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*Bam*H1 POLYMORPHISM IN THE CHINESE, MALAYS, AND INDIANS IN SINGAPORE AND ITS APPLICATION IN THE PRENATAL DIAGNOSIS OF β-THALASSEMIA

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Summary The distribution of restriction fragment length polymorphism (RFLP) at the *Bam*H1 site of the β-globin gene was investigated in the Chinese, Indian, and Malay race in Singapore. The sample comprised of 183 normal individuals and 35 β-thalassemia carriers in which 13 were couples with at least one β-major child. The results from this study indicate that *Bam*H1 polymorphism will be informative in 22% of pregnancies at risk for β-thalassemia major in Chinese, 19% in Malays and 7% in Indians. In prenatal diagnosis using *Bam*H1 polymorphism for one β-major affected family, the fetus was diagnosed to be normal or β-carrier. The validity of *Bam*H1 polymorphism in the exclusion of β-thalassemia major was subsequently confirmed at birth by globin chain biosynthesis.

Key Words RFLP, *Bam*H1, prenatal diagnosis, β-thalassemia

INTRODUCTION

The β-thalassemias are characterized by a reduced output of β-chains of hemoglobin. Couples who are β-thalassemia carriers have a 25% risk of producing a homozygous β-thalassemic child (Weatherall, 1983).

Prenatal diagnosis of β-thalassemias has been carried out using globin chain biosynthesis (Modell, 1983), DNA polymorphisms (Boehm *et al.*, 1983; Huang *et al.*, 1985) and oligonucleotide probes (Rosatelli *et al.*, 1985; Cai *et al.*, 1988). The *Bam* H1 polymorphism located at 3′ to the β-globin gene has been used for prenatal diagnosis of homozygous β-thalassemia in Sardinians (Kan *et al.*, 1980) and Chinese (Chan *et al.*, 1984). The presence of the *Bam* H1 polymorphic site produces a 22 kb fragment instead of the 9.3 kb fragment in normal and β-thalassemia carriers

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while homozygotes produce only the 9.3 kb *Bam*H1 fragment. Therefore presence of the 22 kb fragment indicates a normal or β-thalassemia trait and excludes β-major. The authors present here the distribution of the *Bam*H1 polymorphism in normal individuals and those heterozygous and homozygous for β-thalassemia gene in the Chinese, Indians, and Malays in Singapore. *Bam*H1 polymorphism at the β-globin gene region has not been studied before in the Indian and Malay race.

MATERIALS AND METHODS

Sample. The sample comprised of 80 Chinese (57 normals, 18 β-carriers, 5 homozygotes), 50 Indians (45 normals, 3 carriers, 2 homozygotes), and 53 Malays (32 normal, 14 carriers, and 7 homozygotes). Thirteen families with at least one β-major child each were studied. One family requested prenatal diagnosis at 10 weeks of gestation.

Chorionic villi (CV) was obtained at 10 weeks gestation by a transabdominal approach under ultrasound guidance. DNA extraction from white blood cells and CV was carried out as previously described (Tan *et al.*, 1989).

DNA study. DNA (10 μg) was digested with *Bam*H1 (Amersham International, England), size fractionated in 0.8% agarose and transferred onto Hybond-N membrane (Amersham) by Southern blotting (Southern, 1975). The filter was hybridized overnight with an α-32P dCTP labeled 4.3 kb *Pst* 1 fragment of the β-globin gene (Feinberg and Vogelstein, 1983) and then washed under stringent conditions before autoradiography.

Globin chain biosynthesis. Cord blood was obtained at birth in preservative-free heparin tubes. Globin was labeled with [^{3}H]leucine (Alter, 1983) and globin chains separated by CM-sepharose chromatography (Wong *et al.*, 1988).

RESULTS AND DISCUSSION

Figure 1 shows the three *Bam*H1 genotypes: 9.3/9.3, 9.3/22, and 22/22 kb patterns. The normal genotype (9.3/9.3) was seen in wells 4, 5, 7, 8, 9, and 13. The variant 9.3/32 kb pattern was observed in wells 3, 10, and 12 and the variant 22/22 kb pattern was observed in well 14. DNA from normal individuals or β-thalassemia carriers digested with *Bam*H1 produced all three genotypes. In contrast, DNA from all β-major patients produced only the 9.3/9.3 kb pattern. Table 1 shows the distribution of *Bam*H1 fragments containing the 3′ β-globin in the three races in Singapore. The 22 kb *Bam*H1 site found in 22% of Chinese here was slightly lower than that observed in Hong Kong (29%, Chan *et al.*, 1984). The prevalence of the site was 0.19 in the Malays and 0.07 in Indians.

Thirteen families with homozygous β-thalassemia were studied and six families showed the polymorphic *Bam*H1 site. In the prenatal diagnosis for β-thalassemia,

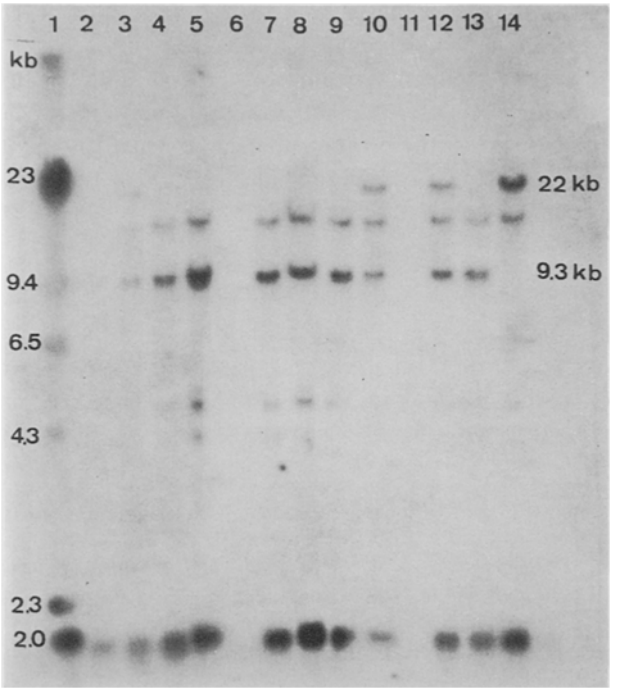

Fig. 1. Autoradiograph of *Bam*H1 digested DNA hybridized with ^{32}P-labeled β-globin gene probe.
lane 4, 5, 7, 8, 9, 13 : 9.3/9.3 kb pattern
lane 3, 10, 12 : 9.3/22 kb pattern
lane 14 : 22/22 kb pattern

Table 1. Distribution of the polymorphic 22 kb *Bam*H1 fragment in three racial groups in Singapore.

		*Bam*H1 pattern			Prevalence	
	n	9.3/9.3	9.3/22	22/22	9.3	22
Normal						
Chinese	57	37	15	5	0.78	0.22
Indian	45	40	4	1	0.93	0.07
Malay	32	24	4	4	0.81	0.19
β-carriers						
Chinese	18	14	4	0	0.89	0.11
Indian	3	2	1	0	0.83	0.17
Malay	14	13	1	0	0.96	0.04
β-homozygotes						
Chinese	5	5	0	0	1	0
Indian	2	2	0	0	1	0
Malay	7	7	0	0	1	0

DNA from the fetus at risk produced both the 9.3 and 22 kb fragments indicating that the fetus was not a β-thalassemia major. The absence of β-major disease in the fetus was confirmed by globin chain biosynthesis using cord blood at birth.

The 22 kb *Bam*H1 polymorphic site can be utilized in prenatal diagnosis in families where one parent has the polymorphic 22 kb fragment. Our results showed the site to be informative in 22% of pregnancies at risk for β-major in Chinese, 19% in Malays, and 7% in Indians.

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