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Beta-Globin Gene Haplotypes in Manipur, North-east India

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ABSTRACT A sample of 204 individuals belonging to five different population units of Manipur, viz. the Meitei, Gangte, Thadou, other Kuki, and Kabui has been analyzed for beta globin gene cluster, considering seven loci to bring out a first hand information on haplotype frequencies of beta globin in the state. A common trend of allelic frequencies observed in the present data are the high frequencies of HindIIε, AvallIβ and HinflIβ and relatively low frequencies of HindlllGγ, HindIIIAγ, HindII5'ψβ and HindII3'ψβ. The frequency of Framework 1(presence of both AvaIIβ and HinfIβ sites) ranges from nil to 17.65% in the present data of Manipur. The frequency of Framework 2 ranges from 33% to 66% and that of Framework 3 from 33% to 55%. One noteworthy observation is the association of HbβEE exclusively with Framework 2, which suggests a similar mutational origin of HbE in South-east Asia and North-east India. Only five beta globin haplotypes are found in the present data. Haplotypes +——+ and +——+-, the most common in Japanese, Cambodians, Koreans, South Chinese, Samoans and Thais, are the most frequent haplotypes in Manipur too.

INTRODUCTION

The beta-globin gene cluster associated with haemoglobin S, E, and beta-thalassemia genes has been studied as a means of searching affinities and common origin among human populations (Antonaraki et al. 1982; Kazazian et al. 1984; Chan et al. 1986; Wainscoat et al. 1986; Hundrieser et al. 1988; Currat et al. 2002). Distribution of haplotypes associated with the βA gene is now known for a number of populations. The data indicate that Africans are the most diverged group, and that the first split separated an African group from an Eurasian one (Antonarakis et al. 1985; Oehme et al. 1985; Maggio et al. 1986; Wainscoat et al. 1986; Ramsay and Jenkins 1987; Shimizu 1987; Hundrieser et al. 1988 a and b; Shimizu et al. 1989; Yongvanit et al. 1989; Chen et al. 1990; Long et al. 1990; Trent et al. 1990; Varawalla et al. 1992; Hewitt et al. 1996; Fucharoen et al. 1997; Kaufman et al. 1998; Villalobos-Arámbula et al. 2000). In north-eastern part of India, Hundrieser et al. (1988) studied the beta globin gene cluster in Assam. The present paper brings out a firsthand information on the frequencies of beta globin gene cluster haplotypes in Manipur.

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AREA AND PEOPLE

The state of Manipur lies between the 23.83^0 and 25.68^0 N latitude and 93.03^0 and 94.78^0 E longitude and has an area of 22,327 sq. km. of which only 8% is the valley. The most populous community of the state is the Meiteis who mainly occupy the valley while the hills are mainly inhabited by two tribal groups, viz. the Kuki and Naga. There are 33 scheduled tribes, who have their own distinct culture and dialects. The present study comprises the Meitei (the major community of Manipur), Thadou and Gangte (as representatives of Kuki tribe), other Kuki tribes and the Kabui (as a representative of Naga tribe).

MATERIAL AND METHODS

About 4ml of intravenous blood samples from 204 healthy and unrelated individuals from the Meitei (75), Gangte (35), Thadou (45), Kabui (12) tribe and other tribes belonging to Kuki group (37) were collected in vecutainer, containing EDTA after obtaining their written consent. The blood samples were collected during July 2006 and April 2007. The Kabui samples were collected from the Tamenglong district, the Gangte, Thadou and other groups belonging to Kuki were collected from Churachandpur district, and the Meitei from Imphal east and west districts of Manipur. Analyses work was done in the DNA Laboratory of Anthropological Survey of India.

DNA was extracted by phenol-chloroform methods (Sambrook et al. 1989) with minor modifications and the seven restriction sites for beta globin gene cluster (HindIIε, HindIIIGγ, HindIIIAγ, HindII5'ψβ, HindII3'ψβ, AvaIIβ and HinfIβ) were amplified using the oligonucleotide primers (Weatherall and Clegg 2001) and the products were digested by respective endonucleases. The digested products were separated in agarose gels containing ethidium bromide. The presence or absence of specific bands was then verified under UV- transilluminator, using appropriate molecular weight marker. The frameworks were identified by the presence (+) or absence (-) of the AvaII restriction site in intron 2 of the beta globin gene and of the BhamHI site located 3' to the beta globin gene. The BhamHI RFLP is located within an L1 repetitive element creating amplification problems and a HinfI RFLP located just 3' to the beta globin gene is used instead, because these two RFLPs have been found to exist in linkage disequilibrium (Semenza et al. 1989). Presence of both restriction sites (++) indicates framework 1, presence of AvaII site together with the absence of the HinfI site indicates framework 2 and the absence of AvaII site and presence of HinfI site indicate framework 3 (Antonarakis et al. 1982). Calculation of allelic frequencies of the seven restriction sites and estimation of haplotype frequencies were estimated following Nei (1987) and Long (1995).

RESULTS AND DISCUSSION

Gene frequencies of the seven polymorphic restriction sites in the beta globin gene cluster of the present sample of five populations of Manipur are shown in Table 1. A common trend of allelic frequencies observed in these populations is the high frequencies of HindIIε, AvalIβ and HinflIβ and relatively low frequencies of HindlllGγ, HindIIIAγ, HindII5'ψβ, and HindII3'ψβ. The frequency of the presence of the HindIIε site in the populations increases from 0.60 in the Thadou to 0.75 in the Kabui, through other Kukis (0.608), Meitei (0.66) and the Gangte (0.729). Frequency of the presence of AvaIIβ site is greatest among the Kabui (0.583) followed successively by the Gangte (0.571), Meitei (0.567), Thadou (0.544) and other Kukis (0.514). The frequency of the presence of HinfIβ is also above 57% in all the other populations except the Kabui who shows only 45.8%. Sample size of the Kabui, of course, is the smallest, only 12 individuals. On the other hand, the greatest frequency of presence of HindIIIGγ site is 0.40, shown by the Thadou, which is followed by other Kukis (0.365), Meitei (0.34), Gangte (0.271) and the Kabui (0.208). The frequency of HindIIIAγ ranges from 0.25

Table 1: Allelic frequencies of seven beta globin loci in five populations of Manipur

	Meitei (N=75)	*Gangte* (N=35)	*Thadou* (N=45)	*Other Kuki* (N=37)	*Kabui* (N=12)	*Total* (N=204)
HindIIε						
—	0.34	0.271	0.4	0.392	0.25	0.352
++	0.66	0.729	0.6	0.608	0.75	0.648
HindIIIGγ						
—	0.66	0.729	0.6	0.635	0.792	0.654
++	0.34	0.271	0.4	0.365	0.208	0.346
HindIIIAγ						
—	0.74	0.757	0.667	0.689	0.75	0.716
++	0.26	0.243	0.333	0.311	0.25	0.284
HindII5'ψβ						
—	0.713	0.814	0.722	0.811	0.75	0.753
++	0.287	0.186	0.278	0.189	0.25	0.247
HindII3'ψβ						
—	0.647	0.729	0.6	0.635	0.75	0.648
++	0.353	0.271	0.4	0.365	0.25	0.352
AvaIIβ						
—	0.433	0.429	0.456	0.486	0.417	0.448
++	0.567	0.571	0.544	0.514	0.583	0.552
HinfIβ						
—	0.353	0.429	0.378	0.338	0.542	0.370
++	0.647	0.571	0.622	0.662	0.458	0.630

in the Kabui to 0.333 in the Thadou. Presence of HindII5'ψβ ranges from 0.187 in the Gangte to 0.287 in the Meitei and that of HindII3'ψβ ranges from 0.25 in the Kabui to 0.365 of Other Kukis.

Frequencies of Beta-globin gene frameworks (F.W.) are displayed in Table 2. Presence and absence in homozygous condition of the beta polymorphic restriction sites AvaIIβ and HinfIβ determines the frameworks. It appears that F.W. 3, the absence of AvaIIβ and presence of HinfIβ sites, shows greatest in other Kuki tribes (0.5556) followed by the Meitei (0.50), Thadou (0.4706), Gangte (0.4615) and the Kabui (0.3333). The frequency of F.W. 2 (presence of AvaIIβ and absence of HinfIβ) ranges from 0.3333 in the Meitei and Other Kukis to 0.6667 in the Kabui, through the Thadou (0.352) and Gangte (0.538). It is noteworthy that five heterozygous HbAE cases and all the homozygous HbEE cases were met exclusively in F.W.2. The beta thalassaemia carriers were, however, found to be associated with F.W. 3. Frequency of F.W.1 (presence of both AvaIIβ and HinfIβ sites) ranges from nil to 17.65% in the populations of Manipur, which is notably lower than the earlier reports of Asian Indians with 52% (Antonarakis et al. 1982; Kazazian et al. 1984; Old et al. 1984; Hundrieser et al. 1988; Varawalla et al. 1992). The high frequencies of F.W.2 and F.W.3 of the present data need further investigation. In a pioneering work, Antonarakis et al. (1982) reported the evidence of two different HbβE associated DNA frameworks in subjects from Cambodia and Thailand. F.W. 3 was exclusively found in Cambodians, suggesting an independent mutational origin of HbE in this population. Other framework of HbE, F.W. 2 was found among the Thai, Laotian and also among some Cambodians. Such an investigation, conducted by Hundrieser et al. in 1988 among the Kachari of Assam did not provide evidence for a separate mutational origin of HbβE in Assam, for the Kachari subjects were, by and large, characterized by F.W. 2. It is pertinent to mention that five heterozygous HbAE cases and all the homozygous HbEE cases were met exclusively in F.W.2. It suggests a common mutational origin of HbE in South-east Asia and north-east India. At the present state of knowledge, the presumption of 'Austroasiatic speaking people as the original owner of HbβE' (Flatz 1967; Das et al. 1971; Flatz et al. 1972) is neither supported by the available evidences, nor the high frequency of HbβE in north-east region can be explained by a possible independent mutational origin of this gene.

Table 2: Frequencies of the beta-globin gene frameworks in five populations of Manipur

Ethnic groups	*Chromosomes*	*Framework 1*	*Framework 2*	*Framework 3*
Meitei	72	0.167	0.333	0.500
Gangte	26	0.000	0.539	0.462
Thadou	34	0.176	0.353	0.471
Other Kuki	36	0.111	0.333	0.556
Kabui	6	0.000	0.667	0.333
Total	174	0.1264	0.3793	0.4943

The beta-globin gene cluster haplotype frequencies in five populations of Manipur are displayed in Table 3. Altogether five beta globin haplotypes are found so far in Manipur. Haplotype +——+, the most frequently encountered in Japanese, Cambodians, Koreans, South Chinese, and Samoans (Antonarakis et al. 1982; Cheng et al. 1984; Chan et al. 1986; Liu et al. 1987; Shimizu 1987; Hundrieser et al. 1988; Shimizu et al. 1989; Trent et al. 1990; Shimizu et al. 1992;), is also found to be most common type in the populations of Manipur. Its frequency is highest in the other Kuki tribes (0.625) followed by the Gangte (0.50), Thadou (0.50) and the Meitei (0.454). Among the Kabui the haplotype +——+-, the most common in Thais (Hundrieser et al. 1988; Yongvanit et al. 1989), is the only representing type. The frequency of haplotype +——+- in the remaining popula-

Table 3: Beta globin haplotype frequencies in five populations of Manipur

Haplotypes	*Polymorphic sites*							*F.W.*	*Meitei*	*Gangte*	*Thadou*	*Other Kuki*	*Kabui*	*Total*
	1	2	3	4	5	6	7							
A	+	-	-	-	-	+	+	1	0.182	0.000	0.250	0.000	0.00	0.085
B	+	-	-	-	-	+	-	2	0.273	0.500	0.000	0.375	1.00	0.371
C	-	+	+	+	+	+	-	2	0.000	0.000	0.250	0.000	0.00	0.029
D	+	-	-	-	-	-	+	3	0.454	0.500	0.500	0.625	0.00	0.486
E	+	-	-	+	-	-	+	3	0.091	0.000	0.000	0.000	0.00	0.029

tions ranges from nil among the Thadou to 0.50 in the Gangte through 0.273 in the Meitei, 0.375 in Other Kuki. The haplotype -+++++- is encountered exclusively among the Thadou tribe with a frequency of 0.25 while the haplotype +—+—+ is found only in the Meitei population (0.091). The Meitei and Thadou exhibit the haplotype +——++ frequencies of 0.182 and 0.25 respectively. This haplotype +——++ is most frequently encountered in Colombian Amerinds, Mediterraneans, Asian Indians, and Mexican Mestizos (Shimizu 2004).

To conclude, it may be recapitulated that the beta-globin gene cluster haplotypes in Manipur show closer similarity with the people of east and south-east Asian populations. A common mutational origin of HbβE in north-east Indian and the South-east Asian populations is also expected.

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