CONCISE REPORT

A Molecular Basis for Hemoglobin-H Disease in American Blacks


We have applied gene counting and restriction endonuclease mapping techniques to the study of two American black families in which there were one or more cases of HbH disease. We found deletions of three of the four normal α-globin genes in individuals with HbH disease. In two of these individuals, the chromosome containing the single α gene could have originated by crossing over between mispaired α genes, resulting in a deletion of about 4.2 kilobases (kb).

The α-thalassemia syndromes are inherited anemias caused by decreased function of one or more α-globin genes. When one, two, three, or all four of the normal α-globin genes are nonfunctional, the respective disease states are termed silent carrier (α-thal-2), α-thalassemia trait (α-thal-1), HbH disease, and α-thalassemia.

Patients with α-thalassemia are usually of Asian, Mediterranean, or black descent. Gene counting experiments using liquid hybridization suggest that one or more α-globin genes are deleted in most, but not all, Asian subjects with α-thalassemia. These deletions in Asians have been confirmed by restriction endonuclease patterns. Recently, both techniques have been used to document genetic compounds of deletions as well as more complex defects in Mediterraneans with HbH disease.

HbH disease is rare in blacks, and affected individuals are reported to have relatively lower percentages of HbH (2%–7%) and a milder condition than Asians with HbH disease. Recently, Dozy et al. reported that one-quarter of a screened black population had α-thal-2 due to deletion of a single α gene. However, none of the 15 black α-thal-1 individuals studied had deletions of both α-loci on one chromosome.

We report quantitative and qualitative studies of the α genes in two black families in which HbH disease is present. Our results indicate that in family members, HbH disease, α-thal-1, and α-thal-2 are due to deletions of 3, 2, and 1 α-globin genes, respectively. Furthermore, at least one of these deletions may have originated by unequal crossing over.

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MATERIALS AND METHODS

Pedigrees for two black families with a-thalassemia are shown in Fig. 1. Individuals A-II-2 and A-II-3 have had symptoms of excessive fatigue all their lives, and their hematocrits have ranged from 28% to 31%. On physical examination, they had no scleral icterus, pallor, or splenomegaly. Patient B-II-1 was found to have a hematocrit of 31% and increased hemolysis. She had no symptoms, signs, or physical findings related to HbH disease.

Mean corpuscular volume (MCV), hemoglobin electrophoresis, and α/β globin synthetic ratios\(^1\) were determined by standard methods. Nuclear DNA was isolated from blood and fibroblast cultures as previously described.\(^3\) DNA was digested with the restriction endonucleases Hpa I, Eco RI plus Bam HI, and Hind III plus Bam HI under conditions specified by the commercial suppliers, and DNA fragments were separated by electrophoresis in 0.8% agarose slab gels.\(^4\) DNA was then transferred to nitrocellulose filters using the methods of Southern\(^15\) as modified by Jeffreys and Flavell.\(^16\)

A probe for human α-globin gene sequences was obtained from the plasmid, JW 101, generously provided by Dr. Bernard Forget.\(^17\) Digestion of plasmid DNA with restriction endonucleases Hinf I and Mbo II yielded a fragment of about 960 base pairs containing the α-globin gene sequences, which was recovered from preparative agarose gels.\(^18\) This fragment was used as an α probe after radiolabeling to a specific activity of approximately 10\(^6\) cpm/μg with α-\(^32\)P-dATP and α-\(^32\)P-dCTP by the “nick translation” function of Escherichia coli DNA polymerase I.\(^19\) Filter hybridization with the α probe was carried out as previously reported.\(^14\) Thereafter, the filters were washed and autoradiographed by procedures described by either Jeffreys and Flavell\(^14\) or Kan.\(^20\)

For gene counting experiments, single-stranded α probe was obtained by hybridizing the double-stranded α probe, described above, with excess mixed globin mRNA. The resulting DNA-mRNA hybrids were eluted from hydroxyapatite (HAP) columns, digested with S\(_1\) nuclease to remove non-α-globin plasmid sequences, and hybridized mDNA was then eliminated by alkali digestion. A constant amount of this single-stranded α probe was hybridized to completion with sonicated genomic DNA (3 mg/ml) from controls and family members. The amount of α probe used was chosen so that normal DNA, containing 4 α genes per diploid cell, would hybridize 50% of the probe at saturation. Under these conditions, the concentrations of probe and complementary genomic sequences are equal, and the percent saturations of the probe expected for genomes with zero, one, two, and three α-globin genes are 0%, 20%, 33%, and 43% by calculation, respectively. Hybridization assays were carried out at

*Experiments involving recombinant DNA were conducted at P2-EK2 containment in accordance with the NIH guidelines.
70°C for 40 hr in a 20-μl mixture of 10 mM Tris-HCl, pH 7.4, 0.9 M NaCl, 1 mM EDTA, and 10 μg/ml oligo-dT. Following hybridization, single- and double-stranded DNAs were eluted from HAP columns, and the percent hybridization was calculated as the ratio of radioactivity in double-stranded DNA to total radioactivity recovered.

RESULTS

The probable phenotypes of family members based on MCV determinations and α:β globin synthetic ratios are shown in Fig. 1. Hemoglobin electrophoresis of fresh blood from individuals A-II-2, A-II-3, and B-II-1 yielded hemoglobin-H concentrations of 4%, 1%, and 10%, respectively.

To ascertain whether these α-thalassemias result from α-gene deletions, the number of α genes was determined by hybridization of the single-stranded α probe to DNAs from members of families A and B (Fig. 2). The range and mean observed for individuals A-II-3 and B-II-1 and an Asian with HbH disease were 19–29, 24%; for A-III-6 (α-thal-1) were 37–38, 38%; for A-III-1 and B-I-2 (α-thal-2) were 37–48, 44%; and for normal controls were 47–54, 50%. Although informative, these results do not allow definitive classification because of the overlap between the ranges for α-thal-1 and α-thal-2 as well as for α-thal-2 and normals.

To clarify the molecular basis of thalassemia in these two black families, nuclear DNA was digested with various restriction endonucleases and analyzed by electrophoresis and hybridization to the α probe. The restriction patterns obtained following double digestion with Eco RI plus Bam HI are shown in Fig. 3A. DNA from a normal control (lane 1) has a 14.0-kilobase (kb) fragment, which contains both α loci. In contrast, α-thalassemia DNA (kindly supplied by Dr. Stuart Orkin; lane 8) has no fragment containing α-globin sequences. Individuals A-III-1 and B-I-2 are presumed to be α-thal-2 (Fig. 1) and to have three α genes (Fig. 2). They have two α-gene-containing fragments (lanes 2 and 5, Fig. 3A), one normal (14.0 kb) and the second smaller than normal (9.8 kb). This pattern is compatible with a chromosome containing two normal α genes and an abnormal homologue containing a single α gene, which yields the 9.8-kb fragment.
Fig. 3. Map of the restriction endonuclease sites in the DNA region containing the α-globin genes as modified from Orkin using our fragment sizes is shown at the top. The 5' and 3' orientation is from left to right, and the α-globin genes are diagrammatically represented by solid rectangles of arbitrary size. Autoradiogram patterns of DNA from a normal control and individuals with α-thalassemia following restriction endonuclease digestion with Eco RI plus Bam HI are shown in (A). Fragment sizes in kilobases (kb) are on the left. Lanes: 1, control; 2, A-III-1; 3, A-III-6; 4, A-II-3; 5, B-I-2; 6, B-II-1; 7, Asian with HbH disease; and 8, Asian α-thalassemia. (B) DNA digestions with Hpa I from the following: 1, A-III-1; 2, A-III-6; 3, A-II-3; 4, B-I-2; and 5, B-II-1.

Individuals A-II-3 and B-II-1 (Fig. 1) and an Asian, all with HbH disease, and a single α gene (Fig. 2), have only a single abnormal 9.8-kb fragment (lanes 4, 6, and 7, respectively; Fig. 3A). Taken as a whole, these data are consistent with deletions of both α genes from one chromosome and one α gene from its homologue. A-III-6, presumed to be α-thal-1 (Fig. 1), has two α genes (Fig. 2). Since his mother, A-II-3, has only the abnormal 9.8-kb fragment, he (lane 3, Fig. 3A) must have received a normal chromosome from his father (A-II-4) and a chromosome containing no α genes from his mother.

That the 9.8-kb fragment in fact contains only one α gene was demonstrated by digestion of the HbH disease DNAs with Hpa I (Fig. 3B). Digestion of normal DNA with Hpa I yields a 4.2-kb fragment, containing the 5' α gene, and a 14.5-kb fragment, containing the 3' α gene. Normal Hpa I patterns are seen using DNA from α-thal-2 individuals A-III-1 and B-I-2 (lanes 1 and 4) due to the presence of a chromosome with intact 5' and 3' α-globin genes. Individual A-III-6 (α-thal-1, lane 2) also has a normal restriction pattern consistent with a chromosome having both α genes and a homologue with neither. In contrast, black individuals with HbH disease, A-II-3 and B-II-1 (lanes 3 and 5), have only a 14.5-kb fragment. The Eco RI plus Bam HI and Hpa I digestions further demonstrate that these individuals with HbH disease have only one α gene.

To gain insight into the nature of the deletion resulting in a single α gene, we
performed DNA digestions with Hind III plus Bam HI (Fig. 4). Following double digestion of DNA from A-III-1 (α-thal-2; lane 2, Fig. 4) and normal DNA (lane 1), we observed the same pattern, namely, bands at 7.8, 4.2, and 3.1 kb (Fig. 3, top). Another black α-thal-2, unrelated to these families (lane 4), also had this normal pattern. However, digestion of DNA from HbH disease subject B-II-1 (lane 3) with Hind III plus Bam HI yielded only two of the normal fragments, 7.8 and 3.1 kb, respectively. Furthermore, the 7.8- and 3.1-kb fragments, but not the 4.2-kb fragment, were observed in a partial Hind III digestion of the Bam HI fragment from the other HbH disease subject, A-II-3. Thus, in both HbH disease subjects, a single α gene was present and the 4.2-kb fragment, which corresponds to sequences between the centers of the two α-globin loci, was absent.

**DISCUSSION**

A combination of α/β globin synthetic studies, gene counting, and specific endonuclease mapping was used to describe the molecular defects in American blacks with α-thalassemia. The restriction patterns obtained suggest that DNA
from all affected family members have a deletion of one or more α-globin genes. While we observed deletions of 3 α genes in blacks with HbH disease, we did not detect the more complex patterns reported in Mediterraneans. 9

Our findings of multiple α-thal-1 individuals who have a normal chromosome and one lacking both α genes contrasts with the report of Dozy et al. 12 However, our results and those of Dozy et al. are not incompatible because our ascertainment of individuals with HbH disease should have selected families with α-thal-1 due to the rare chromosome in which both α genes are either nonfunctional or absent.

From studies of certain Asians with HbH disease, Orkin has suggested that some deletions may have arisen by unequal crossing over between the 5' α locus on one chromosome and the 3' α locus on its homologue. 9 Crossovers occurring in this region should result in the loss of sequences separating the 5' and 3' α-globin genes (Fig. 3, top). Our observations of a normal Hind III plus Bam HI pattern in A-III-1 (α-thal-2) and the absence of the 4.2-kb fragment, corresponding to the sequences between the centers of the 5' and 3' α-globin genes in B-II-1 and A-II-3 (HbH disease), are consistent with this hypothesis. The presence of a single 14.5-kb Hpa I fragment in the two HbH disease samples also agrees with this hypothesis, since an Hpa I site is about 2.5 kb from the 5' end of each α gene (Fig. 3). Further detailed analysis of the α genes and their flanking regions in such individuals should determine the extent of the deletion and its compatibility with a cross over mechanism.

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