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β THALASSEMIC MUTATIONS RECOGNIZED BY DNA MAPPING WITH HPH I AND RSA I IN THE ALGERIAN POPULATION

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By using Hph I and Rsa I restriction enzymes and β globin large intervening sequence as a probe, we have investigated the DNA of 20 Algerian patients with β° or β^{+} thalassemia. In any of them, we detected the nucleotide change which is known to generate an additional Hph I site at the 5' splice junction of the β globin large intervening sequence and which yields a β° phenotype. In one of them, we detected the nucleotide change which is known to generate an additional Rsa I site within the β globin large intervening sequence and which is supposed to yield a β^{+} phenotype. These results indicate that these two types of mutation are relatively rare in the Algerian population.

β-thalassemias are a heterogeneous group of inherited disorders which affect human β-globin chain synthesis (1). This synthesis is absent in β°-thalassemia while it is only reduced in β^{+}-thalassemia (1). In the Algerian population where the frequence of β-thalassemia is relatively high (2), one molecular defect causing a β°-thalassemia phenotype has been identified to date (3). This defect is a substitution in the β-globin gene exon 2 that changes the codon 39 in a nonsense mutation (3).

In Italians and Greeks, other molecular defects have been identified as a cause of β-thalassemia (4-9) and were found linked to specific haplotypes defined by restriction site polymorphisms in the εγδβ globin gene cluster by Orkin et al.(9). Among these defects, some can be spotted directly by restriction mapping of genomic DNA(6,9). The restriction endonuclease Hph I recognizes the normal splice junction at the 5' end of the large intervening sequence (IVS 2) of β-globin gene (6). A substitution which, in this splice junction entails loss of the Hph I site, prevents normal RNA processing and yields a β°-phenotype (10). The restriction endonuclease Rsa I has revealed the existence of a substitution within the IVS 2 of some β^{+}-thalassemic genes. It has been assumed that this substitution generates a new internal splice site in the β mRNA precursor (9). This two mutations are specifically linked to haplotypes III and VII, as defined by Orkin et al.(9).

ABBREVIATIONS IVS 2 : large intervening sequence. kb : kilobases.

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In this study we have investigated, by DNA digestion with Hph I or Rsa I, if these two types of defects are present in Algerian β-thalassemic subjects.

MATERIAL AND METHODS

20 β-thalassemic individuals from various regions of Algeria were studied: 9 were β^+-thalassemic and 11 were β°-thalassemic (11,12). The β^+-thalassemic subjects originated from non-consanguineus parents and therefore could be compound heterozygotes for β^+ and β° thalassemia.

Cellular DNA were extracted from leukocytes or spleen as described previously (13). They were digested by the restriction enzymes Hph I or Rsa I (New England Biolabs), runned in 1% agarose gel, transferred to nitrocellulose filters and hybridized with 32P labelled probe as already described (13,14). The probe used was the β-globin IVS 2 prepared from a normal 5,2 kb Eco RI cloned fragment (3) and subcloned into pBR 322.

RESULTS

Specificity of the β-globin IVS 2 probe has already been demonstrated (6) and was verified in our laboratory (data not shown).

Using this probe and after cleavage of DNA by the enzyme Hph I, a unique fragment of 0,9 kb is revealed when the sequence at the 5' end-IVS 2 splice junction is normal, whereas a unique fragment of 1 kb is revealed when there is a substitution in this sequence (Fig. 1 A). In our study, DNA from the 20 β-thalassemic patients examined, produced normal 0,9 kb fragments after Hph I digestion (Fig. 1 B). Hence the usual Hph I site at the 5' end of IVS 2 of the β globin gene is present in both chromosomes in these individuals.

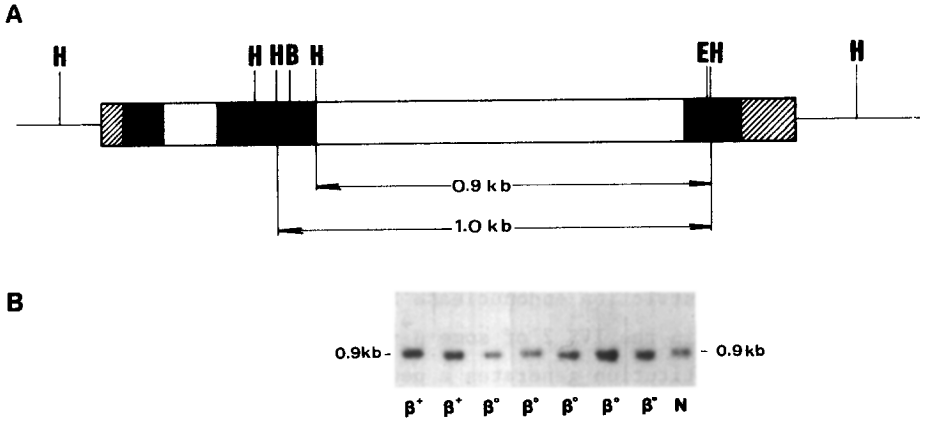

Figure 1 : A : Restriction map of the human β-globin gene. H : Hph I, B : Bam HI, E : Eco RI. Exons are indicated by shaded blocks, introns by open blocks and flanking regions by diagonal shading. After Hph I digestion IVS 2 probe recognizes a fragment of 0,9 kb. in normal DNA and a fragment of 1 kb in DNA with splice junction mutation. B : Autoradiograms of Hph I digested DNA treated as described and hybridized to a 32P labelled β-globin IVS 2 probe. N : normal DNA ; β^+ and β° : DNA from β^+ and β° thalassemic patients.

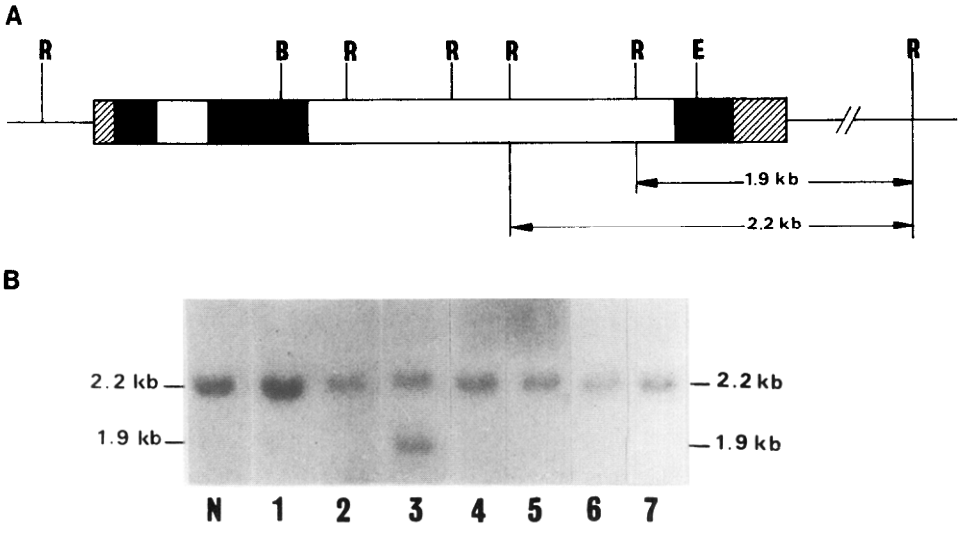

Figure 2 : A : Restriction map of human β-globin gene. R : Rsa I, B : Bam HI, E : Eco RI. Exons are indicated by shaded blocks, introns by open blocks and flanking regions by diagonal shading. After Rsa I digestion, IVS 2 probe recognizes a 2,2 kb fragment in normal DNA and a 1,9 kb fragment in DNA with additional internal splice site. B : Autoradiograms of Rsa I digested DNA treated as described and hybridized to a 32P labelled β-globin IVS 2 probe. N : normal DNA ; 1 to 7 : DNA from β thalassemic patients.

After DNA digestion with the enzyme Rsa I, the same probe reveals a fragment of 2,2 kb when IVS 2 is normal. When an additional Rsa I site is present in IVS 2, this probe recognizes a fragment of 1,9 kb (Fig. 2 A). Among the 20 β-thalassemic patients studied, only one β^+-thalassemic subject produced simultaneously the 2,2 and 1,9 kb fragments (Fig. 2 B). In the other subjects only the normal 2,2 kb fragment was present. Analysis of DNA polymorphisms in the unique subject displaying an additional Rsa I site allowed us to establish that this site was associated to haplotype VII, as in Italians and Greeks (data not shown).

DISCUSSION

Our results show that the defect in β-globin synthesis in 20 Algerian individuals with β° or β^+ thalassemia is not related to an alteration in the nucleotide sequence at the 5' end splice junction of β-globin IVS 2. This suggests that this specific mutation, which is pointed out by Hph I digestion and yields a β°-phenotype in Italians and Greeks (6, 9, 10), is relatively rare in Algerians. Indeed, if it was as frequent in the Algerian as in the Italian and Greek population (9), we should have found, out of the 22 β°-thalassemic chromosomes examined, 1 or 2 chromosomes lacking the Hph I recognition site in β-globin IVS 2.

Our results also show that one β^+-thalassemic subject presents in one of his chromosome, an additional Rsa I site in the IVS 2 of β-globin gene. An additional Rsa I site has also been found in the IVS 2 of some β-thalassemic genes from Italian and Greek origin (9). Sequency of these genes has shown that the additional Rsa I site results from a substitution which generates an internal splice site (9). It is not yet established that this internal splice site is responsible for abnormal RNA processing and thus is the primary cause of β-thalassemia. If it was the case, our finding would correspond to the second molecular defect responsible for β-thalassemia identified in the Algerian population.

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