

# Genetic Variation and Population structure: A study on four populations of Tripura, North East India



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## ABSTRACT

**Back Ground:** Genetic polymorphisms of ABO and RhD blood group and Haptoglobin (HP) types have already been widely used to understand variation and population structure. The present study attempted to understand the variation and population structure on the basis of ABO and RhD blood group polymorphisms and Haptoglobin (HP) as serum protein polymorphism of four Tibeto-Burman speaker groups considered as aboriginal tribes of Tripura. Genetic studies on populations in north-east Indian have been performed less frequently than in the other parts of India, in spite of diversity among these populations. **Aims and Objective:** Best of the knowledge the present study is the first attempt on the variation and population genetic structure concerning the ABO, RhD and HP polymorphism of the four endogamous populations – Tripuri, Hrangkhawl, Rupini and Kolui tribal population of Tripura. **Materials and Methods:** Present study consisted of four hundred (400) participants 100 each from above mentioned population groups. ABO and RhD blood groups were done by antigen-antiserum agglutination test and HP types were ascertained by Polyacrylamide Gel Electrophoresis (PAGE) following standard techniques. Allele frequencies and Fst were calculated by using standard method. **Results:** Examination on ABO blood group revealed higher O allele among the two groups Tripuri and Hrangkhawl followed by A and B alleles. Among the other two groups, Rupini and Kolui, revealed higher A allele and B allele respectively. Higher D allele frequency in the RHD Blood group system has been found for each of the population group. On the other hand, Serum protein marker - HP types demonstrated higher HP\*2 allele frequency than HP\*1 alleles. Calculated very low Fst value (0.02-0.08) of three polymorphic markers (ABO, RhD and HP) were suggestive for low differentiation among these groups, indicating less admixture and prevailing endogamous nature of these population. **Conclusion:** Very low Fst values of three polymorphic markers were suggestive for low differentiation among these groups, indicating least admixture and prevailing endogamous nature of these population. The overall genetic difference revealed highest distance between Tripuri and Hrangkhawl and least distance between Rupini and Kolui.

**Key words:** ABO, RhD, HP, Tripura, Fst, Genetic difference.

## INTRODUCTION

Determining the genetic structure of natural populations forms an important part of population genetics. For example, this has applications in evolutionary biology, conservation and forensics. India occupying the centrestage of Palaeolithic and Neolithic migrations, and somewhat has been under-represented in genome-wide studies of variation.<sup>1</sup> Being at the cross-roads of migration, Indian

populations have undergone complex and ancient admixture events over a long period<sup>2-5</sup> and have been the melting-pot of disparate ancestries originating from different parts of Eurasia and South-East Asia.<sup>5,6</sup> Although the date of entry of modern humans into India remains uncertain but it is reasonable to consider by the middle Palaeolithic period (50,000–20,000 years before present ybp), humans appear to have spread to many parts of India.<sup>7</sup> The contemporary ethnic India is a land of enormous genetic, cultural, and

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linguistic diversity.<sup>8-10</sup> A more recent study exploring Indian genomic diversity demonstrated four major ancestral genetic components in mainland India that included four dominant ancestries in populations from mainland India: Ancestral North-Indian (ANI), Ancestral South-Indian (ASI), Ancestral Tibeto-Burman (ATB) and Ancestral Austro-Asiatic (AAA).<sup>5</sup> On the basis of the literature review on major and contributing publications regarding genetic structure of the North East Indian population, it has been found that studies on the tribal populations of Tripura seems to have been under represented.

In this context, to best of the knowledge, the present study is the first attempt to understand the variation and population structure of the four Tibeto-Burman speaking tribal populations, namely Tripuri, Hrangkhawl, Rupini and Kolui tribal population of Tripura, North East India using the three ABO, RHD and Haptoglobin (HP) polymorphic traits.

## MATERIALS AND METHODS

### Background of studied participants

Tripura, a hilly state in the North-Eastern region of India is the homeland 19 tribal populations. The tribal populations can be divided into two major groups as (i) Ab-original (Tripuri, Reang, Jamatia, Noatia, Lusai, Uchai, Chaimal, Halam, Kukis, Garos, Mog and Chakma) and (ii) Immigrants (Bhil, Munda, Orang, Santal, Lepcha, Khasia, Bhutias). The aboriginal tribal populations of this state have been migrated in this territory from a place in between Tibet, up hills of Burma, like Arakan Hills Tracts and Shan State and adjacent to China. The immigrant tribal populations mostly migrated from their homeland located in the states of Madhya Pradesh, Bihar, Orissa and West Bengal. Some of these tribes are, however, Northern-Frontier tribes who migrated from the states of Bhutan, Meghalaya, Sikkim and North Bengal. The Chakmas and the Mogs are the Arakan Tribes who entered Tripura through the Chittagong Hills Tracts.

Linguistically, the aboriginal tribal populations of the state of Tripura can be divided into three groups, such as Bodo Groups, Kuki-Chin Groups and Arakan Groups. The tribal populations, like Tripuri, Reang, Jamatia, Uchai and Noatias bear Mongoloid physical features and belong to the Bodo linguistic group. The Kukis, Lusai and most of the tribes under Halam tribes linguistically belongs to the Kuki-Chin group and speak in Kuki-Chin language. The Mog and the Chakmas speak in Arakan language. While most of the tribal populations of Tripura follow Hinduism, the Lusai-Kukis embraced Christianity and the Chakmas and Mogs embraced Buddhism.<sup>11</sup>

The present study was carried out among the four endogamous tribal groups, Tripuris, Kolui, Rupini and Hranghaws (sub clan groups of Halam). The area of the study was Kamalasangar and Sipahijola districts of West Tripura. Ethnically, Halam belongs to the Kuki-Chin tribes. Their language is also similar to that of the languages under the Tibeto-Burman family. Halams are also known as Mila Kuki, though they are not at all Kukis in terms of language, culture and living style. Halams are divided into several sub-clans which are referred as "Barki-Halam". The major sub-clans of Halams are (1) Kaloi (2) Kor-Bong (3) Keipeng (4) Bong (5) Saka Chep (6) Thanga chep (7) Dab (8) Bongser (9) Rupini (10) Hrangkhawl (11) Chorai (12) Langai (13) Kaireng (14) Ranglong (15) Naveen and (16) Khulang. The Hrankhaws are one of the sub clans of Halam-Kuki community. On the other hand, Tripuris originated from the Indo-Mongoloid stock and linguistically affiliated to the Tibeto-Burman family. The Tripuris are the largest tribal community and the first group of aboriginal tribal migrants of Tripura.

The study was conducted on 100 participants each from Tripuri, Hrangkhawl, Rupini and Kolui tribal population, and altogether 400 individuals from West Tripura. Prior to the study, verbal consent was taken from each of the participants. Blood samples were collected in EDTA vial and kept in ice buckets and collected samples were transported to the Human Genetics Laboratory, Department of Anthropology, University of Calcutta for further analysis. ABO and RHD blood phenotypes were obtained by standard antigen-antiserum agglutination technique.<sup>12</sup> Polyacrylamide Gel Electrophoresis (PGE) (7%) technique was used to identify the Haptoglobin (HP) phenotypes using standard protocol with slight modification.<sup>13</sup> The allele frequencies for all the polymorphic markers, such as ABO and RHD blood groups including HP were computed by Maximum Likelihood Estimation.<sup>14</sup> Fst statistic<sup>15</sup> was calculated to estimate the genetic differentiation of the studied populations. The test of goodness of fit (chi-square test) based on the observed and expected frequencies of polymorphic markers were calculated in order measure Hardy-Weinberg equilibrium and as well the measure of genetic equilibrium ( $D/\sigma$ ) was computed to understand the excess or deficiency of AB blood group. The overall genetic difference ( $G^2$ ) between any two endogamous groups was calculated<sup>16,17</sup> using standard procedure. The cut off was set as  $p=0.05$ .

## RESULTS AND DISCUSSION

Distribution of phenotypes and allele frequencies of ABO blood group as presented in Table-1, demonstrated higher O allele among the two groups Tripuri and Hrangkhawl followed by A and B alleles. Among the other two groups, Rupini and Kolui, revealed higher A allele and B allele

respectively. Estimated Hardy-Weinberg (HW) equilibrium revealed no deviation for ABO allele frequencies among the four groups. Moreover, the four groups showed significant ( $\chi^2=94.44$ ; 9 df;  $p<0.05$ ) difference in the distribution of ABO blood group phenotypes. The computed positive values of genetic equilibrium ( $D/\sigma$ ) for all these groups indicated no as such deficiency of AB blood group. The distribution of ABO blood group and allele frequencies with higher A blood group than other regions of India and close A and B allele frequency of the four Tibeto-Burman speaker groups was found to be in consistency with earlier studies.<sup>18-20</sup>

Examination on Rh blood group phenotypes (Table 2) revealed highest proportion of D alleles and also found to be in HW equilibrium for all the groups. Comparative distribution of Rh blood group phenotypes between the four groups demonstrated significant ( $\chi^2=24.67$ ; 3 df;  $p<0.05$ ) differences. The distribution of higher frequency of RhD alleles among these Tibeto-Burman speaker groups was found to be in corroboration with earlier studies.<sup>18-20</sup> Estimated allele frequencies (Table 3) of serum protein marker (HP) revealed higher HP\*2 alleles in all the four groups. Rupini, Kolui, Tripuri, demonstrated no significant deviation from HW equilibrium, while Hrangkhawl showed significant ( $\chi^2=15.34$ ; 1 df;  $p<0.05$ ) deviation from HW equilibrium. Furthermore, significant ( $\chi^2=69.59$ ; 6 df;  $p<0.05$ ) difference between

these four groups in terms of the distribution of haptoglobin phenotypes were evident. However, the result of the present study in terms of higher frequency of HP\*1 alleles in comparison to other linguistic groups (except Indo-European) confirms the consistency with the earlier studies.<sup>18-20</sup> The estimated Fst values for three genetic marker traits of the four populations (Table 1, 2 and 3) demonstrated very low values (Range 0.02-0.08), indicating low differentiation<sup>21</sup> among these four ethnic (aboriginal) groups of Tripura. Furthermore, the low values of Fst also signify higher possibility of endogamy among these populations.

Genetic differences as calculated by G<sup>2</sup> analysis<sup>16,17</sup> revealed (Table 4) highest distance between Tripuri and Hranghwal and least distance between Rupini and Kolui. Probable population Structure revealed both Rupinis and Koluis closer to the Tripuris, and Rupinis and Koluis are very close to each other. The available result from the present study indicated the possibility of the origin of Rupini and Kolui group from the major aboriginal group Tripuri.

The result of the present study corroborates with the earlier studies conducted on Tibeto Burman speakers of Tripura in terms of low differentiation<sup>22-24</sup> and high homogeneity on Haplogroups. The overall result obtained from the present attempt delineates higher possibility of endogamy

**Table 1. Distribution of ABO blood group phenotypes and allele frequencies among the four studied population**

Population	(N)	Blood group, (%)				Allele frequencies			Chi <sup>2</sup> df=3	Fst
		A	B	O	AB	A	B	O		
Rupini	100	38 (38.00)	37 (37.00)	6 (6.00)	19 (19.00)	0.357	0.348	0.288	3.4	0.08
Kolui	100	25 (25.00)	55 (55.00)	5 (5.00)	15 (15.00)	0.237	0.475	0.285	6.26	
Tripuri	100	36 (36.00)	24 (24.00)	37 (37.00)	3 (3.00)	0.221	0.148	0.629	2.91	
Hrangkhawl	100	37 (37.00)	22(22.00)	39 (39.00)	2 (2.00)	0.221	0.130	0.640	3.71	

**Table 2. Distribution of RHD blood group phenotypes and allele frequencies among the four studied population**

Population	(N)	Blood groups, (%)		Allele frequencies		Chi <sup>2</sup> 1 df	Fst
		Rh D	Rh d	D	d		
Rupini	100	99 (99.00)	1 (1.00)	0.99	0.01	0.000	0.02
Kolui	100	95 (95.00)	5 (5.00)	0.78	0.22	0.006	
Tripuri	100	86 (86.00)	14 (14.00)	0.63	0.37	0.008	
Hrangkhawl	100	80 (80.00)	20 (20.00)	0.56	0.44	0.020	

**Table 3. Distribution of haptoglobin phenotypes and allele frequencies among the four studied population**

Population	(N)	Haptoglobin group, (%)			Allele frequencies		Chi <sup>2</sup> df=2	Fst
		Hp 1-1	Hp 2-1	Hp 1-1	Hp 1	Hp 2		
Rupini	100	1 (1.00)	14 (14.00)	85 (85.00)	0.08	0.92	0.51	0.06
Kolui	100	1 (1.00)	25 (25.00)	74 (75.00)	0.13	0.87	2.26	
Tripuri	100	6 (6.00)	51 (51.00)	43 (43.00)	0.31	0.68	3.43	
Hrangkhawl	100	1 (1.00)	59 (59.00)	40 (40.00)	0.30	0.70	15.34	

**Table 4. Genetic differences (G<sup>2</sup>) among the four populations of tripura**

	Genetic difference (G <sup>2</sup> ) (Sanghvi and Khanolkar, 1949; Sanghvi, 1953)
Rupini - Kolui	0.0001
Rupini - Tripuri	0.0049
Rupini - Hrangkhawl	0.0043
Kolui - Tripuri	0.0018
Kolui - Hrangkhawl	0.0032
Tripuri - Hrangkhawl	0.0056

among the studied four groups (Tibeto- Burman speakers) of North East India from Tripura and thus indicating no admixtures among these Tibeto Burman speaker groups.

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### Authors Contribution:

**PD-** Collected Data, laboratory analysis, statistically analyzed; **PS-** laboratory analysis, statistically analyzed **DC-** reviewed the literature, helped in preparing first draft of manuscript; **ARB-** Concept and design of the study, data interpretation, critical revision of the manuscript

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