Hum. Hered. 37: 113–121 (1987)

0001–5652/87/0372–0113 $2.75/0

A Genetic Study among the Lepchas of the Darjeeling Area of Eastern India

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Key Words. Lepchas · Polymorphism · Himalaya · Mongoloids · Population genetics · Adaptation · Medium altitude

Abstract. A total of 215 Lepchas (75 Buddhists and 140 Christians) living in the Kalimpong subdivision, Darjeeling district, West Bengal, India, were investigated for the distribution of haemoglobin, serum proteins and red cell enzymes. The gene frequencies were as follows: $Hb^E = 0.02$; $Hp^1 = 0.18$; $Tf^B = 0.007$; $Tf^{DChi} = 0.005$; $Gc^2 = 0.22$; $p^a = 0.18$; $p^c = 0.03$; $PGM_1^2 = 0.18$; $PGM_1^+ = 0.002$; $PGD^c = 0.17$; $AK^2 = 0.02$; $GLO^1 = 0.21$. The most striking features were the complete lack of G6PD deficiency and very high frequency of PGD^C. The remaining loci (serum albumin, lactate dehydrogenase, malate dehydrogenase and glucose-6-phosphate dehydrogenase, phosphohexose isomerase and superoxide dismutase) were monomorphic. The gene frequencies were similar in the Buddhist and Christian Lepchas. The observed average heterozygosity (9 loci) was 0.20 in the entire sample.

Introduction

The populations of the sub-Himalayan regions in the northern frontier of India are examples of Indian populations with a high degree of Mongoloid features. However, they inhabit a large extent of areas bordering the north of India from the east to the west, as, e.g., the Darjeeling area in the east and Nepal in the midwest with Bhutan, Sikkim and Tibet intervening. These pockets of the populated areas are not continuous, rather they are considerably isolated from each other. However, they are in continuity with the different regions at their southern borders. It is expected that there have been varying degrees of admixture between these populations surrounding them. So it is expected that these Mongoloid populations in different geographically isolated areas may be genetically heterogeneous. The Lepchas of the sub-Himalayan region are believed to be original inhabitants of Sikkim and

Table I. Distribution of haemoglobin, serum protein and cell enzyme phenotypes among the Lepchas

Phenotypes	Buddhists		Christians		All		χ^2
	n observed	n expected	n observed	n expected	n observed	n expected	
Haemoglobin (Hb)							
A	72	72.03	134	134.07	206	206.11	
AE	3	2.94	6	5.86	9	8.78	
EE	0	0.03	0	0.06	0	0.09	
Total	75	75.00	140	139.99	215	214.98	0.10
Serum proteins							
Haptoglobin (Hp)							
1-1	1	2.08	3	4.59	4	6.66	
2-1	23	20.82	43	40.80	66	61.66	
2-1M	0	0	1		1		
2-2	51	52.08	89	90.60	140	142.67	
0	0	0	4	4	4	4	
Total	75	74.98	140	139.99	215	214.99	0.76
Transferrin (Tf)							
C	74	74.00	136	136.02	210	210.04	
CB	1	1.00	2	1.96	3	2.98	
CD	0	0	2	1.96	2	1.97	
Total	75	75	140	139.94	215	214.99	0.00
Group-specific component (Gc)							
1-1	46	46.24	83	81.42	129	127.63	
2-1	25	24.51	46	49.16	71	73.72	
2-2	3	3.25	9	7.42	12	10.65	
Total	74	74.00	138	138.00	212	212	0.28
Red cell enzymes							
Acid phosphatase (AP)							
A	5	2.46	4	4.15	9	6.61	
AB	17	21.52	40	38.17	57	59.70	
B	49	47.04	89	87.85	138	134.89	
BC	3	2.40	3	7.16	6	9.60	
CC	0	0	3	0.15	3	0.16	
Total	74	73.42	139	137.48	213	210.96	52.87
6-Phosphogluconate dehydrogenase (6PGD)							
A	53	53.77	93	93.49	146	147.21	
AC	21	19.47	42	41.01	63	60.56	
CC	1	1.76	4	4.50	5	6.23	
O	0	0	1	1	1	1.00	
Total	75	75.0	140	140	215	215.00	0.35

Table I (continued)

Phenotypes	Buddhists		Christians		All		χ^2
	n observed	n expected	n observed	n expected	n observed	n expected	
Phosphoglucomutase (PGM_1)							
1-1	57	58.08	85	82.18	142	140.37	
2-1	18	15.84	42	47.24	60	63.48	
2-2	0	1.08	9	6.79	9	7.18	
6-1	0	0.00	1	0.76	1	0.83	
Total	75	75.00	137	136.97	212	211.86	0.70
Adenylate kinase (AK)							
1-1	72	72.03	136	136.02	208	208.05	
2-1	3	2.94	4	3.95	7	6.89	
2-2	0	0.03	0	0.03	0	0.06	
Total	75	75.00	140	140.00	215	215.00	
Glyoxalase I (GLO I)							
1-1	7	2.61	15	6.72	22	9.26	
2-1	14	22.78	30	46.57	44	69.47	
2-2	54	49.61	89	80.71	143	130.27	
Total	75	75.00	134	134.00	209	209.00	28.82

are now settled in the Darjeeling area at the foot of the Himalaya [Das, 1978]. They are distinct from the Nepalese or Bhutanese indigenous populations of Nepal and Bhutan, respectively. They number about 50,000 and are distributed in the Darjeeling area, Sikkim and Bhutan. Though they are distinct from the Nepalese, there had been a strong Nepalese influence during the Nepalese domination in Sikkim during the 18th century. Originally they were hunter-gatherers, practiced either Christianity or Buddhism and are now settled farmers. The language of the Lepchas belongs to the Tibeto-Burmese family named *Rong*.

Most of the studies on the blood genetic markers in these sub-Himalayan populations have been carried out, in the past, in the Nepalese [Mourant et al., 1976]. Limited studies have been carried out in Bhutan and Tibet [Glasgow et al., 1968; Mourant et al., 1968]. The populations of the eastern region have been studied least. The Lepchas of the eastern region are the typical Mongoloid population of the eastern sub-Himalayan populations and they encompass many small groups among themselves. Earlier studies of blood genetic markers among the Lepchas have been limited to blood group systems and phenylthiocarbamide taste sensitivity [Mourant

Table II. Gene frequencies of haemoglobin, serum protein and red cell enzyme polymorphisms among the Lepchas

Markers	Genes	Buddhists	Christians	All
Haemoglobin	Hb^A	0.9800	0.9787	0.9791
	Hb^E	0.0200	0.0214	0.0209
Serum Proteins				
Haptoglobin	Hp^1	0.1667	0.1838	0.1777
	Hp^2	0.8333	0.8162	0.8223
	Hp '0'	0.0000	0.0286	0.0186
Transferrin	Tf^C	0.9933	0.9857	0.9884
	Tf^B	0.0067	0.0071	0.0070
	Tf^D	0.0000	0.0071	0.0046
Group-specific component	Gc^1	0.7905	0.7681	0.7759
	Gc^2	0.2095	0.2319	0.2241
Red Cell Enzymes				
Acid Phosphatase	p^a	0.1824	0.1727	0.1761
	p^b	0.7973	0.7950	0.7958
	p^c	0.0203	0.0324	0.0282
6-Phosphogluconate dehydrogenase	PGD^A	0.8467	0.8201	0.8294
	PGD^C	0.1533	0.1799	0.1706
	PGD^O	0.0000	0.0071	0.0047
Phosphoglucomutase (locus 1)	PGM^1	0.8800	0.7745	0.8137
	PGM^2	0.1200	0.2226	0.1840
	PGM^0	0.0000	0.0036	0.0024
Adenylate kinase	AK^1	0.9800	0.9857	0.9837
	AK^2	0.0200	0.0143	0.0163
Glyoxalase I	GLO^1	0.1867	0.2239	0.2105
	GLO^2	0.8133	0.7761	0.7895

et al., 1976], and a few serum protein and red cell enzyme systems [Gupta et al., 1981].

The prevalence of malaria is very low at this high altitude as mosquitoes cannot live in the adverse environmental conditions. The adaptive mechanism for selection against malaria operating in haemoglobin and red cell G6PD polymorphisms in the planes should be lacking at high altitudes. We report in this paper a detailed study of the blood genetic markers in a group of Lepchas living in the Kalimpong (altitude 1,225 m) area of the Darjeeling district, West Bengal, India.

Materials and Methods

Samples of blood were collected from 215 Lepchas (75 Buddhists and 140 Christians) of both sexes living in villages around Kalimpong by the finger-prick method described earlier [Saha and Kirk, 1973] into heparinized capillary tubes and onto 3 MM filter paper strips (Whatman). The capillary tubes and dried filter paper strips were place in insulated containers in wet ice in the field 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 kept stored frozen at −20°C until brought to Singapore at wet-ice temperature in a thermos flask. Red cell enzyme typing was carried out using the methods outlined by Saha and Kirk [1973]. Red cell acid phosphatase (AP) was typed using fluorogenic stains and visualized under a UV lamp. Serum proteins were typed by polyacrylamide gel electrophoresis. The gene frequencies were estimated by gene counting and Hardy-Weinberg equilibrium calculated by the χ^2 test.

Results and Discussion

Table I shows the phenotypic distributions of haemoglobin, serum protein and red cell enzyme types in the Buddhist and Christian Lepchas. Christianity has been introduced among the Lepchas very recently by contact with Western missionaries, and they are more likely to have mixed with other populations. We thought it might be interesting to look into the possibility of such an admixture. The gene frequencies in the two religious groups of Lepchas are presented in table II.

Haemoglobin (Hb)

The only variant form of Hb encountered in the Lepchas was HbE. The overall frequency of Hb^E was found to be 0.02 with no difference between Buddhists and Christians. A similar low frequency of Hb^E has been reported among the Nepalese, Bhutanese and Sherpas of Tibet and upper Khumbu [Glasgow et al., 1968; Saha and Banerjee, 1973; Santachiara-Benerecetti et al., 1976]. However, a very high incidence of Hb^E has been reported among the populations of neighbouring Assam, and South-East Asia [Flatz et al., 1972; Goedde et al., 1972; Saha and Banerjee, 1973]. Gupta et al. [1981] did not identify any abnormal Hb in a series of 186 Lepchas from the same area. The Hb^E gene in the present Lepcha populations might have ben introduced from the surrounding populations.

Haptoglobin (Hp)

The frequency of Hp^1 was found to be 0.17 and 0.18 in Buddhist and Christian Lepchas, respectively. Hp^0 was present in very low frequency (0.03) in the Christians only. A slightly higher frequency of Hp^1 (0.24) has been reported among the Sherpas of Kalimpong by Gupta et al. [1981]. The Nepalese have been reported to have

a higher frequency of Hp^1 (0.27) by Sunderland et al. [1979]. A similar frequency of Hp^1 has been reported in Bhutan (0.21) by Glasgow et al. [1968], and also in Bengali Muslims [Saha, in press]. Still lower values of Hp^1 have been reported among the Tibetans [Mourant et al., 1976]. A slightly higher frequency of Hp^1 has been reported in Assamese (0.24) and Khasis (0.21) by Goedde et al. [1972]. One example of a modified form of HP2-1 was detected in Lepcha Christians.

Tranferrins (Tf)

A very low frequency of Tf^B and Tf^D was observed in the present series, which is consistent with the results of Gupta el al. [1981]. Similar low frequencies of Tf^B and Tf^D have been found in populations of India, Sri Lanka and Southeast Asia [Saha, in press, unpubl. data].

Group-Specific Components (Gc)

The frequencies of Gc^1 and Gc^2 amongst the Lepchas were found to be 0.79 and 0.21 for Buddhists, and 0.77 and 0.23 for Christians, respectively. Similar frequencies of *Gc* alleles have been reported in Tibetans and Nepalese by Constans et al. [1985]. The Indian populations including Assam have been reported to have a much higher frequency of Gc^2 [Goedde et al., 1972; Kirk et al., 1963].

Glucose-6-Phosphate Dehydrogenase (Gd)

There was no G6PD-deficient subject encountered in the present series of 215 Lepchas (99 males and 116 females). All the subjects had the common Gd^B phenotype. Red cell G6PD deficiency is widespread among the populations of Assam [Flatz et al, 1972], West Bengal and other populations of India [Saha and Banerjee, 1971; Flatz et al, 1972] and those in the South-East Asian regions.The absence of G6PD deficiency in the Lepchas is not surprising as the malarial parasites are not able to breed at this altitude. No electrophoretic variant of G6PD has been encountered in the present series. In an earlier study among the Bengalis we did not detect any electrophoretic variant, though G6PD deficiency was present [Ghosh et al, 1981]. However, the Angami Nagas living at similar high altitude in North-Eastern India have been reported to have a very high frequency of G6PD deficiency in males (27.06%) and females (15.35%) by Seth and Seth [1971].

6-Phosphogluconate Dehydrogenase (PGD)

The frequency of PGD^C was found to be 0.15 and 0.18 in Buddhist and Christian Lepchas, respectively. A high frequency of PGD^C (0.16) has also been observed in Nepalese by Mourant et al. [1976] and in Bhutanese (0.23) by Glasgow et al. [1968]. However, the neighbouring Khasis, Assamese and Bengalis, and the Mongoloid populations of South East Asia do not have high frequencies of PGD^C [Goedde et al., 1972; Das et al., 1970; Saha, in press; Bhasin et al., 1981]. A high frequency of PGD^C has been reported in New Guinea highlanders, Ethiopians and Bhutanese [Mourant et al., 1976; Glasgow et al. 1968]. Another variant allele, PGD^{Kadar} has been observed in polymorphic frequency (0.04) among the Kadar tribe living in Annamalai Hills in Southern India, which frequency might be due to adaptation [Saha et al., 1974]. They also had no G6PD deficiency.

Acid Phosphatase (p)

The frequencies of p^a, p^b and p^c were found to be 0.18, 0.80 and 0.02 in Buddhists, and 0.17, 0.80 and 0.03 in Christian Lepchas, respectively. Similar frequencies of the acid phosphatase alleles have been reported in different populations of Nepal [Sunderland et al., 1979] and Bhutan [Glasgow et al., 1968]. A slightly higher frequency of p^a has been reported in Indo-Nepalese tribes [Mourant et al., 1968], Tamang and Sherpa [Bangham and Howarth, 1980], Assamese [Goedde et al., 1972] and Bengalis [Das et al., 1970; Saha, in press]. A low frequency of p^c has been reported among the Kiranti of Nepal, Assam, and Bengal.

Phosphoglucomutase (PGM)

The frequencies of PGM^1 and PGM^2 were found to be 0.88 and 0.12 among the Buddhist, and 0.77 and 0.22 in the Christian Lepchas, respectively. An isolated case of PGM6-1 has been observed among the Christian Lepchas. Similar frequencies of PGM alleles have been observed in Nepalese [Sunderland et al., 1979; Mourant et al., 1976] and in Bhutanese [Glasgow et al., 1968]. However, Bangham and Howarth [1980] have reported a lower frequency of PGM^1 (0.63) in Sherpas of Nepal. The Assamese and Bengalis have been found to have slightly lower PGM^1 [Goedde et al., 1972; Das et al., 1970; Saha, in press]. PGM^6 has ben reported in Bengalis by Das et al. [1970].

Adenylate Kinase and Glyoxalase (AK, GLO)

The low frequency of AK^2 (0.02) found in the present Lepcha population is in agreement with the reported low frequency of AK^2 in the populations of Nepal [Sunderland et al., 1969; Bangham and Howarth, 1980]. However, a slightly higher frequency of AK^2 has been reported in other Indian populations including the Assamese [Bhasin et al., 1982; Saha, in press].

The frequencies of GLO^1 and GLO^2 in the Lepchas were found to be 0.19 and 0.81 amongst the Buddhists, and 0.22 and 0.78 amongst the Christians. There was a *significant deviation from the Hardy-Weinberg equilibrium* ($\chi^2_3 = 28.82$) in the distribution of GLO alleles with excess of homozygosity.

Monomorphic Systems

No variation of the lactate dehydrogenase locus was observed in the Lepcha series. Lactate dehydrogenase variants (1–4%) have been observed in all the non-tribal populations of India studied. Five other loci, serum albumin, malate dehydrogenase, phosphohexose isomerase,

Table III. Average heterozygosity at 9 polymorphic loci among the Lepchas

Locus	Buddhists	Christians	All Lepchas
Hb	0.0400	0.0448	0.0419
Hp	0.3067	0.3235	0.3175
Tf	0.0133	0.0286	0.0233
Gc	0.3378	0.3333	0.3349
p	0.2703	0.3094	0.2958
PGD	0.2800	0.3022	0.2944
PGM^1	0.2400	0.3139	0.2877
AK	0.0400	0.0286	0.0326
GLO	0.1867	0.2239	0.2105
Average	0.1905	0.2120	0.2043

phosphoglucomutase (locus 2) and superoxide dismutase were monomorphic.

Average Heterozygosity

The average heterozygosity at 9 polymorphic loci amongst the Lepchas was found to be 0.19 in Buddhists and 0.21 in Christians (table III).

Conclusion

In conclusion it may be said that the Lepchas are genetically related to other Mongoloid populations with low Hb^E, Tf^{DChi}, Gc^2 and p^a. The gene frequencies of the polymorphic loci studied were found to be similar in both the groups excepting a higher PGM^2 among the Christians ($p < 0.05$). This study shows that these two groups are genetically similar. The Lepchas appear to be genetically adapted to high altitude by virtue of their having an absence of red cell G6PD deficiency and high PGD^C.

Acknowledgements

This project was supported by generous grants from National University of Singapore, the Singapore Turf Club and Shaw Foundation. The authors would like to thank Miss Fannie Chan Wai Fen for excellent secretarial help, and Mdm Ho Chiu Han, Miss Jumiah Bte Basair and Miss Esther Chang for technical assistance.

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