Mapping the α-Globin Genes in an Algerian HbH Patient and His Family

By E. Whitelaw, J. Pagnier, G. Verdier, T. Henni, J. Godet, and R. Williamson

The organization of α-globin genes in normal white European, normal Algerian, and α-thalassemic Algerian DNA was examined by restriction endonuclease mapping using HindIII, Hpal, BamHI, EcoRI, BglII, and PstI. The results for normal DNA confirm and add to the findings of Surrey et al.1 and Orkin;2 the two α-genes are approximately 3.0 kb apart. The restriction enzymes BglII and Hpal cut between the two α-genes. Four PstI sites are located: two surrounding each α-gene. The physical maps for a number of Algerian controls (normal α- and β-globin biosynthesis profiles) are identical to that of the European controls. The Algerian α-thalassemic presenting with HbH disease was found to be homozygous for a 3.5–3.7 kb deletion at the α-gene locus, leaving one α-gene per chromosome. The patient’s mother and father are both found to be heterozygous for this deletion. An unaffected sibling carries both normal chromosomes. The deletion could be the result of a Lepore-like crossover fusion event between the two α-globin genes, or of a 3.7 kb deletion of either the entire 5’ α-gene or the entire 3’ α-gene. The Algerian case of HbH disease studied differs from Asian cases in both the mode of inheritance and the molecular pathology of the α-thalassemia mutation. If this type of deletion is the major cause of Algerian α-thalassemia, it would explain the apparent absence of Hydrops feticus in this geographical area.

Thalassemia is a hereditary disorder of hemoglobin synthesis where the production of α-chains and “non-α”-chains is unequal.3 The α-thalassemias are characterized by a reduction or absence of α-chain synthesis. In severe cases, the β-type chain produced in excess may associate with itself, giving rise to the nonfunctional hemoglobin tetramers, hemoglobin Bart (γ4) and hemoglobin H (β4). α-Thalassemia occurs in high frequency in South-East Asian populations4 but also appears in other areas of the world: Greece,5 Italy,4 Turkey,7 and Saudi Arabia.8

The clinical and molecular pattern of α-thalassemia has been defined in the Asian population.9 The nonthalassemic person carries four functional α-genes per diploid chromosome. α-Thalassemia-2, where one of the four genes is nonfunctional, is not associated with any marked clinical symptoms. α-Thalassemia-1, where two of the four α-genes are nonfunctional, is accompanied by microcytosis and an unequal α/β biosynthesis ratio. HbH disease occurs when α-thalassemia-1 and α-thalassemia-2 are both present, i.e., three of α-genes are nonfunctional. Hb Bart hydrops feticus is the homozygous state for α-thalassemia-1; all four α-genes are nonfunctional. Solution hybridization studies on Asian α-thalassemics10-12 have demonstrated that two of the four α-genes are deleted in homozygous α-thalassemia-2, that all are deleted in homozygous α-thalassemia-1 (Hb Bart hydrops feticus), and that three of the four α-genes are deleted in HbH disease. However, α-thalassemia from a number of non-Asian populations does not appear to fit into this genetic pattern. A recent study of the DNA from eight HbH patients from Italy, Turkey, and Israel by restriction mapping has revealed that seven of these possess two or even three α-genes per diploid chromosome.13 This implies that some of these genes must be nonfunctional even though the majority of their coding sequence is present.

Among Northern Algerians, the incidence of α-thalassemia, as determined by the level of Hb Bart’s in cord blood, is approximately 10%.14 Although 12 families with cases of HbH disease have been reported, no case of Hb Bart hydrops feticus has ever been identified. A hemato logic study of one of these families reveals that the mother and father, who are first cousins, both present similar profiles—slight microcytosis and a slightly unbalanced α/non-α-globin chain synthesis (0.85 and 0.92, respectively)—while the patient has pronounced microcytosis, an α/non-α-globin chain synthesis of 0.64, and 5.6% HbH. The patient’s brother presents with a completely normal hematologic picture. This suggests that the patient with HbH disease may be homozygous for an α-thalassemia gene.15

Recent developments in molecular biologic techniques permit the direct analysis of the globin genes present in total cellular DNA. Cellular DNA fragments produced by specific cleavage with restriction endonucleases are separated on the basis of size by agarose gel electrophoresis. The DNA is transferred to nitrocellulose filters, and the fragments containing α-globin genes are located by autoradiography follow-
ing the molecular hybridization of 32P-labeled α-globin gene specific probes to the filters. Using different enzymes separately and in combination, a physical “map” of the α-gene loci and surrounding DNA sequences can be constructed. In this article we have used this technique to study the α-genes of the Algerian family mentioned above. The results confirm that the HbH patient is homozygous for a deletion in the α-gene locus. Using a number of different restriction enzymes we have located the size and position of this deletion.

MATERIALS AND METHODS

Sources and Preparation of Human DNA Specimens

α-Thalassemia was diagnosed using standard hematologic and family studies.14 The HbH patient had 5.6% HbH. High molecular weight DNA was prepared from blood (mother, father unaffected brother, Algerian normals), from spleen (HbH patient), and from placenta (European normals) by standard methods.17

Molecular Procedures

Cellular DNA samples were digested to completion with restriction endonucleases in the standard way. EcoRI was purchased from M.R.E. Porton. HindIII from Boehringer, Mannheim, Germany, and HpaI from Bethesda Research Laboratories, Bethesda, Md. BgIII, PstI, and BamHI were gifts from Ray Dalgleish, Rob Elles, and Janet Arrand (Biochemistry Department, St. Mary’s Hospital Medical School).

After restriction, the DNA was electrophoresed in a horizontal slab gel at 0.8% or 1.0% agarose. Electrophoresis was carried out at 45V for 16 hr. Southern transfers and filter hybridization were carried out as described by Jeffreys and Flavell.17 32P-labeled probes were labeled in vitro by nick translation of the human α-globin cDNA plasmid, pHαGl.18 and JW1Ol.19 These recombinant plasmids were grown and handled under category II conditions as advised by the U.K. Genetic Manipulation Advisory Group.

The 3’-specific and 5’-specific probes were prepared from recombinant plasmid JW1Ol. The plasmid was digested with HindIII and the resulting two fragments were isolated by gel electrophoresis.20 The 0.5-kb fragment contains the 3’ α-globin gene sequence, and the 6.0-kb fragment contains the 5’ α-globin gene sequences (Forget B, personal communication). The specific activity of all 32P-labeled probes were 2.5–10 \times 10^7 dpm/μg.

The filters were autoradiographed using preflash Kodak film with tungsten intensifier screens and stored at -70°C. Exposure time varied from 2 to 4 days.

Hybridization Markers

The recombinant plasmid pHαGl.18 was restricted with BgIII giving fragments of size 6.6 kb, 4.4 kb, 1.8 kb, and 1.0 kb. These crosshybridize with the pHαGl probe and were used routinely as low molecular weight markers. For larger size markers, γDNA restricted with EcoRI and BgIII were used. Prior to hybridization, the filters were cut such that the γDNA slots were hybridized separately with 32P-labeled λDNA (specific activity 10^7 dpm/μg).

RESULTS

A Map of Restriction Sites in and Around the Normal α-Globin Gene Loci

We have used restriction endonucleases EcoRI, BamHI, HindIII, and HpaI in single and double digests to construct the map shown in Fig. 1. This map agrees with that published by Orkin.2 In addition, we have located three BgIII sites and four PstI sites around the α-globin loci.

Location of the BgIII Sites

A single digest with BgIII results in two fragments of about 9.5 kb and 6.6 kb, which hybridize with the human α-cDNA plasmid (Fig. 2). This suggests that either BgIII cuts the DNA between the two α-genes or that BgIII cuts the coding sequence or intervening sequence (non-coding sequence that lies within the coding sequence of the gene) of one gene but not the other. If the former is true, then the central HindIII fragment (3.7 kb) (Fig. 1) should be cut into two after double digestion with HindIII and BgIII. This is so; the 3.7-kb HindIII fragment is cut into a 1.9- and a 1.7-KB fragment (Fig. 2). Since the sum of these two bands is equal in size (within experimental error) to the original 3.7-kb HindIII band, the BgIII site must lie between the two α-genes. The 1.7-kb BgIII/HindIII fragment hybridizes with a 5’ probe (Fig. 2) and not with the 3’ probe (results not shown), while the 1.9-kb fragment hybridizes with the 3’ probe (results not shown) and not with the 5’ probe (Fig. 2). This
a digestion with PstI gives a single band of 1.3 kb (Fig. 3). This suggests that PstI cuts the DNA around each α-gene to give two 1.3-kb fragments per haploid genome. Double digestion with PstI/BglII, PstI/BamHI, and PstI/HpaI (results not shown) all give the same 1.3-kb fragment. PstI/HindIII digestion results in two bands of 0.8 and 0.5 kb. The sum of these two bands is equal to the original 1.3-kb PstI band. Since the 0.8-kb fragment hybridizes only to the 5’ probe and the 0.5-kb fragment only to the 3’ probe (Fig. 3), two PstI sites must lie 0.8 kb to the 5’ side of the central HindIII sites of both α-genes and the other two PstI sites must lie 0.5 kb to the 3’ side of the central HindIII sites of both α-genes.

Location of the PstI Sites

A digest with PstI gives a single band of 1.3 kb (Fig. 3). This suggests that PstI cuts the DNA around each α-gene to give two 1.3-kb fragments per haploid genome. Double digestion with PstI/BglII (Fig. 3), PstI/BamHI, and PstI/HpaI (results not shown) all give the same 1.3-kb fragment. PstI/HindIII digestion results in two bands of 0.8 and 0.5 kb. The sum of these two bands is equal to the original 1.3-kb PstI band. Since the 0.8-kb fragment hybridizes only to the 5’ probe and the 0.5-kb fragment only to the 3’ probe (Fig. 3), two PstI sites must lie 0.8 kb to the 5’ side of the central HindIII sites of both α-genes and the other two PstI sites must lie 0.5 kb to the 3’ side of the central HindIII sites of both α-genes.

α-Gene Organization for an Algerian HbH Patient and Family

The endonuclease BglII generates two fragments containing α-globin genes in DNA from normal subjects; one of 9.5 kb and the other of 6.6 kb (Fig. 2). In DNA from the Algerian HbH patient, it generates a single fragment of 12–13 kb (Fig. 4, Table I). The

![Graph](https://example.com/graph.png)
DNA of each parent shows the normal and abnormal BglII fragments (Fig. 4). This shows that the Algerian HbH patient is homozygous for a 3–4 kb deletion, which includes the central BglII site. The 12–13 kb fragment is equal to the sum of the 2 normal fragments (9.5 kb and 6.6kb) minus the 3–4 kb deletion, i.e., 9.5 + 6.6 – (3–4) kb = 12–13 kb. The parents each carry one normal and one abnormal chromosome. The unaffected sibling shows a normal BglII pattern (Fig. 4) and must have received both normal chromosomes (9.5 kb and 6.6 kb) minus the 3–4 kb deletion, while mother, father, and unaffected sibling have two fragments: 12–13 kb and 4.5 kb (Fig. 4, Table 1). We must conclude that on each chromosome only one α-gene remains intact along with the two PstI sites adjacent to it. It is not possible that both α-genes and the four flanking PstI sites are present, since the intergene deletion would then be only 2.5 kb. All the other data presented here predict a deletion of between 3.5 and 4.0 kb.

HindIII digestion of DNA from the HbH patient is identical to that of DNA from normals, a single 1.3 kb band (Fig. 3, Table 1). PstI/HindIII digestion of the patient’s DNA generates the same 0.8 kb and 0.5 kb fragments that are formed after PstI/HindIII digestion of normal DNA. The 0.8 kb fragment hybridizes only with the 5’ probe and the 0.5 kb fragment only with the 3’ probe (see Fig. 3). We must conclude that on each chromosome only one α-gene remains intact along with the two PstI sites adjacent to it. It is not possible that both α-genes and the four flanking PstI sites are present, since the intergene deletion would then be only 2.5 kb. All the other data presented here predict a deletion of between 3.5 and 4.0 kb.

HindIII digestion of DNA from normals results in three fragments: 12–13 kb, 4.5 kb, and 3.7 kb (Fig. 1). HindIII digestion of DNA from the HbH patient gives two fragments: 12–13 kb and 4.5 kb (Fig. 4, Table 1). From this we can deduce that one intragenic HindIII site has disappeared and that the deletion is approximately 3.7 kb. The HindIII data thus agree with the PstI data in predicting that one α-gene has been deleted on each chromosome.

DISCUSSION

The restriction endonuclease data reveal that the α-thalassemia is associated with a deletion of between 3.5 and 4.0 kb, which includes the central BglII and HpaI sites and one of the α-genes. Furthermore, it is clear that the HbH patient carries this deletion on both chromosomes, while the mother and father only carry it on one chromosome. Since the patient can synthesize qualitatively normal α-globin and appears to be homozygous at the α-locus, one whole gene per haploid genome must be present.

Orkin et al., Dozy et al., and Embury et al. also report deletion-type α-thalassemia genes in both Asian

### Table 1. α-Globin-Specific Fragments From Normal and Algerian HbH DNA Generated by Various Restriction Enzymes

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Normal DNA</th>
<th>Algerian HbH DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BamHI</td>
<td>11–12</td>
<td>8</td>
</tr>
<tr>
<td>BglII</td>
<td>9.5</td>
<td>12–13</td>
</tr>
<tr>
<td>HpaI</td>
<td>12–13</td>
<td>12–13</td>
</tr>
<tr>
<td>HindIII</td>
<td>12–13</td>
<td>12–13</td>
</tr>
<tr>
<td>PstI</td>
<td>1.3</td>
<td>1.3</td>
</tr>
<tr>
<td>PstI/HindIII</td>
<td>0.8</td>
<td>0.8</td>
</tr>
<tr>
<td>EcoRI/HpaI</td>
<td>4.8</td>
<td>5.1</td>
</tr>
<tr>
<td>HindIII/HpaI</td>
<td>4.4</td>
<td>4.4</td>
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The sizes of the fragments are given in kilobases.
and non-Asian populations with similar 3.5–4.0-kb deletions (i.e., 18–20-kb EcoRI fragments). Orkin’s study does not attempt to delineate which of the two genes is deleted. Embury et al. have reported that, at least in the Chinese population, this deletion-type thalassemia gene is the result of a deletion of the 5’ α-gene locus. In this study, the central HpaI site is deleted. It seems that the α-thalassemia gene described here for the Algerian is not the same as that described for the Chinese population.

It has been suggested by Orkin et al. that the deletion type of thalassemia, which is associated with an 18–20-kb EcoRI fragment is the result of an unequal crossover-fusion event between the α-genes: A deletion of approximately 3.7 kb has occurred such that the 3’ end of the 3’ α-gene is attached to the 5’ end of the 5’ α-gene to form one complete gene, analogous to the Hb Lepore gene formed by the unequal crossover of β and δ genes. This could explain the case presented here. There are two other possible explanations. The deletions could span from the 5’ side of the 5’ α-gene to a site between the central BglII site and the beginning of the 3’ α-gene, or the deletion could span from the 3’ side of the 3’ α-gene to between the central BglII site and the beginning of the 5’ α-gene. These possibilities are portrayed diagrammatically in Fig. 5. It is not possible to decide which of these three alternatives is correct by this method until further restriction sites are found at the α-gene locus or until a clone of genomic DNA from this patient is isolated and sequenced.

It is interesting to note that the 5’ and the 3’ PstI sites are the same distance from the intragenic HindIII sites for both α-genes. This raises the intriguing possibility that the event that caused the duplication of the α-genes also caused the duplication of the surrounding extragenic DNA. This could explain the fact that gene deletions of 3.7 kb (the distance between equivalent points within the two α-genes) are frequently observed at this locus. These deletions could be generated by unequal crossover events either within the α-genes or within the homologous extragenic DNA. The larger the area of duplicated DNA, the higher the probability of crossover fusion events.

It is clear that the Algerian HbH studied here has only two α-genes per diploid genome. HbH disease in the Asian population is generally associated with a deletion of three of the four α-genes. Asians with two α-genes deleted (α-thalassemia-1 or homozygous α-thalassemia-2) do not synthesize detectable amounts of HbH. There are a number of possible explanations for this. The Algerian HbH may carry an additional mutation undetected by our techniques, a “nondeletion” thalassemic gene, which results in the decreased production of α-globin from the two remaining loci. Alternatively, by removing DNA proximal to the 3’ or 5’ end of the intact gene, the deletion may reduce gene activity. Nondeletion thalassemic mutations have been reported in non-Asian HbH patients and more rarely in Asian HbH patients, but in these cases they are functionally as effective as a gene deletion. In the case reported here, the evidence suggests homozygosity for the α-gene locus. Therefore, it is probably that the nondeletion α-thalassemic gene is also carried on both chromosomes and if so, it must be associated with only partial loss of gene activity (i.e., α’thal).

If this genotype is common in Algeria, then it would not be surprising the Hb Bart hydrops fetalis, the homozygous form of α-thalassemia-1 in Asian populations, has not been reported. In this particular family, the parents are first cousins but two other Northern Algerian families have been found where HbH also appears to be associated with a homozygous condition. It has been suggested that in some populations, including Algerians, the two α-genes are not equally active. This idea was put forward to explain the fact that heterozygotes for a number of α-variants (HbJ Mexico, HbJ Cape Town) are synthesized not at 25% but at 30–40%, more than one would expect from a mutation affecting only one of four genes and yet, at least for HbJ Mexico, solution hybridization suggests that all four α-genes are present. At the γ-globin locus, the two genes (γ and γ), which are distinguishable by a single amino acid difference, show differential expression. It is possible, therefore, that the α-gene deletion found here in the Algerian HbH occurs in the “high-producer” α-gene, leaving two “low-producer” genes active. This would have a greater effect on the capacity for α-globin synthesis than the deletion associated with Asian α-thalassemia-

![Fig. 5. Map of the α-globin locus of the normal and the Algerian HbH DNA.](image)
where both genes on one chromosome are inactivated, leaving one low producer and one high producer.

By studying different types of α-thalassemias and α-variants at the level of the structural DNA, we should be able to learn a great deal about the structure–function relationships of the human α-globin genes. However, it must be remembered that a gene deletion that is found to be associated with a particular clinical symptom is not necessarily the cause of that clinical symptom. It may prove difficult to determine the primary thalassemic mutation. Comparisons of the fine structure of the DNA in and around the α-genes of normals and α-thalassemics made possible by cloning and sequencing techniques in combination with some in vitro expression system should provide answers to these questions.

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