothills of the Himalayas and are quite distinct from the Nepalese, Bhutanese or Tibetans. They are inhabitants of high altitude. A detailed genetic study of the Lepchas with a description of their ethnological background has been presented elsewhere (Saha *et al.*, 1987).

There have been very limited studies on the distribution of blood groups amongst the Lepchas in the past (Bhattacharjee, 1968; Miki *et al.*, 1960a–c). No study had been carried out in this population on serum protein and red cell enzyme polymorphisms excepting our recent study on the Lepchas (Saha *et al.*, 1987). In this paper we report on the distribution of serum Tf, Gc and red cell PGM1 subtypes in a group of Lepchas practising Buddhism or Christianity.

MATERIALS AND METHODS

The material of study comprised 213 Lepchas (73 Buddhists and 140 Christians of both sexes) living in the district of Darjeeling, West Bengal, India at the foothills of the Himalayas (altitude, 4,000 ft). Blood samples were collected by the finger-prick method described earlier (Saha and Kirk, 1973) into heparinized capillary tubes and onto Whatman 3 MM filter paper strips. The capillary tubes and dried filter paper strips were placed in insulated containers in wet ice and brought to the Indian Statistical Institute, Calcutta. The plasma was separated by centrifuging the capillary tubes at low speed. Both the capillary tubes and filter paper strips were transported to our laboratory in Singapore, at wet-ice temperature.

Transferrin subtypes were determined by isoelectric focusing (IEF) using thin layer polyacrylamide gels (PAG) with LKB Ampholine of pH 3.5 to 10 according to the method of Kühnl and Speilman, (1978, 1979). The serum samples were treated with neuraminidase before isoelectric focusing (Beckman *et al.*, 1980). All the samples with rare variants like Tf^B and Tf^D were re-run on thin layer PAG of pH 5–7 (LKB) for further clarification. The gels were stained with Coomassie Blue. The Gc subtypes were studied by PAG and IEF. The gels contained 2 per cent Pharmalyte of pH 4.0 to 6.5 (Pharmacia). The isoelectric focusing was carried out after pre-run. Filter papers soaked with undiluted serum samples were used. After focusing the gels were fixed in 3 per cent sulfosalicylic acid solution in an ethanol–water mixture (1 : 2). After a few minutes the Gc phenotypes were read against a black background illuminated with fluorescent lamps from the side. After the reading the gels were stained with Coomassie Blue for record. Some of the samples were confirmed by immunofixation with antihuman Gc (Dako). In no case was the result different from that by the sulfosalicylic acid precipitation method.

The red cell PGM1 types were determined by starch-gel electrophoresis (Spencer *et al.*, 1964) and isoelectric focusing on thin layer PAG-Ampholine gels of pH 5–7 (LKB) (Bark *et al.*, 1976; Kühnl *et al.*, 1977). Inserts were cut in size of 1 cm × 5 mm from blood-soaked dried filter paper (3 MM), soaked briefly in distilled water and applied directly on starch-gel and IEF gel, respectively, for electrophoresis and isoelectric focusing. After the run, the gels were stained by substrate mixture described by Spencer *et al.* (1964).

RESULTS AND DISCUSSION

Table 1 shows the observed and expected frequencies of different phenotypes and genes of the transferrin alleles among the Lepchas according to their religion. The phenotypic distribution was at equilibrium in both the groups of Lepchas. The frequencies of Tf^{C1}, Tf^{C2} and Tf^{B} amongst the Buddhists were 0.804, 0.182 and 0.013, while the Christians had slightly different frequencies of these alleles (0.741, 0.244 and 0.007) in addition to Tf^{DChi} (0.007). A similar frequency of Tf^{C2} has been reported in other Mongoloid populations like Japanese (Beckman *et al.*, 1980; Kamboh and Kirk, 1983b) and Chinese (Tan *et al.*, 1982; Kamboh and Kirk, 1983b; Saha, 1985, 1987). The frequencies of Tf^{B} and Tf^{DChi} in the present Lepcha population are also similar to those in the Chinese, Japanese, Nepalese and Bhutanese (Beckman *et al.*, 1980; Kamboh and Kirk, 1983b; Tan *et al.*, 1982; Sunderland *et al.*, 1979; Mourant *et al.*, 1968; Glassgow *et al.*, 1968). The frequencies of *Tf* alleles in the present Lepcha population is not very different from those reported in diverse groups of Indian populations (Saha, 1987; Saha and Tan, 1983; Kamboh and Kirk, 1983b; Reddy *et al.*, 1984; Walter *et al.*, 1981, 1983).

Table 1. Distribution of transferrin subtypes among the Lepcha.

Phenotypes	Buddhists		Christians		All	
	No. obs.	No. exp.	No. obs.	No. exp.	No. obs.	No. exp.
C1-1	49	47.8	75	74.1	124	121.7
C2-1	19	21.7	46	48.8	65	70.8
C2-2	4	2.5	10	8.0	14	10.3
C1-B	2	1.7	2	1.4	4	3.2
C1-D	0	0.0	2	1.4	2	1.6
Total	74	73.7	135	133.7	209	207.6
Gene frequencies						
Tf^{C1}	0.804		0.741		0.763	
Tf^{C2}	0.182		0.244		0.222	
Tf^{B}	0.014		0.007		0.010	
Tf^{DChi}	0.0		0.007		0.005	

Table 2 shows the distribution of observed and expected frequencies of different phenotypes and genes of the Gc alleles in the Lepchas according to their religion. The phenotypic distribution was at equilibrium in both the groups of Lepchas. The frequencies of Gc^{1F}, Gc^{1S} and Gc^{2} have been found to be 0.587, 0.203 and 0.210 among the Buddhists, while those in Christians were 0.539, 0.242 and 0.215. A solitary example of a rare phenotype designated 2'-1F has been encountered in the latter group. It was not possible to identify this new allele due to lack of reference sample. The Japanese and Chinese had a lower frequency of Gc^{1F} and a higher frequency of Gc^{2} (Ishimoto *et al.*, 1979; Matsumoto *et al.*, 1980; Kim and Lewis, 1981; Saha, 1985). However, the Indian population groups have a very low frequency of Gc^{1F} in contrast to the Mongoloid populations (Papiha *et al.*, 1981, 1982; Karlsson *et al.*, 1983; Walter *et al.*, 1984). The Tibetans have been reported to have a lower frequency of Gc^{1F} (0.364) and higher frequencies of Gc^{1S} and Gc^{2} than either Indian populations or other Mongoloid populations (Omoto and Miyake, 1978; Constans *et al.*, 1979; Matsumoto *et al.*, 1980; Kim and Lewis, 1981; Kamboh *et al.*, 1984b; Saha, 1985). The frequency of Gc_1^{F} in the present Lepcha populations is intermediate between the Mongoloid populations (Chinese and Japanese) and Indians. From the above it appears that the subtyping of Gc alleles might prove to be a better discriminatory marker in the study of the ethnogenetics rather than Gc typing by PAG and starch-gel electrophoresis.

Table 2. Distribution of group-specific component subtypes among the Lepcha.

Phenotypes	Buddhists		Christians		All	
	No. obs.	No. exp.	No. obs.	No. exp.	No. obs.	No. exp.
1F	24	23.8	40	37.6	64	61.3
1F-1S	13	16.4	36	33.9	49	50.6
1S	6	2.8 [a]	6	7.6	12	10.4
2-1F	20	17.0	23	30.1	43	47.3
2-1S	3	5.9	15	13.5	18	19.5
2	3	3.1	9	6.0	12	9.1
2'-1F [b]	0	0.0	1	0.6	1	0.7
Total	69	69.0	130	129.3	199	198.8
Gene frequencies						
Gc^{1F}	0.587		0.538		0.555	
Gc^{1S}	0.203		0.242		0.229	
Gc^{2}	0.210		0.215		0.214	
$Gc^{2'}$	0.0		0.004		0.003	

[a] $\chi_1^2=3.52$. [b] See text.

Table 3 shows the phenotypic and genotypic distribution of the red cell phosphoglucomutase (locus 1) in the Lepchas according to their religion. The phenotypic distribution was at equilibrium in both the groups of Lepchas. The frequencies of PGM^{1+}, PGM^{1-}, PGM^{2+}, and PGM^{2-} were found to be 0.706, 0.164, 0.116, and 0.014, respectively, in the Buddhists and 0.714, 0.086, 0.175, and 0.025, respectively, in the Christian group. No other rare allele at the PGM1 locus was detected among the Lepchas. Similar frequencies of PGM1 alleles have been reported in the Japanese (Maneyama *et al.*, 1978; Nishigaki *et al.*, 1982; Kamboh and Kirk, 1983a, 1984a). However, the Thais and Chinese have been reported to have lower frequencies of PGM^{1+} and higher frequencies of PGM^{2-} (Kamboh and Kirk, 1984a; Saha, 1983, 1985). One example of PGM 6-1 has been detected in the Lepchas by starch-gel electrophoresis (Saha *et al.*, 1987). This sample could not be tested by IEF as the sample had been exhausted. Indians in general have been reported to have slightly lower frequency of PGM^{1+} (Papiha *et al.*, 1981, 1982; Saha, 1983; Kamboh and Kirk, 1984a).

Minor differences in the frequencies of some alleles at the Tf, Gc and PGM1 loci have been observed between these two groups. The Buddhists had a higher

Table 3. Distribution of phosphoglucomutase (locus 1) subtypes among the Lepcha.

Phenotypes	Buddhists		Christians		All	
	No. obs.	No. exp.	No. obs.	No. exp.	No. obs.	No. exp.
1^+	35	36.3	73	71.4	108	107.7
1^+1^-	17	16.9	19	17.2	36	34.2
1^-	2	2.0	1	1.0	3	2.7
1^+2^+	14	12.0	33	35.0	47	46.9
1^-2^+	3	2.8	3	4.2	6	7.5
1^+2^-	2	1.4	2	5.0	4	6.4
1^-2^-	0	0.3	0	0.6	0	1.0
2^+	0	1.0	6	4.3	6	5.1
2^+2^-	0	0.2	1	1.2	1	1.4
2^-	0	0.0	2	0.1 [a]	2	0.1
Total	73	72.9	140	140.0	213	213.0
Gene frequencies						
PGM^{1+}	0.705		0.714		0.711	
PGM^{1-}	0.164		0.086		0.113	
PGM^{2+}	0.116		0.175		0.155	
PGM^{2-}	0.014		0.025		0.021	

[a] $\chi_1^2=40.53$ (due to small expected number).

frequency of Tf^{C1} (0.804), Tf^{B} (0.01), Gc^{1F} (0.587), PGM^{1-} (0.164) and lower frequency of PGM^{2+} compared to those in the Christian Lepchas. Tf^{DChi} was present among the Christians (0.01) but not in the Buddhists. There appear to be more foreign gene pools among the Christians which is also supported by earlier observation of increased heterozygosity in the same group (Saha *et al.*, 1987).

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