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A NEW HAPLOTYPE OF THE BETA GLOBIN GENE COMPLEX (HBB^b) IN INDIAN FIELD MOUSE *MUS BOODUGA*

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ABSTRACT

Electrophoretic survey of hemoglobin beta chain (Hbb) polymorphism in Indian pygmy field mouse *Mus booduga* has revealed a new haplotype Hbb^b. Two distinct genes, Hbb-b1 and Hbb-b2 encode for major and minor components of adult Hbb respectively. Previously determined Hbb haplotypes Hbb^s, Hbb^d, Hbb^p and Hbb^{w1} show same electrophoretic mobility of major component. So far variation has been reported only for the mobility in minor component of the different haplotypes except Hbb^s which shows single electrophoretic band. I demonstrate major component of faster mobility in *Mus booduga*. Although major component of Hbb of faster mobility has been shown by induced mutation, it has not been reported in the natural population. Therefore, the presence of distinct mobility major band of hemoglobin beta chain in *Mus booduga* is interesting and has been designated as Hbb^b.

Key words : *Mus terricolor*, *Mus booduga*, Hemoglobin beta chain

INTRODUCTION

Polymorphism of hemoglobin beta-chain (Hbb) is widespread in natural populations of house mouse, *Mus musculus* (Berry, 1978), and five haplotypes Hbb^s,Hbb^d, Hbb^p, Hbb^{w1} and Hbb^{w2} have been characterized (Ranney and Gluecksohn-Waelsch, 1955; Morton, 1962; Whitney III, 1978; Kawashima et al, 1991; Sato et al, 2008; Runck et al, 2009). Hemoglobin beta-chain exists as the beta-globin gene complex; two genes Hbb-b1 (encodes beta-chain subunits of major Hb isoform) and Hbb-b2 (encodes the beta-chain of minor Hb isoform) encode the adult β-globin (beta globin) chains. In Hbb^s, which exhibits a single electrophoretic band, the Hbb-b2 is a complete duplicate of Hbb-b1 while Hbb^d and Hbb^p haplotypes form two bands each. Gene Hbb-b2^d encodes a minor component (d_{minor}) which is different from Hbb-b2^p (p_{minor}), but the major component Hbb-b1^d and Hbb-b1^p (d_{major} and p_{major}) is the same in both haplotypes (Kawashima et al, 1991; Runck et al, 2009). A new haplotype Hbb^{w1}

in *M. musculus* from China was reported by Kawashima et al, 1991, in which the variation was observed in the mobility of the minor band, which showed a slower electrophoretic mobility than those of the Hbb^d and Hbb^p while the mobility of the major components of Hbb^d, Hbb^p and Hbb^{w1} were the same. Nucleotide sequences of the mouse globin beta gene cDNAs in wild derived new haplotype Hbb^{w1} indicated that the Hbb gene complex of the Hbb^p haplotype carries Hbb-$b1^d$ and Hbb-$b2^{w1}$ genes and is probably a recombinant between Hbb-$b1^d$ and Hbb-$b2^{w1}$ haplotypes (Ueda et al, 1999).

Another new Hbb haplotype Hbb^{w2} in a wild-derived house mouse, *Mus musculus,* was reported by Sato et al., 2008. Compared to the previously determined Hbb haplotypes, d, p, and w1, the Hbb-b1 gene and ca. 11 kb of the spacer region of Hbb^{w2} were most similar to the w1 haplotype; however, the remainder of the spacer region and the Hbb-b2 gene were most similar to the d haplotype but may represent a still uncharacterized and divergent haplotype (Sato et al, 2008).

The different haplotypes distinguished by electrophoretic methods, have their characteristic distribution pattern in different populations of house mice. Compared to the world-wide distribution of Hbb^d (Miyashita et al, 1985), Hbb^s is restricted to the European and North and South American populations (Selander et al, 1969a; Selander et al, 1969b; Selander and Yang, 1969; Petras et al, 1969; Roderick et al, 1971; Britton and Thaler, 1978; Bonhomme et al, 1984). The populations from Central Asia (including Israel) and Eastern Asia (including Japan and Thailand) have Hbb^p haplotype (Miyashita et al, 1985; Minezawa et al, 1979; Bonhomme et al, 1989; Boursot et al, 1989). So far, Hbb^{w1} has been observed only in northwestern China (Kawashima et al, 1991). Haplotype Hbb^{w2} was reported by Sato et al., 2008 in a wild-derived house mouse, *Mus musculus,* collected near Lake Balkhash in the Republic of Kazakhstan.

The house mouse *Mus musculus* and the Indian pygmy field mice *Mus booduga* and *Mus terricolor* belong to two sister lineages under the subgenus *Mus. M. booduga* and *M. terricolor* are co-existing sibling species but they differ in site-preference for burrows and in their burrow-patterns (Cheong, 1986; Sharma, 1996; Singh et al, 2009). The three apparently non-overlapping chromosomal species *M. terricolor* I, II & III nevertheless have identical burrow-patterns. Study of the hemoglobin beta-chain (Hbb) haplotypes distinctly showed that Hbb^d and Hbb^p haplotyes occurred predominantly in the Indian house mouse *Mus musculus tytleri* and the *M. terricolor* complex, respectively. Interestingly, *M. booduga* possessed a new haplotype, which has

been designated as Hbb^b. I report here the occurrence of the new haplotype which displays a faster moving major component of hemoglobin beta-chain.

MATERIALS AND METHODS

Wild mice were collected from different places in India by digging burrows in cultivated fields. Individuals of *M. booduga* were collected from Varanasi (V) in the north (81 metres above sea level) and Mysore (M) in the south (770 metres above sea level in between the Eastern and Western ghats, towards south) separated by about 2000 Km. *M. terricolor* I, II and III were collected from Varanasi, Mysore and Chennai (east side of Eastern ghats 8 metres above sea level), respectively. Mysore and Chennai (=Madras) are separated by about 450 Km. House mouse *M. m. tytleri* individuals were collected from houses and shops in Varanasi and Delhi (293 metres above sea level) which are 700 Km apart. This study has been approved by the institutional ethical committee.

Sample Preparation

For hemolysate preparation, blood was collected from each individual separately in eppendorf tubes from supra orbital vein by capillary (heparinised) pricking.

Hemolysate was prepared following the method of Selander et al., 1971 with slight modifications. The blood was centrifuged at 4000 rpm for 10 min at 4^oC to separate the serum. The RBCs pellet was washed twice in approximately 10 volume of 0.85% cold saline. The lysis was carried out by adding deionized water to RBCs (equal volume to that of blood) and half volume of toluene and shaken for 1 minute. The suspension was centrifuged at 22,000 rpm for 20 min at 4^oC and the clear hemolysate (supernatant) was carefully pipetted out in a fresh tube followed by polyacrylamide gel electrophoresis within 24 hrs of preparation.

Electrophoresis

Electrophoresis for Hbb was carried out using a thin-layer 7.5% polyacrylamide vertical gel. Equal amounts of hemolysates were loaded with a loading buffer [0.1% bromo-phenol blue, 40% (w/v) sucrose in distilled water]. Gels were run in 1X TBE buffer [89 mM boric acid, 2.5mM EDTA, pH 8.0] for 4-5 hours at a constant 250 V at 4^oC.

Staining

Gels for hemoglobin were stained in 0.2% Commassie Brilliant Blue R [0.2 gm CBB in 100 ml of 45% methanol and 10% acetic acid] for 4-6 hours at

room-temperature. Gels were then diffusion destained in destaining solution [45% methanol and 10% acetic acid] repeatedly until background stain was removed. The gels were fixed in 7% acetic acid.

RESULTS

Electrophoresis of hemolysates of the house mouse *M. m. tytleri*, the field mice *M. booduga* and the three chromosomal species *M. terricolor* I, II, III revealed that the distribution of hemoglobin haplotypes was very characteristic. The *M. terricolor* complex studied from northern as well as southern India was found to have only the Hbb^p haplotype compared to predominant Hbb^d haplotype (frequency 0.9333 & 0.833) and a less frequent Hbb^p in the two widely separated house mouse populations (Table 1). *M. booduga* exhibited a less frequent Hbb^p and a predominant haplotype, different from Hbb^d and Hbb^p. The electrophoretic pattern of this haplotype showed a faster moving major component which, to the best of our knowledge, has not been observed in other cases (Fig. 1). This haplotype has been designated as Hbb^b. The haplotype Hbb^s was absent in these populations of mice studied from the subcontinent (Table 1).

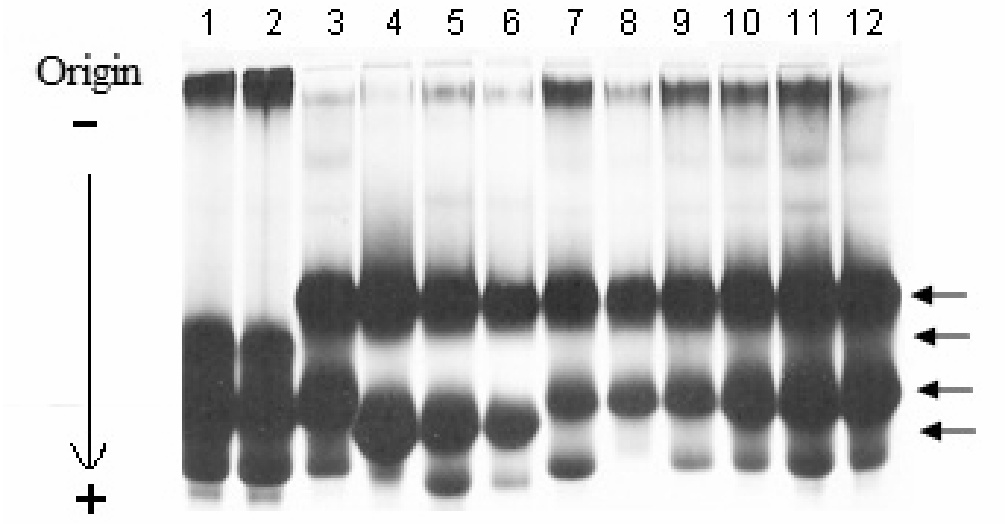

Fig. 1 : Electrophoregram showing the band pattern of hemoglobin β – chain. Lanes 1, 2 *M.m.tytleri* (V); lanes 3, 4 *M. booduga* (M); lanes 5, 6 *M. booduga* (V); lanes 7, 8 *M. terricolor* I; lanes 9, 10 M. terricolor II; lanes 11, 12 *M. terricolor* III. Please note major band of faster mobility in lanes 4-6 (arrow).

Table 1. Allele frequencies of Hbb (Hemoglobin beta– chain) haplotypes in Indian house and wild pygmy field mice with sample size (N) in parenthesis.

Loci	Tytleri (Varanasi)	Tytleri (Delhi)	Booduga (Mysore)	Booduga (Varanasi)	Terricolor I	Terricolor II	Terricolor III
Hbb							
d	0.933	0.833	-	-	-	-	-
p	0.067	0.167	0.067	-	1.000	1.000	1.000
b	-	-	0.933	1.000	-	-	-
(N)	(15)	(12)	(15)	(19)	(18)	(15)	(19)

DISCUSSION

Of the typical three haplotypes $Hbb^{s,}$ Hbb^{d}, $Hbb^{p,}$ present in different house mouse populations, Hbb^{d} has been reported to be very common throughout the world.The two other haplotypes (Hbb^{p} and Hbb^{s}) have been found to have restricted distribution (Miyashita et al, 1985; Selander et al, 1969a; Selander et al, 1969b; Selander and Yang, 1969; Petras et al, 1969; Roderick et al, 1971; Britton and Thaler, 1978; Bonhomme et al, 1984; Minezawa et al, 1979; Bonhomme et al, 1989; Boursot et al, 1989). In all the three haplotypes the variation observed was in the mobility of the minor band and/or its absence. Kawashima et al., 1991 reported a new haplotype Hbb^{w1} in *M. musculus* from China in which the variation was observed in the mobility of the minor band while the mobility of the major components of Hbb^{d}, Hbb^{p} and Hbb^{w1} were the same. In the present study also Hbb^{d} was found to be the predominant haplotype in house mouse *Mus musculus tytleri* and Hbb^{p} as the predominant haplotype in the three chromosomal species of *M. terricolor* complex. But *M. booduga* showed the presence of a distinct mobility variation in the major band of the hemoglobin beta-chain.The variation in mobility of the major band in any of the haplotypes has not been reported from natural populations in the mouse systems. Therefore, the presence of a distinct mobility variation in the major band of hemoglobin in *M. booduga* is interesting. An increased anodal mobility of the major component of a structurally altered Hbb as a result of a mutation induced by alkylating agent ethylnitrosourea, has been reported by Peters et al, 1985 which has been found to be associated with increased oxygen affinity.

Natural populations of house mice are often polymorphic for distinct two-locus haplotypes that differ in levels of functional divergence between duplicated beta-globin genes, Hbb-b1 and Hbb-b2 (Runck et al, 2009). The beta-globin gene family of house mice (genus *Mus*) represents an especially promising system for evaluating the effects of gene conversion on the functional divergence of duplicated genes. The various alpha- and beta- chain hemoglobin isoforms of house mice are characterized by different O_2–binding affinities (Newton and Peters, 1983; D'Surney and Popp, 1992). Modifications of hemoglobin function often play a key role in adaptation to high-altitude hypoxia (Perutz, 1983; Monge and Leon-Velarde, 1991; Poyart et al, 1992; Storz, 2007) as well as in the occupied burrows of rodents where hypoxic/hypercapnic situation exists (Singh et al, 2009; Shams et al, 2004; 2005). Surveys of hemoglobin variation in different populations of house mouse and field mouse may be especially informative about the role of selection in shaping patterns of functional variation at globin genes.

House mice colonized the New World within the past several hundred years in conjunction with human movement and settlement (Auffray, 1990; Guenet and Bonhomme, 2003), and they have succeeded in colonizing a diverse range of environments. Survey of the allelic distribution at Hbb locus in *Mus musculus* populations of Asia, Europe, North and South America showed a clear pattern of geographical distribution of the typical three kinds of haplotypes (Miyashite et al, 1985; Runck et al, 2009). Patterns of geographical variation in beta-globin allele frequency may reflect adaptation to different climatic conditions.

Converging points of evidence from phylogenetic studies showed Hbb-b1 and Hbb-b2 genes in *Mus* species to exhibit full range of evolutionary outcomes in terms of the levels of interparalog divergence. While two identical Hbb paralogs on the Hbb^s haplotype (shared by *Mus domesticus, Mus musculus,* and *Mus spretus*) represent a classic example of concerted evolution, the two distinct Hbb paralogs on the Hbb^d, Hbb^p, Hbb^{w1}, and Hbb^{w2} haplotypes (shared by multiple species in the subgenus *Mus*) are distinguished by a number of functionally important amino acid substitutions. The variation in the level of functional divergence between Hbb-b1 and Hbb-b2 may underlie its important physiological variation (Runck et al, 2009).

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