Unequal Crossing-Over: A Common Basis of Single \( \alpha \)-Globin Genes in Asians and American Blacks With Hemoglobin-H Disease

By John A. Phillips III, Terry A. Vik, Alan F. Scott, Keith E. Young, Haig H. Kazazian, Jr., Kirby D. Smith, Virgil F. Fairbanks, and Harold M. Koenig

The \( \alpha \)-globin genes of five black Americans, two Chinese, and five Filipinos with HbH disease (an \( \alpha \)-thalassemia state in which there is a single functional \( \alpha \) gene) were analyzed by restriction endonuclease techniques. All subjects were found to have one chromosome 16 lacking both \( \alpha \) genes and another containing a single \( \alpha \) gene. The \( \alpha \)-globin genes of five black Americans, two Chinese, and five Filipinos with HbH disease (an \( \alpha \)-thalassemia state in Blood, 1066 as the mechanism of origin of the single \( \alpha \) gene in these individuals. Restriction endonuclease patterns of the DNA obtained from all 12 subjects were identical and compatible with unequal crossing-over as the mechanism of origin of the single \( \alpha \) gene in these individuals.

The normal human chromosome 16 contains two \( \alpha \)-globin genes (\( \alpha_{\alpha} \)), the centers of which are separated by about 3700 nucleotides (3.7 kb). Abnormal chromosome 16s bearing a single functional \( \alpha \)-globin gene lead to mild \( \alpha \)-thalassemia syndromes. For example, Dozy et al. have reported that 16% of chromosome 16s in American blacks have a single \( \alpha \) gene (\( \alpha -/\alpha - \)). Furthermore, hemoglobin screening studies of Asian newborns suggest that deleted or nonfunctional \( \alpha \)-globin genes occur in 3%-7% of Chinese and Malaysians and 20%-31% of Thais.

Chromosome 16s with a single \( \alpha \)-globin gene can be studied in HbH disease, an \( \alpha \)-thalassemia state in which there is only one functional \( \alpha \) gene (\( \alpha -/\alpha - \)) per diploid genome. The data of Orkin et al. on a single Asian subject with HbH disease suggest that his single \( \alpha \) gene could have originated by unequal crossing-over between \( \alpha \) genes. However, Embury et al. have reported another Asian with a single \( \alpha \) gene in whom the \( 5' \) \( \alpha \) gene was deleted but most of the sequences between the genes were retained, thereby precluding unequal crossing-over as a mechanism for the deletion. Unequal crossing-over as a common basis for single \( \alpha \) genes is also challenged by Orkin’s report of dysfunctional, but nondeleted, \( \alpha \) genes in Mediterraneans, Turks, and Israelis with HbH disease.

To determine the common mechanism(s) producing single \( \alpha \)-globin genes, we studied the \( \alpha \) genes in a series of subjects selected solely on the basis of their having HbH disease. All 12 subjects (5 American blacks, 2 Chinese, and 5 Filipinos) had deletions of three \( \alpha \)-globin genes. Furthermore, restriction endonuclease analyses of the single remaining \( \alpha \)-globin gene were identical in all subjects and compatible with crossing-over between mispaired \( \alpha \) genes as their mechanism of origin.

MATERIALS AND METHODS

HbH disease was confirmed by at least four of the following six criteria: (1) microcytosis (mean corpuscular volume of less than 60 femtoliters); (2) markedly decreased \( \alpha/\beta \)-globin synthetic ratios (0.2-0.4); (3) normal amounts of HbA, and HbF; (4) presence of HbH in fresh hemolysates; (5) absence of iron deficiency; and (6) consistent family studies.

Mean corpuscular volume (MCV), hemoglobin electrophoresis, and \( \alpha/\beta \)-globin synthetic ratios were determined by standard methods. Nuclear DNA was isolated from blood, digested with restriction endonucleases, and DNA fragments were separated by electrophoresis in 0.8% agarose slab gels. The DNA was then transferred to nitrocellulose filters and hybridized with the \( \alpha \) probe. Thereafter, the filters were washed and autoradiographed.

A probe for human \( \alpha \)-globin gene sequences was obtained from the plasmid JW101, generously provided by Dr. Bernard Forget. For filter hybridizations, the fragment containing \( \alpha \)-globin gene sequences was radiolabeled to a specific activity of approximately 10^6 cpm/\mu g with \( \alpha ^{32} \)P-dATP and \( \alpha ^{32} \)P-dCTP by the “nick translation” function of Escherichia coli DNA polymerase I. For gene counting experiments, single-stranded a probe was prepared, and a constant amount of this probe was hybridized to completion with sonicated genomic DNA (3 mg/ml) from controls and individuals with HbH disease. Hybridization conditions and method of quantitation were as previously reported. The relative amount of \( \alpha \) probe used was chosen so that normal DNA, containing four \( \alpha \) 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 percentages of hybridization of the probe expected for genomes with 0, 1, 2, and 3 \( \alpha \)-globin genes are 0%, 20%, 33%, and 43%, respectively.

*Experiments involving recombinant DNA were conducted at P2-EK2 containment in accordance with the NIH guidelines.

Submitted February 19, 1980; accepted March 10, 1980.

Address reprint requests to John A. Phillips III, M.D., Department of Pediatrics, Johns Hopkins Hospital, Baltimore, Md. 21205.

© 1980 by Grune & Stratton, Inc.

0006-4971/80/5506-0015$01.00/0
RESULTS

The number of α-globin genes remaining in the HbH disease subjects was ascertained by determining the percentage of single-stranded α probe annealed by their genomic DNAs. DNAs from all subjects examined annealed an amount of α probe consistent with their having a single α-globin gene per diploid genome (Fig. 1). Thus, all subjects had one chromosome 16 lacking both α genes and another containing a single α gene (−/−α).

Restriction endonuclease analysis was used to determine the origin of the deletions on these chromosome 16s bearing the single remaining α gene. Following Bam HI digestion and agarose gel electrophoresis, normal DNA yielded a single fragment (14.4 kb) that contained both α loci (Fig. 2A). In contrast, all HbH disease DNA samples had a 10.4-kb fragment, and DNA from a black α-thal-2 (αα/−α) individual had 14.4- and 10.4-kb fragments. This implies that the DNA fragment containing the single remaining α gene in these individuals is approximately 4 kb smaller than normal (Fig. 3). After digestion with Bam HI and Hind III (Fig. 2B), normal DNA yielded fragments of 7.4, 3.8, and 3.0 kb. DNA from all HbH disease subjects yielded only two of the normal α-gene-containing fragments (7.4 and 3.0 kb). All subjects lacked the fragment of approximately 3.8 kb, which corresponds to sequences between the centers of the two normal α-globin genes (Fig. 3).

Double digestion with Eco RI and Hpa I showed that the 4.8 and 4.1 kb α-gene-containing fragments in normal DNA were replaced by a single 5.1-kb fragment in HbH disease DNAs (Fig. 2C). This pattern is compatible with loss of the intergenic Hpa I site and a deletion of 3.8 kb (4.8 + 4.1 = 5.1 kb) and precludes a simple deletion of either the 5' (such as reported by Embury) or 3' α gene (Fig. 3).

To further verify the location of this 3.8-kb deletion in HbH disease DNAs, Hind II digestions were done. The normal 3.6- and 1.5-kb fragments, containing sequences 5' to the 5' α gene and 3' to the 3' α gene, respectively, and an additional 1.2-kb fragment of unknown origin were seen in both control and HbH disease DNAs (Fig. 2D). However, the two fragments (3.1 and 0.8 kb) that correspond to sequences between the center of the 5' and 3' α genes in normal DNA...
were absent in all HbH disease DNAs. The Hind I1 data were confirmed by patterns observed following double digestion with Hpa I and Hind III, which showed the normal 5' (3.5 kb) and 3' (4.5 kb) α-gene-containing fragments but absence of the sequences between the centers of the genes (3.1- and 0.8-kb fragments). These data are summarized in Fig. 3. In addition, recent data on three more unrelated HbH disease subjects (two American blacks and one Chinese) yielded identical restriction patterns (data not shown).

**DISCUSSION**

Taken as a whole, the restriction endonuclease patterns in all 12 subjects indicate that they have a single α-gene-containing chromosome (Fig. 1) with a deletion of approximately 3.8 kb of DNA that is indistinguishable by this analysis from the distance between the centers of the two α-globin structural genes in normal DNA (Figs. 2 and 3). We found no examples of differences in α gene number as reported in Mediterraneans, Turks, Israelis, and recently in Cypriots with HbH disease and no molecular basis for clinical differences in severity of HbH disease in different racial groups. Furthermore, the presence of normal restriction sites 5' and 3' to the center of the single remaining α gene with loss of the intergenic Hpa I and Hind II sites suggests that the 3.8-kb deletion corresponds to the sequences between the centers of the normal 5' and 3' α genes (Fig. 3). This pattern is compatible with an unequal or nonhomologous cross-over mechanism, as shown in Fig. 4. This mechanism agrees with both Orkin's data of DNA from a single Asian with HbH disease and the recent report of individuals bearing the reciprocal chromosome 16 that has three α genes.12 Disparate deletions of just the 5' α gene (as reported by Embury1) or the 3' α gene are excluded by restriction patterns seen following Hpa I plus Eco RI, Hind II, and Hpa I plus Hind III digestions (Figs. 3 and 4). For example, a simple 5' α gene deletion, as reported by Embury1, would yield the following restriction patterns: Hpa I plus Eco RI, 4.8-kb fragment; Hind II, 3.1- plus 1.5-kb fragments; and Hpa I plus Hind III, 3.1- plus 4.5-kb fragments.

The compatibility of our results from 12 subjects (unselected for gene number or deletion pattern) representing two different racial groups (American blacks and Asians) with unequal crossing-over suggests that this may be a common mechanism for deletion of one of the normally linked α-globin genes. However, these data cannot exclude the possibility of spontaneous deletions in our subjects, which are equal in size to the distance between the centers of the two α genes and include the intergenic Hpa I and Hind II sites and the 5' α gene (Fig. 3). To eliminate this unlikely possibility, probes for the intergenic sequences derived from genomic DNA are required.

**REFERENCES**

Unequal crossing-over: a common basis of single alpha-globin genes in Asians and American blacks with hemoglobin-H disease

JA 3d Phillips, TA Vik, AF Scott, KE Young, HH Jr Kazazian, KD Smith, VF Fairbanks and HM Koenig