Polymorphism of the Hinf I Restriction Site Located 1 Kb 5' to the Human β-Globin Gene

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Summary. Mapping of the DNA from 14 Mediterranean subjects indicates a genetic variation in an Hinf I recognition site located 1 kilobase 5' to the β-globin gene. This Hinf I site was found associated with eight β-thalassemic genes and 11 normal β genes, and hence is not specifically linked to β-thalassemia.

Introduction

Several restriction site polymorphisms distributed in the human β-globin gene cluster have been described. Kan and Dozy (1978) initially reported a polymorphic Hpa I restriction endonuclease site 3' to the β-globin gene which generates a 13 kilobase (kb) fragment in association with sickle genes (βs), instead of a 7.6 kb fragment in association with normal β-genes. Subsequently Jeffreys (1979) and Tuan et al. (1979) reported polymorphic fragment in association with normal β-genes. Subsequently Jeffreys (1979) and Tuan et al. (1979) reported polymorphic fragment in association with sickle genes (βs), instead of a 7.6 kb site 3' to the β-globin gene which generates a 13 kilobase (kb) fragment.

In this paper we describe another variation in DNA sequence adjacent to the β-globin gene. This variation was first observed by comparing the fine restriction endonuclease map of a cloned normal β-globin gene and a cloned βs-thalassemic gene (Gorski et al., unpublished results). An Hinf I site located 1 kb 5' to β-globin gene was present in the βs-thalassemic clone but absent in the normal one. A similar observation has been reported since by Moschonas et al. (1982) after the sequencing of several clones of normal and β-thalassemic genes. This additional Hinf I restriction site was found in the cellular DNA of the subjects studied by both group of authors, thus excluding the possibility of a cloning artefact. By investigating the DNA of several subjects of Mediterranean origin, whether β-thalassemic or not, we have confirmed that the Hinf I site 1 kb 5' to the β-globin gene is polymorphic and showed that its presence is not specifically associated with β-thalassemic genes.

Materials and Methods

Fourteen subjects of Italian and Algerian origin were studied. The genotype of these subjects was established on genetic, hematologic, and biochemical grounds. Five were normal, four had homozygous βs-thalassemia (Belhani et al. 1980), one had homozygous βs-thalassemia, one was homozygote for an α-thalassemia (White et al. 1980), two had sickle cell anemia (Henni et al. 1981), and one was a compound heterozygote for β-thalassemia and hemoglobin Lepore.

High molecular weight DNA was extracted from leukocytes, spleen, or placenta of these subjects as described previously (Jeffreys and Flavell 1977). The restriction enzymes used came from Boehringer Mannheim or BRL and were used under the conditions recommended by the commercial suppliers. One hundred µg of DNA of each individual were digested with Eco R I and enriched in fragments of 5.2 kb by electroelution. This enrichment was done to prevent hybridization of the fragments originating from the δ-globin gene with the radioactive probe used. This became unnecessary when it was inferred from the complete sequence of the β and δ-globin genes that little homology exists between the 5' flanking regions of these two genes (Spritz et al. 1980; Lawn et al. 1980). DNA, whether enriched or not, was cleaved with Hinf I, run on a 2% agarose gel, and transferred to a nitrocellulose filter according to the method of Southern (1975). The filters were then hybridized to a 32P-labeled probe as described previously (Jeffreys and Flavell 1977). The probe used was the 1.9 kb Bam HI clone isolated from the 5.2 kb Eco R I clone (Gorski et al. 1982) and subcloned in our laboratory in pBR322. It was labeled by nick translation to specific activities between 80 and 200 106 dpm/µg.

Results

Figure 1 shows the restriction map of the Hinf I sites near and within the β-globin gene, as can be establish from the β-globin gene sequence (Lawn et al. 1980) and fine mapping of the 5' flanking region (Gorski et al. unpublished results). When the Hinf I polymorphic site is present, the 1 kb Hinf I fragment is replaced by two fragments of 0.3 and 0.7 kb. On autoradiogram of cellular DNA digested with Hinf I and hybridized with the 1.9 kb Bam HI probe, only the fragment of 0.7 kb is detected under the conditions used (Fig. 2). The presence of a single band of 0.7 kb signifies that the Hinf I polymorphic site is present on both chromosomes (Fig. 2, lane 2) whereas the presence of two bands of 0.7 and 1 kb indicates that this site is carried over under the heterozygous state (Fig. 2, lane 1).
Fig. 1. Restriction enzyme map of the human $\beta$-globin gene indicating Eco RI (E), Bam HI (B), Pst I (P), Hpa I (Hp), Hinf I (H) sites. The Hinf I polymorphic site reported in this paper is marked by an asterisk. Two fragments of 0.3 and 0.7 kb are generated when this site is present, only one fragment of 1 kb when this site is absent. The clear areas within the rectangle indicate the IVS and the block areas the coding sequences.

Table 1. Polymorphic restriction enzyme sites in the DNA of the 14 subjects examined

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Hinf I site</th>
<th>$\gamma_\gamma$ Hind III site</th>
<th>$\gamma_\beta$ Hind III site</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>+/+</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>$\beta^+$ thalassemic</td>
<td>+/-</td>
<td>+/+</td>
<td>+/-</td>
</tr>
<tr>
<td>$\beta^+$ thalassemic</td>
<td>+/-</td>
<td>+/+</td>
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<tr>
<td>$\beta^+$ thalassemic</td>
<td>+/-</td>
<td>+/+</td>
<td>+/-</td>
</tr>
<tr>
<td>$\alpha$-thalassemic</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
<tr>
<td>$\beta$ homozygote</td>
<td>+/-</td>
<td>+/-</td>
<td>+/-</td>
</tr>
</tbody>
</table>

* Indicates the presence of cleavage at a particular site
- Indicates absence of cleavage

Region corresponding to the location of the polymorphic Hinf I site is deleted on the Lepore chromosome.

As shown in Table 1, the polymorphic Hinf I site was found to be present on the two homologous chromosomes of seven subjects (four normals, two $\beta$-thalassemics, one $\alpha$-thalassemic) and on only one chromosome of five subjects (one normal, four $\beta$-thalassemics), but was absent on the chromosomes of the two subjects with sickle cell anemia. The Hind III sites within the IVS 2 of $\gamma_\gamma$ and $\gamma_\beta$-globin genes previously studied (Kohen 1981) in these 14 subjects are also shown in Table 1. It is interesting to note that the polymorphic Hinf I site and the polymorphic Hind III site within the $\gamma_\gamma$-globin gene are present in 70% and 64% of the 28 chromosomes studied respectively, whereas the polymorphic Hind III site within the $\gamma_\beta$-globin gene is present in only six chromosomes (i.e. 21%). It may also be pointed out that the two subjects with sickle cell anemia, who were shown to carry the Hpa I 13 kb fragment on both chromosomes (Henni et al. 1981), display an homozygous polymorphic pattern. An homozygous polymorphic pattern is also observed in the subject with $\alpha$-thalassemia who was shown to be, on the other hand, homozygous for an $\alpha$-globin gene deletion (Whitelaw et al. 1980).

Discussion

The Hinf I restriction site located 1 kb 5’ to the $\beta$-globin gene was found to be present in 19 of the 28 chromosomes examined. These results ascertain the polymorphism of this Hinf I site and suggest that this polymorphism is relatively frequent in the Mediterranean population. More extensive analysis is required to define the exact incidence of the Hinf I site in Italians and Algerians. However, the results of Table 1 which show that the $\gamma_\gamma$ Hind III site is present on 18 chromosomes out of 28 indicates that the frequency of the Hinf I site may be close to that of the $\gamma_\gamma$ Hind III site in this population (Little et al. 1980).

The Hinf I site was associated in eight cases with a $\beta$-thalassemic gene and in 11 cases with a normal $\beta$-globin gene. Its presence is therefore not linked to $\beta$-thalassemia in contrast with the situation observed with the Bam HI and Hpa I polymorphic site which are frequently associated with $\beta^0$-thalassemic genes (in Sardinians) and sickle genes (in Blacks and Algerians), respectively. The additional polymorphic site found in the $\beta$-globin gene cluster may, however, facilitate the definition of the chromosome haplotypes associated with specific $\beta$-thalassemic mutations (Orkin et al. 1982). Combined analyses of the Hinf I and of the two Hind III sites have already been sufficient in our study to determine that each of the five subjects with homozygous $\beta$-thalassemia had nonidentical homologous chromosomes and therefore might be carriers of two different molecular defects in the $\beta$-globin gene region.