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A Study of Erythrocyte Enzyme Polymorphisms Among People of Kulu District, Himachal Pradesh, India

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ABSTRACT To help complete the genetic map of north Indian hill state of Himachal Pradesh, data on various polymorphic red cell enzyme systems are presented among people inhabiting its central district of Kulu. Blood samples from three main endogamous caste populations of the area viz., the Brahmin (n=114), Rajput (n=234) and Koli (n=101) were collected randomly from unrelated subjects. Haemolysates were typed for a battery of seven erythrocyte enzyme polymorphisms viz., ADA, AK1, ESD, PGM1, ACP1, GLO1 and GPI using standard electrophoretic techniques. Results showed that there were appreciable differences among the present castes in the distribution of the first three polymorphic enzymes (ADA, AK1, ESD) while no significant heterogeneity was discernible in the next three (PGM1, ACP1, GLO1); some GPI variants have been reported for the first time from the state. The Rajput as well as the Koli groups studied here from Kulu district showed similarities with their respective counterparts inhabiting the neighbouring district of Kinnaur. This suggests possible gene flow between these two contiguous districts of Himachal Pradesh.

INTRODUCTION

People of north Indian state of Himachal Pradesh have been the subject of serological anthropological research for several decades now (Bhasin et al., 1992; 1994, Bhasin and Walter, 2001: Singh et al., 1994) but biochemical genetics investigations focusing on the distribution of various serum protein and erythrocyte enzyme polymorphisms among them began only in early eighties of the last century (Singh et al., 1982 ; Papiha et al., 1982; Chahal et al., 1982; Bhasin et al., 1983). Besides these, some other such studies on remote populations (particularly tribals) inhabiting higher mountainous ranges in the northern districts of Kinnaur (Papiha et al., 1984) and Chamba (Chahal et al., 1991; Papiha et al., 1996) and some on caste populations of southern districts of Shimla (Sarin and Chahal, 2001) and Solan (Sarin and Chahal, 2002) have been reported. However, populations inhabiting the central district of Kulu and certain other districts of the state still remain uninvestigated except one study of Bhasin et al. (1983) on caste and tribal groups of Lahaul-Spiti and Kulu districts on blood groups, serum proteins and red cell enzyme markers. The present study aims at establishing the biochemical genetic constitution of the Brahmin, Rajput and Koli-three main endogamous caste populations of the district by investigating the distribution of as many as 7 erythrocyte enzyme polymorphisms viz., adenosine deaminase (ADA), adenylate kinase locus 1 (AK1), esterase D (ESD), phosphoglucomutase locus 1 (PGM1), acid phosphatase locus 1 (ACP1), glyoxalase locus 1 (GLO1) and glucosephosphate isomerase (GPI) in their representative samples. The study will help complete the genetic map of Himachal Pradesh.

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SUBJECTS, MATERIAL AND METHODS

To collect material (blood samples) for this study a field work was undertaken in Kulu district of Himachal Pradesh in the month of October 2003. The subjects, selected at random, were student volunteers, both boys and girls, attending various educational institutes located in rural areas of the district to ensure that only persons of local origin i.e. those born and brought up there were included. Blood samples were collected from a total of 449 not closely related caste students comprising 114 Brahmin, 234 Rajput and 101 Koli subjects attending the Government Senior Secondary Schools at Raison, Nagar, Haripur, Katrain and Kinza and Government High Schools at Shirad and Jagatsukh. From each student about 3/4 ml finger prick blood sample was drawn using sterile blood

lancet and collected in a microcentrifuge vial containing EDTA.K_2 as an anticoagulant. During collections, the samples were temporarily stored in a refrigerator (at 4-10^{O}C) in a chemist's shop in Kulu town and later personally carried to Patiala in a thermocool container stuffed with ice.

Within 24 hours of their arrival in the laboratory at Patiala blood samples were processed for preparation of haemolysates as detailed in Bhasin and Chahal (1996). Standard biochemical methods were employed for electrophoretic typings of various red cell enzymes. ADA and AK1 were electrophoresed together on the same agarose gel (1% w/v) and stained simultaneously as described by Murch et al. (1986). Similarly, ESD and PGM1 were also typed together on agarose gel and stained as detailed in Wraxall and Stolorow (1986). ACP1 was studied following the technique of Wraxall and Emes (1976), with a modification i.e., agarose replaced starch as a medium of separation. Mixed agarose/ starch gel (1% / 0.5% w/v) technique of Scott and Fowler (1982) was used for GLO1 typing. GPI types were studied using the buffer system of the original report (Detter et al., 1968), but instead of starch electrophoresis was performed in agarose gel and staining was done by the modified method of Papiha and Chahal (1984).

RESULTS AND DISCUSSION

The findings of the present biochemical genetic investigation are presented in Tables 1 and 2; the former lists phenotype data of the seven red cell enzyme systems studied and the latter corresponding allele frequencies. Statistical treatment of the phenotype data revealed that barring only a couple of instances i.e. ADA for the Koli ($\chi^2 = 4.358$, d.f. 1, $0.05 > p > 0.025$) and ESD for the Brahmin ($\chi^2 = 4.948$, d.f. 1, $0.05 > p > 0.025$) in no other system was there any significant departure from Hardy - Weinberg expectations in any of the three caste groups considered. This demonstrated that the Brahmin, Rajput and Koli populations inhabiting the central district of Kulu in Himachal Pradesh are in genetic equilibrium.

Adenosine Deaminase (ADA) Polymorphism: All the three common electrophoretic phenotypes of red cell enzyme ADA were encountered in each of the three endogamous caste groups examined here from Kulu district (Table 1). As for allele frequencies, the second common allele of the system *ADA*2* ranged from a low of 0.0743 in the Koli to a high of 0.1711 in the Brahmin (Table 2). Inter-group comparisons in the present caste populations revealed statistically significant differences between the Brahmin on one hand and the Rajput ($\chi^2 = 6.478$, d.f. 2, $0.05 > p > 0.025$) as well as the Koli ($\chi^2 = 9.014$, d.f. 2, $0.025 > p > 0.01$), on the other.

An earlier study reported from the district, albeit based on smaller samples (Bhasin *et al*., 1983), found somewhat similar results for ADA system, except for a rather low *ADA*2* frequency (0.0490) in their Brahmin material (n=51). Ironically, the frequency of the allele observed in the present representative Brahmin sample (n=114) is in fact the highest so far reported in any population group of Himachal Pradesh. It is interesting to note that the *ADA*2* frequencies

Table 1: Phenotype numbers of various polymorphic red cell enzyme systems in different caste populations studied from Kulu district of Himachal Pradesh

Enzyme system	*Pheno-type*	*Brahmin (n=114)*	*Rajput (n=234)*	*Koli (n=101)*
ADA	1	80	192	88
	1,2	29	37	11
	2	5	5	2
	Total	114	234	101
AK1	1	90	223	77
	1,2	24	11	22
	2	-	11	1
	Total	114	234	101
ESD	1	84	133	46
	1,2	24	84	44
	2	6	17	11
	Total	114	234	101
PGM1	1	46	120	51
	1,2	54	89	40
	1,7	2	3	1
	2	12	22	9
	Total	114	234	101
ACP1	A	6	22	10
	A,B	31	81	40
	A,C	1	-	-
	B,C	6	-	1
	B	70	131	50
	Total	114	234	101
GLO1	1	2	18	9
	1,2	45	79	40
	2	66	137	52
	Total	113	234	101
GPI	1	111	231	100
	1,4	2	-	-
	1,5	-	-	1
	1,7	1	1	-
	1,9	-	2	-
	Total	114	234	101

found in the Rajput and Koli groups in this study are very similar respectively to various Kanet (Rajput) groups and Koli population inhabiting the neighbouring district of Kinnaur (Papiha et al., 1984).

For comparison, data on ADA polymorphism are available from Kulu and certain other districts of Himachal Pradesh. The present Brahmin showed statistically significant differences with groups of the caste reported earlier from districts of Kulu ($\chi^2 = 8.266$, d.f. 2, $0.05 > p > 0.01$) (Bhasin et al., 1983), Kangra ($\chi^2 = 7.243$, d.f. 2, $0.05 > p > 0.025$) (Papiha et al., 1982) and Solan ($\chi^2 = 24.239$, d.f. 2, $p < 0.0005$) (Sarin and Chahal, 2002). On the other hand, no such differences were observed for the other two caste populations of this study. Thus, when the present Rajput group was compared with any earlier samples of the caste reported from Kulu, Chamba, Shimla and Solan districts or any of the 5 Gaddi Rajput samples of Chamba district (Papiha et al., 1982, 1996; Chahal et al., 1982) or even any of the 4 Kanet (Rajput) groups of Kinnaur district (Papiha et al., 1984), no such differences were observed. Similarly, the present Koli material failed to show any appreciable differences with either of the two previous samples of this Scheduled Caste population studied from Kulu (Bhasin *et al*., 1983) as well as Kinnaur (Papiha et al., 1984) districts.

Adenylate Kinase Locus 1 (AK1) Polymorphism: The results of the electrophoretic typings of red cell AK locus 1 enzyme revealed that the studied hill caste groups showed the presence of two common phenotypes AK1 1 and 1,2 while AK1 2 could be found only in the Koli sample (Table 1). The distribution of the phenotypes of the AK1 system was very heterogeneous among the present groups, the Rajput showing highly significant differences with both the Brahmin ($\chi^2 = 22.659$, d.f. 1, $p < 0.0005$) and the Koli ($\chi^2 = 27.882$, d.f. 2, $p < 0.0005$).

The range of the frequency of the *AK1*2* allele in the present material from Kulu district was from a low of 0.0235 in the Rajput to as high as 0.1188 in the Koli (Table 2). It may be pointed out that barring a mongoloid Kanet (Rajput) group reported by Papiha et al. (1984), the incidence of this allele observed in the present Rajput group from Kulu district of Himachal Pradesh is the lowest recorded in any population group of the state. Again there is similarity between the Rajput of Kulu district and various Kanet (Rajput) groups of neighbouring Kinnaur district as well as between the Koli of these two districts.

Table 2: Allele frequencies in Kulu populations

Enzyme system	*Allele*	*Allele frequency*		
		Brahmin	*Rajput*	*Koli*
ADA	*ADA*1*	0.8289	0.8996	0.9257
	*ADA*2*	0.1711	0.1004	0.0743
AK1	*AK1*1*	0.8947	0.9765	0.8812
	*AK1*2*	0.1053	0.0235	0.1188
ESD	*ESD*1*	0.8421	0.7479	0.6733
	*ESD*2*	0.1579	0.2521	0.3267
PGM1	*PGM1*1*	0.6491	0.7094	0.7079
	*PGM1*2*	0.3421	0.2842	0.2871
	*PGM1*7*	0.0088	0.0064	0.0050
ACP1	*ACP1*A*	0.1930	0.2671	0.2970
	*ACP1*B*	0.7763	0.7329	0.6980
	*ACP1*C*	0.0307	-	0.0050
GLO1	*GLO1*1*	0.2168	0.2457	0.2871
	*GLO1*2*	0.7832	0.7543	0.7129
GPI	*GPI*1*	0.9868	0.9936	0.9950
	*GPI*4*	0.0088	-	-
	*GPI *5*	-	-	0.0050
	*GPI*7*	0.0044	0.0021	-
	*GPI*9*	-	0.0043	-

Unlike in the former system, in AK1 the Brahmin of Kulu showed no appreciable differences with their counterparts reported earlier from Kulu, Kangra, Chamba, Shimla and Solan districts of Himachal Pradesh. On the other hand, the present Rajput were significantly different from each and every group of the caste reported in literature from the state inhabiting Kulu district ($\chi^2 = 6.508$, d.f. 2, $0.05 > p > 0.025$), Kangra district ($\chi^2 = 15.887$, d.f. 2, $p < 0.0005$), Chamba town ($\chi^2 = 9.672$, d.f. 2, $0.01 > p > 0.005$) and Chuwari tehsil ($\chi^2 = 11.721$, d.f. 2, $0.005 > p > 0.001$), both of Chamba district, Shimla district ($\chi^2 = 16.279$, d.f. 2, $p < 0.0005$) and Solan district ($\chi^2 = 16.161$, d.f. 2, $p < 0.0005$). Interestingly the present Rajput group was also highly differentiated from all the four Gaddi Rajput samples of Chamba and Kangra districts (χ^2 range 13.276-24.800, d.f. 2, $p < 0.0005$) but none of the four Kanet (Rajput) samples reported from Kinnaur district. Like in ADA, in this enzyme system also the Koli were found to be homogeneous with the other two samples of this Scheduled Caste studied earlier from Kulu and Kinnaur districts of Himachal Pradesh.

Esterase D (ESD) Polymorphism: For the only known polymorphic human red cell esterase, esterase D (ESD), the phenotype distribution exhibited that the common types of the enzyme

i.e. ESD 1, 1,2 and 2 were present in each of the three caste groups studied (Table 1). Contingency χ^2 test revealed that as in ADA in ESD system also the Brahmin were significantly different from the Rajput ($\chi^2 = 9.397$, d.f. 2, $0.01 > p > 0.0005$) and more so from the Koli ($\chi^2 = 17.739$, d.f. 2, $p < 0.0005$).

The frequency of the *ESD*2* allele observed in the Koli sample (0.3267) was almost twice the value found in the Brahmin sample (0.1579) (Table 2) and a review of literature revealed it to be the highest so far reported in any indigenous population of Himachal Pradesh. With a frequency of 0.2521 the present Rajput sample showed similarities with the Kanet (Rajput) of Kalpa (0.254) and Nachar (0.268) areas in neighbouring district of Kinnaur (Papiha et al., 1984).

Inter-group comparisons showed that like in ADA, in ESD enzyme system also the present Brahmin were heterogeneous with two samples of the caste reported from Kangra ($\chi^2 = 16.234$, d.f. 2, $p < 0.0005$) and Solan ($\chi^2 = 8.603$, d.f. 2, $0.025 > p > 0.01$) districts. Furthermore, both the Rajput and Koli groups of this study were homogeneous with every sample of the respective caste, including various Kanet (Rajput) groups for the former, studied earlier from Himachal Pradesh.

Phosphoglucomutase Locus 1 (PGM1) Polymorphism: The electrophoretic typing of red cell phosphoglucomutase locus 1 (PGM1) enzyme showed that in the present material apart from the common phenotypes (PGM1 1, 1,2 and 2) found in the Brahmin, Rajput and Koli, examples of a rare phenotype PGM1 1,7 were also detected in each of these three hill caste populations studied here from Kulu district of Himachal Pradesh (Table 1). In fact, this variant has been widely reported earlier also in different other caste populations of the state (Papiha et al., 1982, 1996; Chahal et al., 1991; Sarin and Chahal, 2001, 2002). Unlike the previous three enzyme systems, there was no statistical evidence for phenotypic heterogeneity in PGM1 system among the present caste groups, albeit with their high *PGM1*2* frequency (0.3421) the Brahmin stand out from the other two studied caste populations (Table 2). The value of the allele found in the present Rajput (0.2842) is similar to that of the Kanet (Rajput) of Nachar (0.268) in neighbouring Kinnaur district (Papiha et al., 1984).

As for PGM1 enzyme polymorphism the Brahmin and Rajput of Kulu district were found to be homogeneous with all their respective counterparts reported earlier from different districts of Himachal Pradesh, save a comparison between the present Rajput and the Gaddi Rajput sample from Kangra district ($\chi^2 = 6.179$, d.f. 2, $0.05 > p > 0.025$) (Papiha *et al*., 1982). However, differences were statistically significant between the Koli of this study and an earlier sample of this Scheduled Caste studied from Kinnaur district ($\chi^2 = 6.276$, d.f. 2, $0.05 > p > 0.025$) (Papiha et al., 1984).

Acid Phosphatase Locus 1 (ACP 1) Polymorphism: The variation of erythrocyte acid phosphatase locus 1 (ACP1) isozymes observed in the material from Kulu district revealed the presence of three most common phenotypes of the enzyme system viz., ACP1 A, A,B and B in each caste group studied; phenotype A,C was found only in the Brahmin while B,C was detected both in the Brahmin and Koli samples (Table 1). Like in PGM1, in this enzyme marker also there was homogeneous distribution of the phenotypes in the studied groups.

Table 2 shows that the *ACP1*A* allele incidence varied from a low of 0.1930 in the Brahmin to a high of 0.2970 in the Koli while the *ACP1*C* allele was found to be totally absent in the Rajput but was present in the other two castes with trace values.

Barring inter-group comparisons between the present Brahmin and that of Chamba town ($\chi^2 = 8.455$, d.f. 2, $0.025 > p > 0.01$) and also that between the present Rajput and a Kanet (Rajput) sample from Pooh area of Kinnaur district ($\chi^2 = 14.038$, d.f. 2, $0.001 > p > 0.0005$), all other pairwise χ^2 comparisons were statistically non significant. This indicated homogeneous distribution of ACP1 enzyme system in people of Himachal Pradesh.

Glyoxalase Locus 1 (GLO1) Polymorphism: Glyoxalase locus 1 (GLO1) typing results exhibited that like ADA, AK1 and ESD systems, in this enzyme system also only the common phenotypes (GLO1 1, 1,2 and 2) were found (Table 1). Although inter-group comparisons revealed no appreciable differences between the present caste groups for this enzyme marker, the χ^2 values recorded between the Brahmin and Rajput ($\chi^2 = 5.399$, d.f. 2, $0.10 > p > 0.05$) as well as between the Brahmin and Koli ($\chi^2 = 5.740$, d.f. 2, $0.10 > p > 0.05$) were on the verge of achieving significance.

The frequency of the *GLO1*1* was found to be lowest in the Brahmin (0.2168) and highest in the Koli (0.2871) (Table 2), the latter being also the highest value of this allele in any population of Himachal Pradesh studied for glyoxalase locus 1 polymorphism (Chahal et al., 1991; Papiha et al., 1996; Sarin and Chahal, 2001, 2002). No comparable data are available on this enzyme system from the neighbouring Kinnaur district but the *GLO1*1* frequency recorded in the present Rajput sample of Kulu district (0.2457) was similar to that reported in samples of this caste from Chuwari (0.244) and Churah (0.265) tehsils as well as in Gaddi Rajput of Chuwari (0.254), all of Chamba district (Papiha et al., 1996).

For comparison, glyoxalase locus 1 polymorphism data are available from the Brahmin of Chamba town in Chamba district as well as of Shimla and Solan districts and each of them showed homogeneous distribution with the present material of the caste from Kulu district. The Rajput of this study were also similar to most of the Rajput and Gaddi Rajput groups reported for this enzyme system from Himachal Pradesh, save the Rajput of Shimla ($\chi^2 = 11.211$, d.f. 2, $0.005 > p > 0.001$) and Solan ($\chi^2 = 9.453$, d.f. 2, $0.01 > p > 0.005$) districts. No comparative GLO1 data are available for the Koli population from the state.

Glucose Phosphate Isomerase (GPI) Polymorphism: Results of the electrophoretic analysis of erythrocyte enzyme glucose phosphate isomerase (GPI) in the present study revealed that the common type GPI 1 was occasionally accompanied with variants such as GPI 1,4, 1,5, 1,7 and 1,9 (Table 1). The rare phenotype GPI 1,4 was found only in the Brahmin, GPI 1,5 only in the Koli, GPI 1,7 in both the Brahmin and Rajput but not in the Koli and GPI 1,9 in the Rajput only.

Several studies have reported the occurrence of GPI variants in people of Himachal Pradesh (Chahal et al., 1982, 1991; Papiha et al., 1984, 1996; Papiha and Chahal, 1984; Sarin and Chahal, 2001, 2002). The most common variant found in the state has been GPI 1,3 encountered in as many as 15 out of 21 population groups so far investigated for this enzyme system. Variants like GPI 1,5 and GPI 1,8 have also been detected but only in sporadic groups.

It may be pointed out that in the present investigation, no example of GPI variant 1,3 was found but variants like GPI 1,4, 1,5, 1,7, and 1,9 were detected and barring 1,5, all for the first time from Himachal Pradesh. Thus this study has provided evidence for the occurrence of some hitherto unreported GPI variants in the indigenous populations of this north Indian hill state. The overall incidence of GPI variants in people of Kulu district of Himachal Pradesh was 1.5% which was somewhat higher than the national average (about 1%, Papiha and Chahal, 1984).

In conclusion, the present biochemical genetics investigation among the hill people inhabiting Kulu district of north Indian state of Himachal Pradesh demonstrated that while the caste differences were appreciable in the distribution of some of the erythrocyte enzyme systems (ADA, AK1, ESD), there was similarity in some others (PGM1, ACP1, GLO1). GPI variants 1,4, 1,7 and 1,9 have been reported here for the first time from the state. Overall, the present three endogamous caste populations of the Brahmin, Rajput and Koli of Kulu district showed similarities with their counterparts inhabiting some other districts of Himachal Pradesh for which comparable data are available. In particular, the Rajput and Koli of this study showed affinities respectively with various Kanet (Rajput) groups and the Koli population reported earlier from the neighbouring contiguous district of Kinnaur in the state.

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