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Brief Communication

β-Thalassemia Due to a Deletion of the Nucleotide Which Is Substituted in the β^S-Globin Gene

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SUMMARY

Sickle-cell anemia results from an A→T transversion in the second nucleotide of codon 6 of the β-globin gene. We now report an uncommon β-thalassemia gene that contains a deletion of this nucleotide. Thus, one mutation (GAG→GTG) produces sickle-cell anemia, while the other (GAG→GG) eliminates β-globin production. These data establish that different alterations affecting one specific nucleotide can produce either an abnormal hemoglobin or β-thalassemia. Moreover, the nucleotide sequence comprising codons 6–8 of the β-globin gene appears to be particularly susceptible to mutations affecting nucleotide number.

INTRODUCTION

Sickle-cell anemia is an inherited hemolytic anemia with a high prevalence in malaria-infested regions of the world, including Africa, the Mediterranean basin, Arabia, and parts of India [1]. The same mutation, an A→T transversion in the second nucleotide of codon 6 of the β-globin gene, is responsible for the disease in all ethnic groups [2, 3]. The mutation results in the substitution of valine, encoded by GTG, for glutamic acid, encoded by GAG, at the sixth amino acid of the β-globin chain. The amino acid substitution, in turn, produces hemoglobin S ($\alpha_2\beta_2^S$) and leads to the pathological consequences of the disease.

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On the other hand, β-thalassemia is an inherited disorder characterized by reduced or absent β-globin production [4]. This condition is also prevalent in malaria-infested regions, but, in contrast to sickle-cell anemia, it results from any of several different mutations that reduce β-globin synthesis [5]. The majority of these mutations are nucleotide substitutions that act by disrupting RNA splicing, translation, or transcription. The remainder are insertions or deletions of a few nucleotides that affect translation by causing frameshifts in ribosome reading ([6] and S. H. Orkin and H. H. Kazazian, Jr., unpublished data, 1983).

We now report a β-thalassemia gene that is characterized by a deletion of one nucleotide. That nucleotide is the same nucleotide that undergoes substitution in the β^S gene. This discovery reveals the similarity between mutations producing abnormal hemoglobins and those that cause β-thalassemia.

MATERIALS AND METHODS

Restriction Endonuclease Analysis of Genomic DNA

Nuclear DNA was isolated from the leukocytes contained in 10–15 ml of EDTA-anticoagulated blood [7]. Five μg of DNA was digested with Mst II using conditions recommended by the commercial suppliers. Southern blot analyses of the resulting DNA fragments were done as described [8, 9]. The radioactive probe used was the 1.8-kilobase (kb) Bam HI-Bam HI fragment that contains the 5′ half of the β-globin gene [10].

Cloning of the β-Globin Gene and Partial DNA Sequencing

The β-globin genes of a β-thalassemia-trait father, carrying the appropriate mutant gene in a haplotype I chromosome, were cloned in 7.5-kb Hind III restriction fragments of genomic DNA in bacteriophage Charon 28 [11]. Phage were screened by the Benton-Davis procedure [12] using probe prepared by nick-translation from a plasmid containing the Bam HI-Eco RI fragment encompassing IVS-2 of the normal β gene. Two positive clones were identified, and one lacked the Mst II site at codons 5–7 of the β gene. The β-globin gene of this clone was subcloned as a 3.7-kb Bgl II-Pst I fragment in pBR 322 for DNA sequence analysis. Sequencing in the 3′ direction from the Hinf I site at codons 3–5 of the β gene was carried out by the Maxam and Gilbert procedure [13].

RESULTS

Recently, it was shown that the restriction endonuclease Mst II has a recognition site at codons 5–7 of the normal β^A gene, but that this Mst II site is obliterated by the β^S mutation in codon 6 [10, 14, 15]. Mst II can be used to carry out prenatal diagnosis of sickle-cell anemia because the 5′ end of the β^A gene is cut into 1.1-kb and 0.2-kb fragments while that of the β^S gene remains in a 1.3-kb fragment [11, 14, 15].

To determine the incidence of β-globin genes producing a false positive Mst II test, we screened 150 β^A genes in black Americans, 101 β^A genes in Italians and Greeks, 43 β-thalassemia genes in Greeks, and 58 β-thalassemia genes in Italians. No abnormal Mst II fragments were observed among the β^A genes or the Greek β-thalassemia genes. However, three Italian β-thalassemia genes (5%) yielded a 1.3-kb β-gene fragment after Mst II digestion (fig. 1). This fragment is identical to that produced after cleavage of the β^S gene.

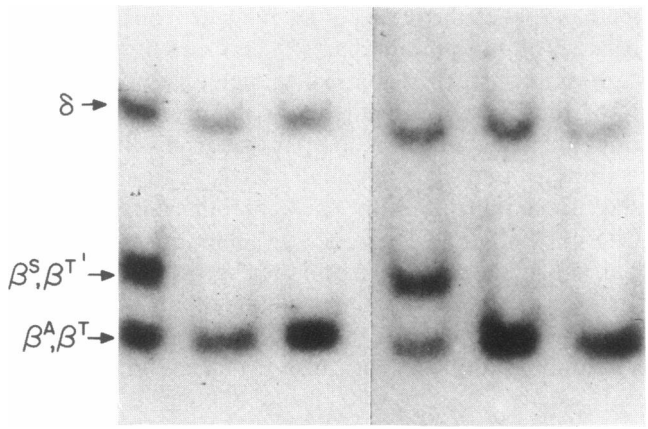

FIG. 1.—A β-thalassemia gene lacks the Mst II restriction site at β codons 5–7. Five μg of leukocyte DNA from Italian β-thalasssemics was digested with Mst II and subjected to blot hybridization with a ^{32}P-labeled Bam HI fragment that includes the 5′ half of the β gene. *The far left lane* contains DNA from a heterozygote for hemoglobins A and S, and *the remaining lanes* contain DNA from Italians with β-thalassemia major. $\beta^{T'}$ refers to the β-thalassemia gene that lacks the Mst II site (*fourth lane from the left*). β^T refers to the remaining β-thalassemia genes shown that contain the Mst II site at β codons 5–7. A constant Mst II fragment derived from the δ gene is labeled δ.

All of these Mst II-positive β-thalassemia genes were present in Italian-Americans whose families had emigrated from Southern Italy. We previously showed an association between β-thalassemia mutations in Mediterraneans and DNA polymorphism patterns at eight polymorphic restriction sites in the β-globin gene cluster [6]. These DNA polymorphism patterns were designated haplotypes I–IX. Of the Mst II-positive genes, one was associated with haplotype I, one with haplotype V, and one with haplotype IX. We previously showed that the predominant mutation associated with haplotype I is the IVS-1 mutation described by Spritz et al. [16]. One of 26 (4%) Italian β-thalassemia genes associated with haplotype I demonstrated the Mst II-positive allele. Likewise, only one of 18 (5%) β-thalassemia genes associated with haplotype V demonstrated a 1.3-kb Mst II fragment. In contrast, we found only three β-thalassemia genes associated with haplotype IX chromosomes in Italians, and one of these is an Mst II-positive allele. A nonsense codon at position 39 has been found in the second chromosome of this type [6], and the mutation of the third has not been characterized.

To determine whether the obliterated Mst II site at codons 5–7 was due to a rare DNA polymorphism or a mutation that reduced the expression of this gene, one of the genes lacking an Mst II site (haplotype I) was cloned and subjected to partial DNA sequencing. The sequence of codons 5–7 in this DNA was CCTGGGAG instead of the normal β^A sequence of CCTGAGGAG. Thus, this β-thalassemia gene is a frameshift mutation, deletion of A in codon 6 of the β gene. As a result of this mutation, a nonsense codon, TGA, is generated at codon 18 of the β-globin gene.

DISCUSSION

This deletion represents the sixth identified frameshift mutation producing β-thalassemia. Frameshift mutations (small deletions and insertions) account for

26% of β-thalassemia mutations characterized to date ([5, 6] and S. H. Orkin and H. H. Kazazian, Jr., unpublished data, 1982). The present mutation is especially noteworthy because of its location. As many of the different mutations producing β-thalassemia have been elucidated, it has become apparent that these mutations are very similar to those that produce abnormal hemoglobins. In fact, some mutations, such as those that produce β^{E} and $\beta^{Knossos}$ [17, 18], lead to both β-thalassemia and an abnormal hemoglobin. This present mutation emphasizes again the similarity between mutations that affect β-globin synthesis and those that affect β-globin structure because it involves the same nucleotide that is substituted to produce the most important abnormal hemoglobin, hemoglobin S.

Three other mutations in codons 6–8 of the β-globin gene affect nucleotide number (fig. 2). One of these eliminates the trinucleotide GAG that serves as codon 6 or codon 7 and leads to an unstable hemoglobin: hemoglobin Leiden ($\alpha_2\beta_2^{6 \text{ or } 7 \rightarrow 0}$) [19]. The remaining two mutations are frameshifts that produce β-thalassemia. One of these is a deletion of AA in codon 8 [20], and the other is an insertion of G between codons 8 and 9 (S. H. Orkin and H. H. Kazazian, Jr., unpublished data, 1982). Only 17 mutations affecting nucleotide number are known in the β-globin gene. Of these, 11 are deletions of one or more codons that lead to abnormal hemoglobins and six are deletions or insertions that produce frameshifts in ribosome reading. Since four (24%) of these 17 mutations are located in the nine nucleotides comprising codons 6–8, this region appears to be particularly susceptible to mutations affecting nucleotide number. The nucleotide sequence in question, GAGGAGAAG, contains several AG, GA, and GAG repeats. Such short-repeating sequences have been implicated as prone to deletions and insertions by a mechanism involving slippage in DNA replication [21].

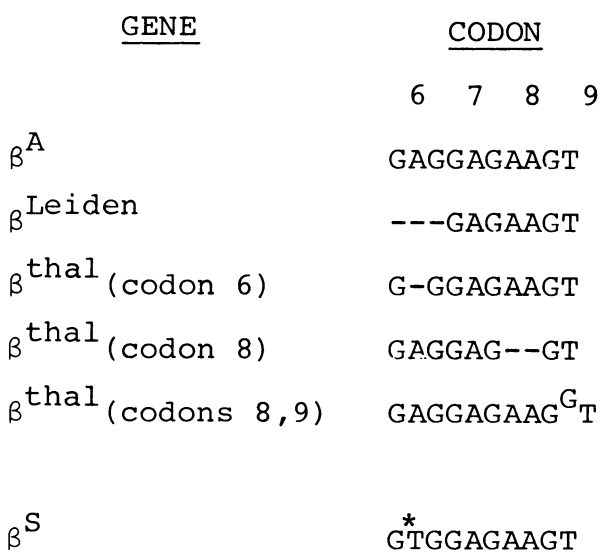

FIG. 2.—Deletions and insertions in codons 6–9 of the β-globin gene. *The mutation in the β-thalassemia gene reported here (codon 6, GAG→GG) is shown below that of* β^{Leiden}, and *the mutation in the* β^{S} *gene (codon 6, GAG→GTG) is also shown for comparison.*

The practical importance of the mutation described here lies in the possibility for error in the prenatal diagnosis of sickle-cell anemia when the father of a fetus is not available for genotype analysis. A prenatal test result indicating sickle-cell anemia in the fetus of an AS woman may actually be the result of the combination of the β^S gene of the mother and the β-thalassemia gene reported here.

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