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Gene Diversity at Three Tetrameric STR Loci Among Eight Ethnic Populations of West Bengal and Manipur, India

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KEY WORDS 3 STR Loci: HUM HPRTB, HUM F13B and HUM LPL; Caucasoid and Mongoloid populations; Heterozygosity.

ABSTRACT The nature and extent of genetic variation at 3 STR loci (HUM HPRTB, F13Band LPL) were investigated among 8 population groups of West Bengal and Manipur regions of India. Of which, two groups from West Bengal belong to Caucasoid and six (one in WB and five in Manipur) belong to Mongoloid stock. Mongoloid shows similarities in some alleles and differences in some other alleles in STR loci. For example, Manipur Muslims show differences in STR allele frequency when compared to other four regional populations. Similarly Garo, one of the Mongoloid populations of West Bengal differ in allele frequency from their counterparts in Manipur region. Heterozygosity values are higher for Caucasoid than Mongoloid group. The overall gene differentiation for STR loci is 5.3%. The clustering pattern for the eight populations shows distinct clusters for Caucasoid and Mongoloid group, whereas Manipur Muslims stand apart from others. The genetic affinities based on DNA markers do not completely disagree with the results obtained from classical genetic markers.

INTRODUCTION

Peopling of India is characterized by conglomeration of wide diversity of populations which can be identified by ethnic, cultural, linguistic and racial stocks; a rare situation to find in other countries. To understand the genetic history, possible origin and affinities of the Indian populations is one of the challenging tasks and is a major objective of anthropological genetics. Previous studies based on classical biological characters (especially sero-genetic etc.) among regional, ethnic and specific populations have shown wide genetic variation characterized by gradients of gene frequency of uneven pattern of distribution or a 'bumpy biological surface'. It depicts very high or low frequency of a few characters scattered in some populations (e.g., Roychaudhury 1983; Mujumder and Mukherjee 1993; Malhotra and Vasulu 1993; Bhasin et al. 1994; Papiha 1996; Bhasin and Walter 2001). Attempt to explain the causative factors of this uneven distribution have shown that geographical continuity and ethno-historical factors are the primary explanatory variables, besides population structure play an important role.

For example, the Bengal region forms connecting link between the northeastern, eastern to western parts of the continent. Historically different ethnic and racial populations from different neighboring regions have settled and thus represent mosaic picture of variety of ethnic elements. . For example, the population in northern parts of the region is Mongoloid affiliated, whereas in southern region the populations in its southern border shows affinities especially with Australoid and Caucasoid racial elements (Roychoudhury 1992). Whereas the northern eastern parts, though settled by a variety of ethnic population of different origin from the neighboring countries, most of them belong to Mongoloid racial stock. However some populations of Caucasoid origin, especially some Hindu caste populations who have settled from other parts of India. During the past decades several workers have studied the genetic diversity using classical genetic polymorphism to understand genetic structure of these populations so as to unravel the affinities, origin and the influence of population structure (Mukherjee et al. 1987; Chakroborty, R et al. 1986, 1987; Deka et al. 1988, Roychoudhury 1992 and others). In this regard, it is of interest to investigate the utility and stability of DNA polymorphism to address the Indian situation: Do different sets of hyper variable loci give similar

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findings? How exactly the findings based on DNA polymorphism differs or contradicts those based on classical sero-genetic data. These problems can be now best addressed more rigorously with DNA polymorphism. Therefore, the objective of the present study is to analyze the genetic structure based on: 1. Variation on three STR loci (HUMHPRTB, HUMF13B and HUMLPL) in 8 populations; three from West Bengal and five from Manipur in Northeast regions. 2. To assess the usefulness of STR markers in assessing the genetic affinities among closely related regional populations. 3. To test the finding that Mongoloid populations show genetic similarity with respective of their geographical contiguity irrespective of their origin or language affiliation. And they are distinct from Caucasoid populations.

MATERIALS AND METHODS

Population: The study consists of eight populations, three from West Bengal and five from Manipur, Northeast region, India. Blood samples were collected from a total of 245 healthy individuals from two higher castes groups: Kayastha (114) and Brhamin (51) from southern and the third from Garo (80), a Mongoloid affiliated population from northern region of West Bengal. In case of Manipur in Northeast region, 379 blood samples were collected from different parts of Manipur Valley, especially from higher caste group Meitei (102), Naga (76), Kuki (66), Hmar (60) and from Manipuri Muslim group (66). The above samples were collected from unrelated volunteers in EDTA containing vacutainers. The medical history of each donor was recorded. Blood was aliquoted (700µL) and stored at –20º C prior to DNA extraction.

DNA Extraction: DNA was extracted from aliquoted blood samples following a phenol-chloroform method (Comey et al. 1994). Briefly, the samples were incubated in 50mM Tris-HCl, 150mM NaCl and 100mMEDTA Na2, with the addition of SDS (1.25%) and 0.03mg/mL proteinaseK, and precipitated with absolute ethanol after two extractions with phenol: chlorofrom:isoamyl alcohol (24:1), respectively. The quantity of DNA in each sample was estimated using the slot-blot procedure described by Waye et al. (1989) and by Hoefer's DyNA Quant 200 Flurometer. Wherever blood samples were collected as a stain (control), DNA was extracted using the Chelex extraction procedure (Singer-Sam et al. 1989). Ten-to-fifteen ng of DNA was used for PCR.

PCR Amplification: The amplification of HPRTB, F13B and LPL were performed using the Gene Print Kit according to the manufacturer's recommendations (Promega Corporation, Madison, WI, 1998) . The primers for the STRs are as follows:

HPRTB 5'	ATGCCACAGATAATACACATCCCC 3'	(forward)
HPRTB 5'	CTCTCCAGAATAGTTAGATGTAGG 3'	(reverse)
F13B 5'	TGAGGTGGTGTACTACCATA 3'	(forward)
F13B 5'	GATCATGCCATTGCACTCTA 3'	(reverse)
LPL 5'	CTGACCAAGGATAGTGGGATATG 3'	(forward) Set:1
LPL 5'	GGTAACTGAGCGAGACTGTGTCT 3'	(reverse)
LPL 5'	ATCTGACCAAGGATAGTGGGATATA 3'	(forward) Set:2
LPL 5'	CCTGGGTAACTGAGCGAGACTGTGTC 3'	(reverse)

The PCR was carried out in 25 µl reaction volumes containing 5-10 ng template DNA and 0.25 unit of Taq DNA polymerase. These were placed into a Perkin Elmer 2400 thermal cycler and were subjected to denaturation at 96ºC for 2 min. and then to 10 cycles of denaturation at 94ºC for one minute; primer annealing at 60ºC for 1 mininute and primer extension at 70ºC for 1.5 minutes. Then PCR was continued for 20 cycles of denaturation at 90ºC for 1 minute; primer annealing at 60ºC for one minute and primer extension at 70ºC for 1.5 minute.

Typing: 5µl of the PCR product was mixed with 4 µl of loading dye containing 10mM NaOH, 95% Formamide, 0.05% Xylene Cyanol FF. The samples were denatured on Perkin Elmer Termal Cycler 2400 for 2 minute and quick chilled on ice. 6 µl of the denatured samples were loaded on to the gel (4%, 7M Urea, 0.5X TBE, 0.4 mm thick). Electrophoresis was carried out on SQ3 Electrophoresis apparatus (Pharmacia Biotech) at constant power of 50 watt and ambient temperature. Electrophoresis was stopped after appromimately one and half hour when the Xylene Cyanol dye front was found 7 cm from the anode. The gel plates were opened and the gel adhered to the shorter glass plate was treated with fixing solution (10% Acetic acid). The gel was subsequently treated with staining solution (0.001% AgNO3; 0.002% v/V 37% Formaldehyde) and developed with developer solution (0.002% v/V 37% Formaldehyde, 6% Sodium Carbonate and 400 ul of 10 mg/ml Sodium Thiosulphate). The development was stopped with 10% Acetic Acid solution when the allele designations were de-

termined by comparison of the sample fragments with the allelec ladders supplied with the Gene Print Kit.

Statistical Analysis: The frequency of alleles for each STR was calculated for each genotype in the sample set (i.e., the gene count method). Average heterozygosity and its standard error for each population were computed as described by Nei (1973). The expected numbers of distinct homozygous and heterozygous and their standard error were calculated according to the method described by Chakraborty et al. (1988, 1991). Possible divergence from Hardy-Weinberg expectations (HWE) was determined by calculating the unbiased estimate of the homozygosity test (Nei and Roychoudhury 1974), the likelihood ratio test (Chakraborty et al. 1991; Edward et al. 1992; Weir 1992) and the exact test (Guo et al. 1992). Independence among the six PCR-based loci was determined by examining whether or not the observed variance of the number of heterozygotes loci in the population sample is within its confidence interval under the assumption of independence (Brown 1980). Genetic diversity and genetic distance were estimated by the help of 'Genetic Distance and Phylogenetic Analysis Software' (Nei 1973 and Nei et al. 1983) and the phylogenetic trees were drawn by unweighted group-method with arithmetic mean (UPGMA) (Sneath and Sokal 1973).

RESULTS

Variation in STR Loci: Variation in allele frequencies of HUMHPRTB locus among the three West Bengal and five Manipur populations are shown in Table 1. Of the 12 alleles, allele no.6 and no.17 are absent in all the eight populations. The West Bengal group shows the highest frequency in allele no.12 (0.226 to 0.351) whereas the Manipur group shows the highest frequency for allele no. 13 (0.272 to 0.400). Garo which is Mongoloid in northern parts of West Bengal nearer to northeast region show an intermediate frequencies and is more similar to Manipur populations. The frequencies clearly show distinct differences between the Caucasoid and Mongoloid stock. For example, it shows the absence of alleles 7, 8, 15, 16 in almost of all the five Manipuri populations. Table 2 shows the allelic variation in F13B. Allele no 10 is the highest and common frequency in all the populations, except in Manipur Muslim (0.111) and it ranges from 0.44 to 0.60 (Hmar). Allele 12 is absent in all the populations whereas, allele no.11 is absent in the entire six Mongoloid group. The other two-allele no. 8 and 9 show wide and similar variation in both the regional populations. Variation in LPL allele frequencies is shown in Table 3. This shows clear differences in allele frequencies between Caucasoid and Mongoloid groups and peculiarities of the Garo and Meitei two intermediately groups in both the regions. All the populations show the absence of allele frequency for 7 and 8. The Manipur populations show the absence of allele frequencies of no. 13 and 14 except for Meitei caste group. Though the allele no.10 is the common variant in the two regions, but the Manipur populations show the highest frequency (above 0.7) than theWest Bengal group. Test of homozygosity, likelihood ratio test, and exact test were examined for the STR loci and the results show no detectable deviation from H-W expectation. The observed frequencies of the most common genotypes of the STR loci for eight populations are shown in Table 4. The values range from 0.157 in Brahmin to 0.611 in Muslim.

Table 1: Variation in STR loci of HUMHPRTB in West Bengal and Manipur populations

		HPRTB locus											
Population	*N*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*	*16*	*17*
WEST BENGAL													
Kayastha	228	0.000	0.000	0.000	0.013	0.114	0.087	0.351	0.148	0.216	0.054	0.013	0.00
Brahmin	102	0.000	0.019	0.000	0.009	0.068	0.107	0.343	0.147	0.137	0.127	0.039	0.00
Garo	160	0.000	0.023	0.015	0.078	0.054	0.031	0.226	0.265	0.156	0.140	0.007	0.00
MANIPUR													
Meitei	204	0.000	0.045	0.000	0.045	0.000	0.090	0.181	0.272	0.136	0.181	0.045	0.00
Naga	156	0.000	0.000	0.000	0.025	0.050	0.050	0.300	0.400	0.150	0.025	0.000	0.00
Kuki	132	0.000	0.025	0.075	0.100	0.075	0.025	0.175	0.250	0.150	0.125	0.000	0.00
Hmar	120	0.000	0.000	0.000	0.033	0.066	0.050	0.250	0.333	0.166	0.100	0.000	0.00
M.Muslim	132	0.000	0.000	0.000	0.027	0.222	0.111	0.361	0.194	0.055	0.000	0.028	0.00

Table 2: Variation in F13B locus in West Bengal and Manipur populations

		F13B locus									
Population	*N*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*HWE*	*LRT*	*ET*
WEST BENGAL											
Kayaastha	114	0.098	0.000	0.147	0.288	0.433	0.021	0.000	0.214	0.128	0.268
Brahmin	51	0.031	0.010	0.148	0.319	0.478	0.010	0.000	0.474	0.937	0.849
Garo	80	0.000	0.007	0.195	0.304	0.492	0.000	0.000	0.235	0.101	0.100
MANIPUR											
Meitei	102	0.000	0.022	0.159	0.340	0.477	0.000	0.000	0.419	0.530	0.409
Naga	76	0.000	0.000	0.125	0.200	0.675	0.000	0.000	0.377	0.713	0.846
Kuki	66	0.000	0.000	0.200	0.325	0.475	0.000	0.000	0.606	0.275	0.394
Hmar	60	0.000	0.000	0.150	0.250	0.600	0.000	0.000	0.446	0.629	0.834
M.Muslim	66	0.000	0.000	0.527	0.361	0.111	0.000	0.000	0.411	0.520	0.402

HWE: Homozygosity test, LRT: Likelihood Ratio Test, ET: Exact Test.
M. Muslim: Manipur Muslim.

Table 3: Variation in LPL locus in West Bengal and Manipur populations

		LPL locus									
Population	*N*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*HWE*	*LRT*	*ET*
WEST BENGAL												
Kayaastha	114	0.00	0.00	0.061	0.330	0.292	0.246	0.069	0.000	0.214	0.128	0.268
Brahmin	51	0.00	0.00	0.042	0.446	0.191	0.191	0.106	0.021	0.538	0.136	0.079
Garo	80	0.00	0.00	0.000	0.640	0.078	0.218	0.039	0.023	0.916	0.210	0.211
MANIPUR												
Meitei	102	0.00	0.00	0.000	0.590	0.113	0.159	0.090	0.045	0.494	0.721	0.463
Naga	76	0.00	0.00	0.000	0.750	0.025	0.225	0.000	0.000	0.342	0.377	0.639
Kuki	66	0.00	0.00	0.000	0.700	0.100	0.200	0.000	0.000	0.520	0.535	0.346
Hmar	60	0.00	0.00	0.000	0.716	0.667	0.216	0.000	0.000	0.789	1.000	1.000
M.Muslim	66	0.00	0.00	0.000	0.722	0.166	0.111	0.000	0.000	0.674	0.890	1.000

HWE: Homozygosity test, LRT: Likelihood Ratio Test, ET: Exact Test.
M. Muslim: Manipur Muslim.

The LPL loci show a high of 0.5 value among the four Manipur populations, except in case of Meitei. The match probability values for the three loci ranges between 0.0011 to 0.00089.

Genetic distances (DA) were computed to investigate the genetic affinity between the eight populations based on the three STR loci along with the average heterozygosity (arranged on the diagonal) values and are shown in Table 5. The distance values range from 0.0085 (between Naga and Kuki) to 0.1235 (between Kayastha and M.Muslim). Both Kayastha and Brahmin populations, the two Caucasoid stock show high distance values with the rest Mongoloid group. The dendrogram depicting the different levels of clustering of related populations with respect to

Table 4: Most frequent genotype observed at the 3 STR loci in West Bengal and Manipur populations

Population		*STR Locus*			*p* (χ *1/100)*	**p-squared* (χ *1/1000)*
	N	*HPRTB*	*F13B*	*LPL*		
WEST BENGAL						
Kayastha	114	0.216	0.281	0.185	1.12	0.126
Brahmin	51	0.157	0.361	0.234	1.32	0.175
Garo	80	0.234	0.359	0.312	2.62	0.686
MANIPUR						
Meitei	102	0.182	0.454	0.363	2.99	0.899
Naga	76	0.300	0.400	0.500	6 .0	3.6
Kuki	66	0.200	0.300	0.550	3.3	1.1
Hmar	60	0.266	0.333	0.500	4.42	1.96
M.Muslim	66	0.167	0.611	0.500	5.1	2.6

*p-squared: Represents the Match probability for the most frequent Genotype

Table 5: Average Heterozygosity (diagonal) and Pair wise genetic distance (Da) based on the 3 STR loci among eight populations from West Bengal and Manipur regions*

Population	*Kayastha*	*Brahmin*	*Garo*	*Meitei*	*Naga*	*Kuki Hmar*	*M.Muslim*
Kayastha		0.739	(0.027)				
Brahmin		0.0240	0.729	(0.047)			
Garo		0.0819	0.0449	0.665	(0.085)		
Meitei		0.1028	0.0433	0.0250	0.690	(0.070)	
Naga		0.1042	0.0922	0.0423	0.0823	0.535	(0.099)
Kuki		0.1035	0.0844	0.0190	0.0676	0.0454	0.649

*The figures on the diagonal are estimates of Average Heterozygosity with standard Error (in parenthesis)

STR loci considered shows two main clusters. The least differentiated populations are Naga and Hmar followed by Garo and Kuki, which form one main subcluster. The Meitei stands apart from the four neighboring populations. The second cluster includes Kayastha and Brahmin from West Bengal, which form a separate cluster and distinctly away from the four Manipur populations. Muslim population is distinct from all the populations and is separated out from others. Table 6 shows the locus wise and average gene diversity (Ht, Hs and Gst) indicating the degree of differentiation among the two regional populations. The populations show a considerable amount of average heterozygosity (65%). The average Gst value is 0.0532. The extent of degree of genetic differentiation varies with the population and for the locus. The highest value is 0.0825 for F13B and the lowest is 0.0267 for HPRTB locus.

Table 6: Estimates of gene diversity among the eight populations from West Bengal

And Manipur regions.

	HPRTB	*F13B*	*LPL*	*Average*
Ht	0.8079	0.6900	0.6164	0.7048
Hs	0.7864	0.6330	0.5824	0.6672
Gst	0.0267	0.0825	0.0551	0.0532

DISCUSSION

The results of the present study based on three STR loci confirms the other studies based on classical genetic markers; especially that Caucasoid affiliated populations (Brahmin, Kayastha etc.) are separate from Mongoloid population. And further the Mongoloid affiliated populations of West Bengal are separate from those in Northeast region (Roychoudhury 1992). Allele frequencies show distinct differences for some specific populations possibly suggesting the influence of population structure variables, especially admixture, migration etc. The Mongoloid affiliated population Garo in West Bengal shows intermediary frequencies in some of the

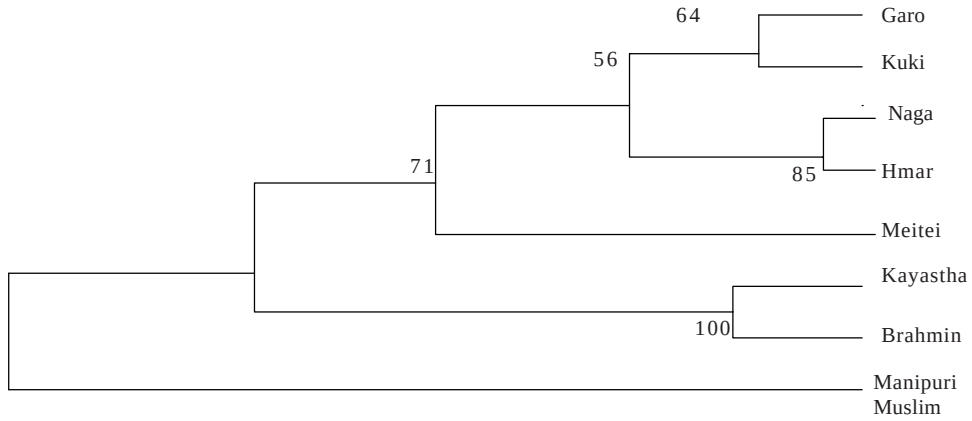

Fig. 1. Genetic affinities between 8 populations from West Bengal and Manipur Regions based on three STR loci (HUMHPRTB, HUMF13B and HUMLPL) by DA distance and UPGMA clustering method

alleles of STR loci. One of the interesting aspects of the results is about the Muslim population in Manipur. Unfortunately there were no available records about the history, or origin of the population. It is not known from where they came from or is they local people converted in the recent past etc. STR loci show some distinct features of Manipur Muslim possibly suggesting that they are genetically different from other local populations. It can be inferred that they may be a migrant group from nearby region in the past and have remained as distinct ethnic group through social, cultural and marriage regulations. This possibly accounts for some of the allelic differences observed in the study. For example, they show least frequency (0.028) of allele 16 and absence of allele 15 at HPRTB locus; lowest frequency (0.111) for allele 10 at F13B locus. Similar such results have also been found by other studies in different ethnic populations in other regions of the continent. For example, based on mtDNA variation Bamshad et al. (1996) have investigated the influence of population structure on the genetic variation of among four castes and infer that the effects of social structure on mtDNA variation are much greater than those based on traditional markers. Balakrishnan et al. (1996) investigating the affinities of the Iyer, a Brahmin population of Tamil Nadu, based on HLA haplotypes found that they are differ from other Brahmin population and possibly their ancestors must have migrated from Central Asia or Eurasian steppes. Papiha et al. (1996) have found the Gst values based on VNTR loci is within the range found for classical markers studied from different populations of the Indian subcontinent. Mukherjee et al. (1999) studying the variation on 4 STR loci among eight population groups infer that the findings are not completely in agreement with the findings based on classical genetic markers, especially that geographic proximity has a greater influence between populations than socio-cultural proximity.

The dendrogram based on STR loci for the above 8 populations show similar clustering which separates Caucasoid and Mongoloid populations thus validating the usefulness of the hyper variable loci for investigating the genetic affinities of regional populations. It shows Manipur Muslim as a separate group away from the two sub-clusters. STR sequences are extremely polymorphic loci that occur mostly in all the non-expressed regions and show high mutation rates. As such the STR loci is expected to show better resolution of genetic affinities and origin of the closely related regional and continental populations. Based on the available historical, cultural and linguistic information about the populations studied, it can be said that the clustering pattern based on STR loci is more confirmatory. The present study (based on STR loci) shows better clarity of the genetic affinities of the regional populations with geographic proximity. Whereas a similar study based on different set of STR loci among the 8 regional populations from a wider region that include Orissa, Uttara Pradesh and West Bengal showed poor affinity with either geographic proximity or socio-cultural proximity (Mukherjee 1999). Such variance results based on (different sets of) STR loci is indeed could be due to several reasons, for example it could be due to the nature of populations considered for the study. The selection, type and number of STR loci considered for the study is important in this regard. Though it is expected that the findings of the study may not differ much on the type of STR loci selected, but it is interesting to verify the expected proposition? Since for some forensic purposes certain type of STR loci are selected and for genetic variation different type of loci are selected (as can be observed from the published results).

As far as we know, it is the first report of three populations of West Bengal on three (HUMHRTB, F13B and LPL) STR loci among the caste groups. As such we do not have the comparative analysis with other populations. Though there were some reports on STR (and VNTR) loci but they were based on different set of loci (for example, Balakrishnan et al. 1996 and Mukherjee et al. 1999). We have also studied the inheritance of the studied set of multiple loci for STR in 32 families belonging to eight populations and the results showed no deviation from the expected data. The generated fragments were successfully inherited across the generations. No mutation across any of the studied loci was detected in the studied samples.

The results show higher levels of average heterozygosity for the two Caucasoid populations (73%) than the Mongoloid populations. While higher levels of heterozygosity for different set of DNA polymorphic data in the Caucasoid population is also recently reported (for example, Martinovic et al. 1999), but the observed heterozysoity levels (53% to 66%) for Mongoloid for the studied DNA loci of the study, are similar to the values for Cambodian (69.4), Filipino (68.6%) etc., for 9

autosomal microsattelite loci (Parra et al. 1999). But the present values are below the reported values for other Mongoloid population. For example, Robinson et al. (1996) have reported an average hetrozygosity of 76.3% (based on three DNA loci) for immigrant Vietnamese and Hongkong Chinese in Australia. Though the heterozygotes levels differ with respect to the type of loci used for the study, it also reflects the influence of population structure variables, especially marriage pattern, migration which need to be investigated for understanding the genetic diversity of the populations. The coefficient of gene differentiation Gst, which is a measure of the relative magnitude of genetic contribution that can be attributed to subdivision also differs between the type of DNA marker. The average Gst coefficient for three STR loci is 5.3% for the studied populations. Overall the Gst values are similar to other populations studied for Indian and World populations and it is similar to or equivalent to the values found in case of classical markers (Deka et al. 1994; Robinson et al. 1996, Das et al. 1996 etc.). But the values for the present study are lower that other such studies on Mongoloid population.

Investigating the genetic relationship based on available classical gene frequency data between four sets of populations, (of different castes and ethnicity) in eastern India, Roychoudhury (1992) observed that "all the Mongoloid affiliated populations in eastern India show genetic similarity with respect to geographical proximity, no matter whether they originated from the same tribal group or same linguistic family in the past". The available historical records suggest that different Mongoloid populations, especially in Manipur and other nearby states in northeast region are believed to be the migrants from Mynmar (Burma) and adopted different life styles in their pursuit for survival in their adopted region in the recent past. Recent studies based on STR loci also show clustering of northeastern populations with Chinese and other neighboring Mongoloid populations (Reddy et al. 2001) and form a separate cluster in comparison with other Indian populations (Dutta and Kashyap 2001; Dutta et al. 2002)

The genetic similarity possibly suggests common genetic affinity and least genetic differentiation irrespective of cultural differences among them, but show distinct differences with the Caucasoid populations of the region. However, Mukherjee et al. (1999) studying the genetic variation based on four STR loci among eight population groups in east India has observed the contradictory results, where the results obtain does not completely agree with the geographic similarity. The present study based on different set of DNA polymorphism supports the findings of Roychoudhury and do not completely agree with the findings of Mukherjee et al. (1999).

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