

# A New Polymorphism in the Human $\beta$ -Globin Gene Useful in Antenatal Diagnosis

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**ABSTRACT** A new polymorphism in the  $\beta$ -globin gene is described, using the restriction enzyme Asu I. A radioactive probe specifically representing the large intervening sequence (IVS 2) of the  $\beta$ -globin gene has been used to detect this polymorphism. Normally, a 0.8-kilobase fragment containing  $\beta$ -IVS 2 is generated by Asu I; however, a 1.0-kilobase fragment is seen in association with 18% of  $\beta^A$ -genes, and 38% of  $\beta$ -thalassemia genes in an Israeli population studied. By contrast, the Asu I polymorphism has rarely been seen in blacks examined to date. An additional Asu I change is seen in the  $\delta$ -globin gene with a  $\delta$ -IVS probe. The  $\beta$ -Asu I polymorphism is shown to be useful in the antenatal diagnosis of  $\beta$ -thalassemia.

## INTRODUCTION

Polymorphisms of nucleic acid sequences within and adjacent to the  $\beta$ -globin gene complex have recently afforded a new approach to the antenatal detection of  $\beta$ -globin chain variants and  $\beta$ -thalassemia ( $\beta^T$ )<sup>1</sup> (1-5). Kan and Dozy (1) initially reported a polymorphism in the DNA sequence for an Hpa I restriction endonuclease site 5 kilobases (kb) 3' to the  $\beta$ -globin gene with the sickle mutation ( $\beta^S$ ). A nucleotide change in the Hpa I site generates a 13.0-kb fragment in association with ~70% of  $\beta^S$  genes in blacks, whereas >90% of  $\beta^A$  genes are contained in a normal 7.6-kb fragment (1, 2). When the linkage phase is known in a pedigree, the Hpa I polymorphism will allow antenatal diagnosis of sickle cell disease (SS) in ~40-50% of couples at risk.

Another polymorphic site in the large intervening sequence (IVS 2) of both the  $\gamma$ - and  $\delta$ -genes is recog-

nized by the enzyme Hind III (3). Use of the Hind III polymorphism in conjunction with Hpa I has increased the feasibility of prenatal diagnosis to >80% in AS couples (4). Another polymorphic site 3' to the  $\beta$ -gene is recognized by the enzyme Bam HI (5). A nucleotide change in the Bam HI site leads to the presence of a 22-kb fragment rather than the normal 8.3-kb fragment. In a Sardinian population with a high incidence (originally reported as 9.3 kb) of  $\beta^0$ -thalassemia, this polymorphism is potentially useful because the 22-kb fragment is associated with one-third of  $\beta^A$ -genes, whereas the  $\beta^0$ -thalassemia gene is only found with an 8.3-kb fragment (5).

A  $\beta$ -cDNA probe was used to detect globin gene-containing restriction fragments in all of these previous studies. We now report the use of probes representing the  $\delta$ - and  $\beta$ -IVS 2 regions to detect additional polymorphisms in the  $\delta$ - and  $\beta$ -genes. We have found a polymorphism of an Asu I site in the  $\beta$ -IVS 2 of both  $\beta^A$ - and  $\beta^T$ -genes in Israeli, Jewish, and Arab populations, and lack of this polymorphism in United States black  $\beta^A/\beta^A$  and  $\beta^S/\beta^S$  populations. The polymorphism is also associated with the  $\beta^A$ -gene in a small West African population studied. This polymorphism may be useful in antenatal diagnosis of homozygous  $\beta$ -thalassemia in Israeli populations, where it is found in significant frequency. We also report a change in an Asu I site in the  $\delta$ -structural gene of two Italian and one Iranian patients with  $\beta^+$ -thalassemia, and a patient with HbH disease; this site was not found to be polymorphic in the Israeli or black populations studied.

## METHODS

Peripheral blood samples from  $\beta^0$ - and  $\beta^+$ -thalassemia homozygotes and heterozygotes of Kurdish Jewish, or Arabic origins, and normal individuals from heterogeneous Jewish groups (Sephardic, Ashkenazi, and Kurdish), were obtained from Israeli subjects. Normal HbA samples from West Africans were from Mali subjects. The Israeli  $\beta^+$ - and  $\beta^0$ -thalassemia homozygotes all exhibit clinical features of severe homozygous  $\beta$ -thalassemia, and most have been studied at the level of

*Received for publication 20 April 1981 and in revised form 22 June 1981.*

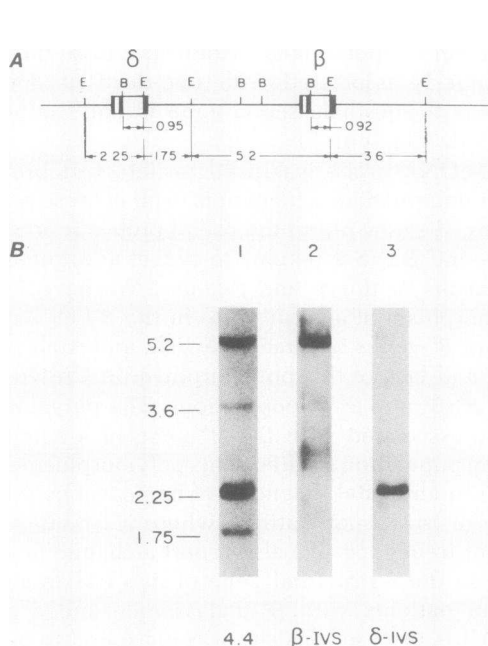
<sup>1</sup>Abbreviations used in this paper:  $\beta^A$ , normal  $\beta$ -globin gene;  $\beta^S$ ,  $\beta$ -globin gene with the sickle mutation;  $\beta^T$ ,  $\beta$ -thalassemia gene; IVS 1, small intervening sequence; IVS 2, large intervening sequence; kb, kilobase; SS, sickle cell disease.

globin synthesis and  $\beta$ -mRNA content (6, 7). Heterozygotes have the classically high HbA<sub>2</sub> of the  $\beta$ -thalassaemia-trait.

High molecular weight DNA was prepared from blood samples as described (8). 10  $\mu$ g of DNA were cleaved with Asu I or Bam HI, according to specifications of the manufacturer, and electrophoresis was performed using 0.8 or 1.0% agarose gels. The DNA fragments were transferred to nitrocellulose filters, hybridized to [<sup>32</sup>P]DNA probes, and autoradiography was performed as described (8). The probes used were a 0.92-kb  $\beta$ -IVS 2 and a 0.95-kb  $\delta$ -IVS 2 specific probe, prepared in our laboratory by isolation of Eco RI/Bam HI digested fragments from cloned  $\delta$ - and  $\beta$ -globin genes and subsequent subcloning in pBR 322 (9). Each of the probes was isolated from pBR 322 after growth, and labeled by nick translation to specific activities between 1 and  $8 \times 10^8$  cpm/ $\mu$ g (9).

## RESULTS

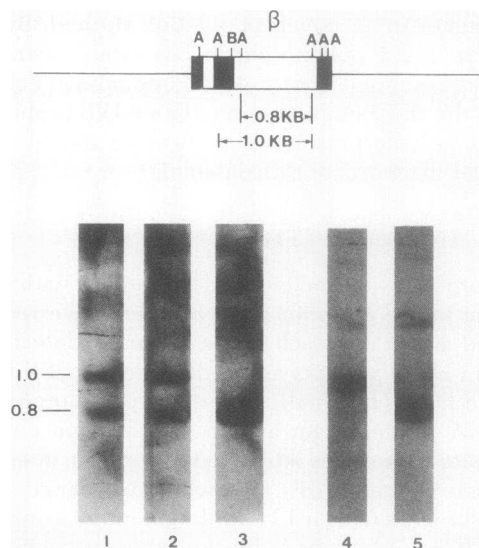
**Specificity of  $\delta$ - and  $\beta$ -IVS probes.** Specificity of the  $\delta$ - and  $\beta$ -IVS probes was demonstrated by hybridization to Eco RI and Eco RI/Bam HI digested DNA from normal individuals. As shown in Fig. 1, the  $\beta$ -IVS probe only hybridizes to the 5.2-kb fragment, and the  $\delta$ -IVS only hybridizes to the 2.25-kb fragment, indicating their specificity for these Eco RI fragments. When normal DNA is cleaved with both Eco RI and



**FIGURE 1** Specificity of hybridization of  $\delta$ - and  $\beta$ -IVS 2 probes. (A) Restriction enzyme map of the human  $\delta$ - and  $\beta$ -gene complex indicating Eco RI (E) and Bam HI (B) sites. The clear areas within the rectangles indicate the IVS, and the black areas, the coding sequences. The  $\delta$ -IVS 2 is contained in a 2.25-kb fragment and the  $\beta$ -IVS 2 in a 5.2-kb fragment when DNA is digested with Eco RI. (B) Autoradiographs of Eco RI-digested DNA hybridized with various probes (for details see text). Lane 1: 4.4-kb <sup>32</sup>P-cloned DNA probe; lane 2: <sup>32</sup>P-labeled  $\beta$ -IVS 2 probe; lane 3: <sup>32</sup>P-labeled  $\delta$ -IVS 2 probe. Only the 5.2-kb fragment hybridizes in lane 2, and only the 2.25-kb fragment in lane 3.

Bam HI, 0.92-kb  $\beta$ -IVS 2 and 0.95-kb  $\delta$ -IVS 2 fragments are generated, which hybridize specifically to the  $\beta$ -IVS 2 or  $\delta$ -IVS 2 probe, respectively (data not shown). Cross-hybridization between these probes and other regions of the  $\beta$ - or  $\delta$ -genes not containing IVS 2,  $\gamma$ -globin coding, or IVS nucleotide sequences, is not observed.

**Asu I polymorphism in the  $\beta$ -IVS.** The enzyme Asu I recognizes the sequence GCNCC (10), and cleaves at 5' and 3' sites within the  $\beta$ -IVS (11), generating a 0.8-kb fragment in normal DNA (Fig. 2). Loss of the 5' Asu I site would be expected to generate a 1.0-kb fragment, whereas loss of the 3' Asu site would produce a 0.87-kb fragment. In a series of Israeli DNA studied, an Asu I polymorphism, generating a 1.0-kb fragment is observed in 51% of patients (Table I). To determine which Asu I site is lost in this DNA, an Asu I/Bam HI double digestion was performed. As seen in Fig. 2, the Asu I/Bam HI digest cleaves the 1.0-kb fragment to 0.8 kb. This indicates that the 5'  $\beta$ -IVS Asu I site is altered, as Bam HI, which normally cleaves 30 nucleotides 5' to this site, cuts down the 1.0-kb fragment to 0.8 kb. The Asu I fragment size would not have been affected



**FIGURE 2** Asu I fragments hybridizing to  $\beta$ -IVS 2 probe. At the top is a diagram of the  $\beta$ -globin gene with its Asu I (A) and Bam HI (B) sites. The light areas are the IVS, and the dark areas the coding regions. The normal 0.8-kb fragment is shown. The loss of the Asu I site at the 5' end of IVS 2 leads to a 1.0-kb fragment. The lower part of the figure shows autoradiographs of cellular DNA digested with Asu I hybridized to <sup>32</sup>P-labeled  $\beta$ -IVS 2 probe (see text for details). Lanes 1 and 2 show heterozygotes for 0.8- and 1.0-kb Asu I fragments; lane 3 shows a 0.8-kb homozygote. Lane 4 is a homozygote for the 1.0-kb fragment and lane 5 shows the result of double digestion of the DNA in lane 4 with Asu I and Bam HI showing shortening of the 1.0-kb fragment to 0.8 kb as expected if the 1.0-kb fragment is generated by loss of an Asu I site at the 5' end of  $\beta$ -IVS 2. The bands >1.0 kb seen in lanes 2-5 are not reproducible in different experiments and are artifacts.

TABLE I

Israeli	Asu I genotypes		
	0.8/0.8 kb	0.8/1.0 kb	1.0/1.0 kb
$\beta^T$ -homozygotes	4	7	1
$\beta^T$ -heterozygotes	7	7	0
$\beta^A$ -homozygotes	7	4	0
	18 (48.6%)	18 (48.6%)	1 (2.8%)

by Bam HI digestion if the 3'  $\beta$ -IVS Asu I site were altered.

In Israelis, the normal 0.8-kb Asu I fragment is observed with 82% of  $\beta^A$ -genes and 62% of  $\beta^T$ -genes, and the 1.0-kb fragment is seen in 18% of  $\beta^A$  and 38% of  $\beta^T$ -genes (Table II). The polymorphism is found in all Jewish groups studied: Kurdish Jews: 7 of 11  $\beta^T\beta^T$ , 6 of 12  $\beta^A\beta^T$ , and 1 of 2  $\beta^A\beta^A$ ; Ashkenazi: 2 of 5  $\beta^A\beta^A$ , Sephardic: 1 of 2  $\beta^A\beta^A$ . Among the Arabs the polymorphism was found in 1  $\beta^T\beta^T$  and 1 of 2  $\beta^A\beta^T$ . By contrast, in a New York black population, only the 0.8-kb fragment is seen in association with all  $\beta^A$  and  $\beta^S$  genes. However, in a small group of West African (Mali) DNA, the 0.8-kb fragment is seen in 20 of 22 and the 1.0-kb fragment in 2 of 22  $\beta^A$ -genes.

**Asu I site heterogeneity in the  $\delta$ -structural gene.** The nucleotide sequence of the normal  $\delta$ -gene indicates Asu I sites in the second and third coding regions of the gene generating a 1.1-kb fragment containing the  $\delta$ -IVS (12) (Fig. 3). However, in this study we note instead a 3.0-kb Asu  $\delta$ -fragment in all Israeli patients and American and African blacks studied. This 3.0-kb fragment is produced by Asu I cleavage at the 5' site in the second coding sequence of the  $\delta$ -gene, and a site 1.5 kb 3' to the  $\delta$ -gene. The 3' site is confirmed by an Asu I/Pst I digest that cleaves the normal 3.0-kb to a 1.6-kb and a 1.4-kb fragment (Fig. 3). We have observed a 1.1-kb fragment in DNA of two  $\beta^+$ -thalassemics

TABLE II

$\beta^T$ :	Asu I gene frequency	
	0.8 kb	1.0 kb
Israeli		
$\beta^S$	9	1
$\beta^+$	4	4
$\beta^?$	2	4
Total	15 (62%)	9 (38%)
Normals ( $\beta^A$ )	27 (82%)	6 (18%)
United States blacks		
$\beta^S$	51 (100%)	0 (0%)
$\beta^A$	19 (100%)	0 (0%)
West African blacks		
$\beta^A$	20 (91%)	2 (9%)

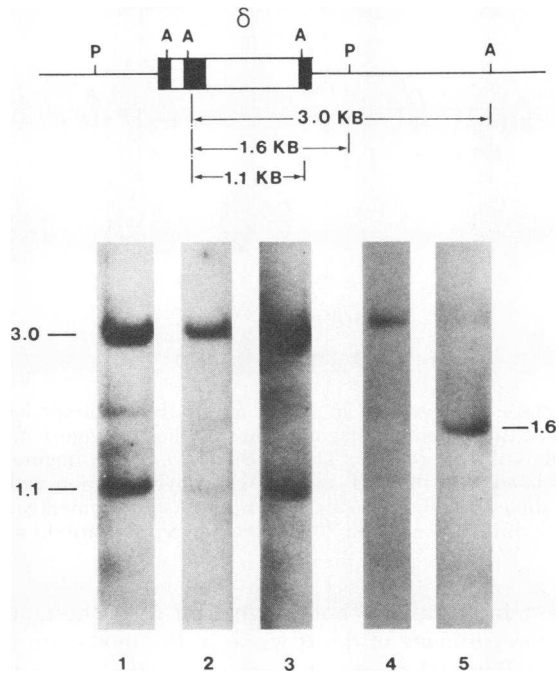


FIGURE 3 Asu fragments hybridizing to  $\delta$ -IVS 2 probe. At the top is a diagram of the  $\delta$ -gene with Asu I (A) and Pst I (P) sites shown. The light regions are the IVS, and the dark are the coding regions. At the bottom of the figure are autoradiographs of cellular DNA digested with Asu I, and hybridized to  $^{32}$ P-labeled  $\delta$ -IVS 2 probe. Lanes 1 and 3 show heterozygosity for 1.1- and 3.0-kb fragments. Lane 2 is homozygous for the 3.0-kb fragment. Lane 4 is another homozygote for the 3.0-kb fragment, and lane 5 is a double digestion of the DNA in lane 4 with Asu I and Pst I, and shows shortening of the 3.0-kb fragment to 1.6 kb as expected, if the 3.0-kb fragment is generated by a site 3' to the 3' end of the  $\delta$ -gene.

of Italian origin: an Iranian  $\beta^+$ -thalassemic, and a patient with HbH disease (Fig. 3).

**Use of the Asu I polymorphism in the prenatal diagnosis of  $\beta^T$ .** The presence of a polymorphic Asu I site in the  $\beta$ -IVS is seen in 51% of the Israeli population studied and is associated with both the  $\beta^A$ - and  $\beta^T$ -genes. When the linkage phase is known in a pedigree, the Asu I polymorphism alone should be useful in the prenatal diagnosis of homozygous  $\beta$ -thalassemia in ~23% of couples at risk. (This calculation is based on the frequencies of  $\beta^A/\beta^T$ -heterozygotes from observed gene frequencies which are:

$$\beta_{1.0}^A\beta_{0.8}^T = 0.112; \quad \beta_{0.8}^A\beta_{1.0}^T = 0.312;$$

$$\beta_{0.8}^A\beta_{0.8}^T = 0.508; \quad \beta_{1.0}^A\beta_{1.0}^T = 0.068.)$$

The 22-kb Bam HI polymorphism has a frequency of 12% in Israeli  $\beta^A$  genes in this study (data not shown). Fig. 4 shows the results in an actual Israeli pedigree where a combination of Bam HI and Asu I polymorphism would allow detection of a homozygous  $\beta^T$ -fetus

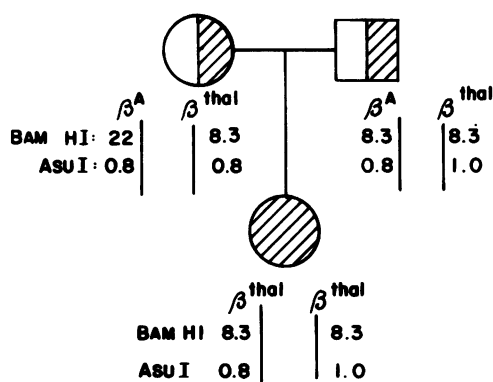


FIGURE 4 Pedigree of an Israeli family. Both parents have  $\beta^T$ -trait (indicated by diagonal lines). A homozygote female child is the propositus. The restriction enzyme fragments associated with Bam HI digestion, and hybridization with a  $^{32}\text{P}$ -labeled 4.4-kb probe are shown; the Asu I fragment sizes after hybridization with a  $^{32}\text{P}$ -labeled  $\beta$ -IVS probe are shown.

at risk antenatally. A homozygote for  $\beta^T$  in the family indicates linkage of the  $\beta^T$ -gene in the mother to the 8.3-kb Bam HI fragment, and of the  $\beta^T$ -gene in the father to the 1.0-kb Asu I fragment. Thus, homozygotes for  $\beta^T$  in fetuses at risk would have an 8.3/8.3 kb Bam HI and a 0.8/1.0 kb Asu I genotype (Fig. 4).  $\beta^T$ -heterozygotes would either have 8.3/22.0-kb Bam HI, 0.8/1.0-kb Asu I, or 8.3/8.3-kb Bam HI, and 0.8/0.8-kb Asu I; normals would have an 8.3/22.0-kb Bam HI and 0.8/0.8-kb Asu I.

## DISCUSSION

Polymorphisms for nucleic acid sequences in the  $\beta$ -globin gene complex (1–5), in mitochondrial DNA (13), and in human single copy genomic DNA unassociated with any known gene (14) are associated with either the gain or loss of restriction enzyme sites or DNA rearrangements. Mendelian inheritance has been confirmed in all polymorphisms studied to date associated with the  $\beta$ -globin gene complex, and have been shown to be useful in prenatal detection of  $\beta$ -globin hemoglobinopathies (15, 16). The Asu I polymorphism described in this paper can now be added to the Bam HI, Hpa I, and Hind III polymorphisms previously described. The high frequency of this polymorphism in affected populations makes it particularly useful; its association with both the  $\beta^A$ - and  $\beta^T$ -genes, however, requires individuals homozygous for either normal or  $\beta^T$ -genes to define the linkage phase of the polymorphism in families under study.

The Asu I site recognizes the nucleotide sequence GGNCC, which occurs at a frequency of 1/256 nucleotide pairs in a random sequence of DNA. Such site alterations occur in the  $\beta$ -globin gene in the  $\beta$ -IVS 2 sequence, and in the third coding sequence of the

$\delta$ -structural gene. These silent substitutions in the  $\beta$ -IVS 2 and the  $\delta$ -structural gene are in agreement with reports of lesser nucleotide sequence conservation in the IVS 2, third coding region, and 3' flanking sequences of most mammalian  $\beta$ -like genes analyzed (17), as compared with the first and second coding regions, the small IVS (IVS 1), and the 5' flanking sites.

The  $\beta$ -thalassemias are a heterogeneous group of molecular disorders. The  $\beta^0$ -genes have been characterized by deletions (18, 19), nonsense mutations (20, 21),<sup>2</sup> and 5'  $\beta$ -IVS 2 splice site defects (22). The molecular defects responsible for  $\beta^+$ -thalassemia genes have been less well characterized. A single base change in the IVS 1 of a cloned human globin  $\beta$ -gene from a patient with  $\beta^+$ -thalassemia has been reported, and may be the cause of the  $\beta^+$ -thalassemia genotype in this patient (23).

The Asu I  $\beta$ -IVS polymorphism is reported here in association with both the  $\beta^A$ - and  $\beta^T$ -genes in a heterogeneous Israeli population. The polymorphic 1.0-kb fragment was found in all Jewish groups (Kurds, Sephardics, and Ashkenazi) and in Arabs, but was not found in American blacks, and was in low incidence in West African blacks. The Asu I  $\beta$ -IVS polymorphism has also been detected in three Italian pedigrees at risk for  $\beta^T$ , and affords potential complete antenatal diagnosis in conjunction with another polymorphic loci in two of three pedigrees analyzed (data not shown). There is a higher frequency of the 1.0-kb associated with the  $\beta^+$ -thalassemia gene as compared with the  $\beta^0$ -thalassemia gene (Tables I and II). The  $\beta^0$ -thalassemia gene is associated with the 0.8-kb fragment in 9 of 10 genes, and suggests linkage disequilibrium and a marker for the  $\beta^0$ -thalassemia gene in this population. It may also be consistent with different genetic origins of the  $\beta^0$ - and  $\beta^+$ -thalassemia genes. More extensive population analysis is required to define this with greater statistical certainty.

## ACKNOWLEDGMENTS

We wish to thank Helen Schreiner for her technical assistance, and Una Collins for preparing the manuscript.

This work was made possible by grants from National Institutes of Health (AM-25274); National Science Foundation (24120), Cooley's Anemia Foundation (312-2581), the March of Dimes Birth Defects Foundation (NF-1-664), and The New York Community Trust. Dr. Driscoll was supported by grant GM-07419 and Dr. Baird was supported by grant AM-07373 from the National Institutes of Health.

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