Two Different Molecular Organizations Account for the Single $\alpha$-Globin Gene of the $\alpha$-Thalassemia-2 Genotype

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ABSTRACT The $\alpha$-thalassemia-2 ($\alpha$-thal-2) genotype or mild $\alpha$-thalassemia gene consists of a single structural $\alpha$-globin gene on the chromosome that normally bears two $\alpha$-globin genes. We used blot hybridization to investigate variation in the molecular organization of this genotype and to determine the distributions of these variations in the world population. Two different patterns of gene organization responsible for the $\alpha$-thal-2 genotype were found: the first was the result of a 4.2-kilobase pair deletion involving the normal 5' $\alpha$-globin gene (leftward deletion $\alpha$-thal-2 genotype), and the second probably the result of a crossover deletion of a DNA fragment bridging the two normal $\alpha$-globin genes (rightward deletion $\alpha$-thal-2 genotype). The rightward deletion was found in all 9 Black subjects, all 8 Mediterranean subjects, and 4 of 13 Chinese subjects. The leftward deletion was found in four and the nondeletion $\alpha$-thalassemia lesion was found in five of the nine remaining Chinese subjects. It is likely that these deletions are related to specific DNA sequences that determine DNA recombinational events.

INTRODUCTION

The $\alpha$-thalassemia syndromes are a group of inherited anemias characterized by diminished or absent $\alpha$-globin synthesis (2, 3), which in Blacks (4), Chinese (5, 6), and Mediterraneans (7) is usually caused by deletion of the $\alpha$-globin structural genes.

The inheritance of $\alpha$-thalassemia is determined by two different types of $\alpha$-thalassemia genes, the more severe of which is called the $\alpha$-thalassemia-1 ($\alpha$-thal-1) gene and the milder of which is called the $\alpha$-thalassemia-2 ($\alpha$-thal-2) gene (8). Restriction endonuclease mapping has shown that the latter is composed of a single $\alpha$-globin locus (9, 10) and that the former consists of a chromosome devoid of $\alpha$-globin genes (10), as contrasted with the two $\alpha$-globin loci of the normal $\alpha$-globin genotype (10, 11). The particular clinical syndromes found in a population containing $\alpha$-thalassemia are determined by the relative frequency of the $\alpha$-thalassemia genotypes segregating within that group. In the Black population, the $\alpha$-thal-2 genotype occurs so frequently (12–14) and the $\alpha$-thal-1 genotype so infrequently that most cases of $\alpha$-thalassemia trait are due to homozygous $\alpha$-thal-2 (14); and hydrops fetalis due to homozygous $\alpha$-thalassemia has never been reported. In the Asian and Mediterranean populations are found the $\alpha$-thal-1 as well as the $\alpha$-thal-2 genotypes (7, 10), and this accounts for the presence of hydrops fetalis due to homozygous $\alpha$-thal-1 in these groups.

We examined the variations in the physical organization and the worldwide population distribution of the $\alpha$-thal-2 genotypes. We found two varieties of this genotype, one of which was the result of a deletion.

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Abbreviations used in this paper: $\alpha$-thal-1, $\alpha$-thalassemia-1; $\alpha$-thal-2, $\alpha$-thalassemia-2; Hb-H, hemoglobin-H; kb, kilobase pairs.
involving the normal 5' α-globin locus, which we have called the leftward deletion of α-thal-2 genotype, and the other of which was due to a deletion possibly involving both normal α-globin loci by a homologous but unequal crossing-over, which we have called the rightward deletion α-thal-2 genotype. We screened DNA containing the single α-globin locus from 30 subjects of three different races and found the leftward deletion only in 4 of 13 Chinese subjects with hemoglobin-H (Hb-H) disease, the rightward deletion in all 9 Black subjects with homozygous α-thal-2, in all 8 Mediterranean subjects with Hb-H disease, and in 4 of the Chinese subjects with Hb-H disease. The other five Chinese subjects had the nondeletion type of Hb-H disease (10, 15).

METHODS

The Chinese and Mediterranean subjects with Hb-H disease had microcytic hemolytic anemia (2, 3) and evidence of Hb-H within their erythrocytes, as tested by brilliant cresyl blue staining (16) or by Hb electrophoresis (2). The Black subjects with α-thalassemia trait due to homozygous α-thal-2 were identified by screening DNA samples to determine their α-globin genotypes (14). DNA was prepared from peripheral blood leukocytes as previously described (17).

Restriction endonucleases Eco RI, Xba I, Bam HI, Sac I, Bgl II, and Mbo II were purchased from New England Biolabs, Beverly, Mass., and Hpa I and Hind III were purchased from Bethesda Research Laboratories, Rockville, Md. 7-μg aliquots of DNA were digested for 4 h under conditions recommended by the manufacturer. When digests with more than one enzyme were done, the first enzyme was inactivated at 68°C for 10 min after the initial digestion, and the DNA was ethanol-precipitated and redissolved in the buffer appropriate for the second digestion. After the last digestion, the DNA was phenol-extracted, ethanol-precipitated, and redissolved for electrophoresis.

The hybrid plasmid JW101, containing a synthetic copy of the human α-globin coding sequences (18) (a gift from Dr. B. Forget of the Yale University School of Medicine, New Haven, Ct.), was cultured and isolated (19) according to the containment conditions described in the National Institutes of Health Guidelines for Recombinant DNA Research. A 1.6-kilobase pair (kb) DNA fragment containing the α-globin insert was isolated and nick-translated as described (10) to provide hybridization probe.

After restriction endonuclease digestion, DNA samples were electrophoresed through 0.8% agarose gels, transferred onto nitrocellulose filters (Schleicher and Schuell, Inc., Keene, N. H.), hybridized, and autoradiographed using a method modified by Kan and Dozy (17) from that of Southern (20).

RESULTS

Normal DNA generated α-globin gene-specific fragments of 4.2 and 14 kb when digested with Sac I and of 12.5 and 7.0 kb when digested with Bgl II (Fig. 1). Thus, as with Hpa I (10, 11), these enzymes cleave between the normal α-loci. DNA containing the leftward deletion α-thal-2 genotype, known to contain only the normal 3' α-globin gene (10), generated just

![Figure 1](imageurl)  
**Figure 1** Autoradiographs show α-globin gene-specific restriction fragments. DNA was digested with enzymes indicated at the top and was from the following subjects: (1) nonthalassemic, (2) Hb-H disease with leftward deletion α-thal-2 genotype, and (3) Hb-H disease with rightward deletion α-thal-2 genotype. Sizes of the fragments in kilobase pairs shown in the margins were determined from size markers of λ-DNA X Hind III (21).

the 14-kb Sac I fragment and the 7.0-kb Bgl II fragment (Fig. 1). Thus, the normal 3' α-globin gene also resides on these fragments. DNA containing the rightward deletion α-thal-2 genotype generated a single Sac I fragment of ~14 kb and a single Bgl II fragment of ~16 kb. Thus, these α-thal-2 genotypes can clearly be distinguished by α-globin gene mapping using digestion with the enzyme Bgl II alone.

As seen in Fig. 2, the enzyme Xba I produced a single α-globin gene-specific DNA fragment from DNA containing the normal α-globin genotype (~18 kb) or containing either of the two α-thal-2 genotypes (~14 kb). The fragment from the leftward deletion α-thal-2 genotype was ~0.5 kb smaller than the fragment from the rightward deletion α-thal-2 genotype.

A series of double digests was performed using Xba I with Hpa I, Sac I, or Bgl II in order to use the established Hpa I cleavage sites (10, 11) to determine the location of the Xba I, Sac I, and Bgl II cleavage sites. From normal DNA the α-globin gene-specific fragments produced by Xba I and Hpa I were 4.2 and 3.9 kb; by Xba I and Sac I, 4.2 and 3.5 kb; and by Xba I and Bgl II, 12.5 and 2.6 kb (Fig. 2). Thus, Xba I does not cleave the normal 5' fragments produced by these enzymes but does cleave their 3' fragments so that the distance from the most 3' Xba I site to the intergenic sites are 3.9 kb for Hpa I, 3.5 kb for Sac I, and 2.6 kb for Bgl II. In each of these double digestions, DNA containing the leftward deletion α-thal-2 genotype generated a fragment equal in size to the 3' fragment from normal DNA (Fig. 2), confirming that this deletion involved the normal 5' α-globin gene and did not alter
the intergenic cleavage sites of Hpa I, Sac I, and Bgl II. In each of these double digestions, DNA containing the rightward deletion \(a\)-thal-2 genotypes generated a single \(\alpha\)-globin gene-specific fragment equal in length to the sum of the lengths of the two normal fragments minus 3.7 kb (Fig. 2): 4.4 kb for Xba I and Hpa I, 4.0 kb for Xba I and Sac I, and 11.4 kb for Bgl II and Xba I.

Hind III cuts within the \(\alpha\)-globin structural gene (22) to produce three \(\alpha\)-globin-specific fragments from normal DNA (10, 11), so that digestion with this enzyme in combination with one of the enzymes known to cut between the normal \(\alpha\)-globin loci (Hpa I, Sac I, and Bgl II) produces four \(\alpha\)-globin gene-specific fragments. Such digestions were performed to identify which DNA fragments had been deleted in the two \(a\)-thal-2 genotypes. Fig. 3 shows that normal DNA generated \(\alpha\)-globin-specific fragments of 17, 3.7, and 4.5 kb in digestion with Hind III alone, and that DNA containing either \(a\)-thal-2 genotypes generated fragments of \(\sim17\) and 4.5 kb. The fragments generated from normal DNA by each of the double digestions are shown in Fig. 3 and are in 5' to 3' order: 3.5, 0.7, 3.0, and 4.5 kb for Hind III + Hpa I; 3.1, 1.1, 2.6, and 4.5 kb for Hind III + Sac I; and 10.5, 2.0, 1.7, and 4.5 kb for Hind III + Bgl II. In Table I these fragments are labeled A, B, C, and D in 5' to 3' order, and the presence or absence of the fragments is tabulated. With the leftward deletion \(a\)-thal-2 genotype, only fragments C and D were detected, and with the rightward deletion \(a\)-thal-2 genotype, only fragments A and D were detected. These data confirm that the leftward deletion \(a\)-thal-2 genotype is missing the fragments generated from the normal 5' \(\alpha\)-globin locus, and show that the rightward deletion \(a\)-thal-2 genotype is missing the two fragments generated from the normal intergenic sequences, as mapped in Fig. 4.

In Fig. 4, the normal sequences deleted in the creation of the leftward \(a\)-thal-2 genotype are indicated by the upper shaded grey area which spans from the normal 5' Hpa I site to the normal intergenic Hpa I site. However, these mapping data do not identify exactly which of the normal Hpa I and Sac I sites remain in this \(a\)-thal-2 genotype. The 4.2-kb deletion could have involved either but not both of these two normal Sac I sites and either but not both of these two normal Hpa I sites. A possible location of the normal sequences deleted to create the rightward deletion \(a\)-thal-2 genotype is indicated by the lower shaded grey area. The findings that this deletion is the same size as the intergenic distance and that it involves the intergenic sequences are compatible with the deletion having been caused by a homologous but unequal crossing-over. The mapping data locate the site of this deletion within a target area defined by one intergenic Bgl II site and one intergenic Hpa I site, and this location is compatible with a crossover having occurred either within the \(\alpha\)-globin structural gene, resulting in a fusion product analogous to the Hb Lepore gene (23–26), or adjacent to the structural gene, in which case the products would map the same but would contain no fusion gene.

To determine the worldwide distribution of the two \(a\)-thal-2 genotypes defined here, DNA samples from
The fragments of each double digestion are shown and are labeled A, B, C and D in 5' to 3' order. The presence (+) or absence (−) of each fragment is indicated for the two α-thal-2 genotypes.

13 Chinese subjects with Hb-H disease, 9 Black subjects with homozygous α-thal-2, and 8 Mediterranean subjects with Hb-H disease were analyzed by single Bgl II digestion, and the results are shown in Table II. The leftward deletion α-thal-2 genotype was found only in 4 of the 13 Chinese Hb-H disease subjects. The rightward deletion α-thal-2 genotype was found in all eight Mediterranean Hb-H disease subjects, four of the Chinese Hb-H disease subjects and all nine Black homozygous α-thal-2 subjects (the last group accounting for 18 chromosomes with this genotype). The nondeletion α-thalassemia lesion with α-globin genes at both normal loci (10) was found in five Chinese subjects with Hb-H disease.

**DISCUSSION**

Either interchromosomal or intrachromosomal crossover events could result in the deletions that cause the α-thal-2 genotypes. The leftward deletion would be the result of either type of crossover occurring between the DNA sequences designated as area 1 in Fig. 5. These are highly homologous sequences (27), bounded on both stretches of chromosome by an

![Figure 4](image-url)

**FIGURE 4** Physical maps showing the restriction endonuclease sites around α-globin genes of the normal and both α-thal-2 genotypes. The leftward deletion α-thal-2 genotype is shown above, the normal α-globin genotype in the middle, and the rightward deletion α-thal-2 genotype below. The direction of transcription is shown by the horizontal arrow with the 5' to 3' orientation of DNA indicated. Cleavage sites of the restriction endonucleases including previously established sites (12, 16) are indicated by the arrows. The shaded grey areas indicate the normal α-globin DNA sequences that were deleted to create α-thal-2 genotypes, and the limits of the accuracy with which these locations have been determined is explained in the text.
TABLE II
Prevalence of the α-Thal-2 Genotypes in Chinese, Black, and Mediterranean α-Thalassemia Populations

<table>
<thead>
<tr>
<th></th>
<th>Leftward deletion genotype</th>
<th>Rightward deletion genotype</th>
<th>Nondeletion (α-thal-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chinese Hb-H disease</td>
<td>4</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Black homozygous α-thal-2</td>
<td>0</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td>Mediterranean Hb-H disease</td>
<td>0</td>
<td>8</td>
<td>0</td>
</tr>
</tbody>
</table>

Because Bgl II digestion allowed distinction of the α-globin genotype (Fig. 2), this enzyme was used to assess the α-globin genotype responsible for the α-thalassemia syndromes listed.

Hpa I and a Sac I site. The rightward deletion would be the result of either type of crossover occurring between the DNA sequences designated as area 2 in Fig. 5. These, too, are highly homologous sequences (27), bounded on one stretch of chromosome by an Hpa I site and on the other by a Bgl II site. The detection of the apparent reciprocal crossover product of an interchromosomal event causing the rightward deletion, a chromosome bearing three α-globin loci (28, 29), suggests that the rightward deletion is the result of an interchromosomal crossover.

The finding of the rightward deletion α-thal-2 genotype in all three races commonly afflicted with α-thalassemia suggests that the determinants of this deletion, possibly specific DNA sequences that regulate DNA recombination (30), are widespread in the population of the world. Although the Chinese population demonstrated remarkable molecular diversity in having the nondeletion α-thalassemia lesion and both the leftward and the rightward deletion α-thal-2 genotypes, the similarity in the physical maps of the four leftward deletion α-thal-2 genotypes suggests that the Chinese may also have specific DNA sequences that determine the site of this deletion.

The two normal human α-globin genes are part of a gene cluster (27), several features of which are characteristic of a multi-gene family involved in the coincidental evolutionary process of homologous but unequal crossing-over in the maintenance of homology (31, 32). These characteristics are a degree of homology among members that is sufficient to promote unequal alignment a common phenotypic function among members, evidence of expansion and contraction of the gene family size (such as variation from zero to three α-globin genes per chromosome), and the fixation of one member of the gene family (9, 10, 27–29). Thus, the same process may be responsible both for the maintenance of homology in this gene cluster and for the deletions that cause α-thalassemia.

FIGURE 5 Recombinant DNA events that would result in the two α-thal-2 genotypes. (A) Interchromosomal crossover deletions related to unequal alignment. The leftward deletion α-thal-2 genotype would result from a crossover event occurring in shaded area 1. The 0.4-kb area in which this crossover could occur is bounded by the Hpa I and Sac I sites that flank the 5′ α-locus. The rightward deletion α-thal-2 genotype would result from a crossover event occurring in shaded area 2. This 2.1-kb area is bounded by the intergenic Bgl II site of the chromosome shown above and the intergenic Hpa I site of the chromosome shown below. If the crossover occurred within the structural genes, the single locus of this α-thal-2 genotype would be a Lepore-like fusion product. (B) Intrachromosomal crossover deletions. The leftward deletion α-thal-2 genotype would result from an intrachromosomal crossover event occurring in shaded area 1. This 0.4-kb area in which this crossover could occur is bounded by the Hpa I and Sac I sites flanking the 5′ α-locus. The rightward deletion α-thal-2 genotype would result from an intrachromosomal crossover event occurring in shaded area 2.

It has been postulated that the frequent occurrence of α-thalassemia in malarial endemic areas is due to evolutionary selection of genes that afford protection against death from malaria (33). This proposal recently has been supported by data suggesting a mechanism of such protection: the diminished growth of Plasmodium falciparum in α-thalassemic erythrocytes is related to inadequate host cell defense against the oxidative stress generated by parasitic growth (34). In addition to the evolutionary selection of protection from malaria, another determinant of the high frequency of α-thalassemia genes may be specific DNA sequences that determine DNA recombination (30). The relationship between the degree of homology within a multi-gene family and the rate of crossing-over within the family (32) suggests that the frequent finding of α-globin gene deletions may be related to the α-globin genes having both extensive flanking sequence homology that extends at least as far as 3 kb 5′ from
each normal human α-globin gene (27) and only small intervening sequences (27, 35). Thus, the potential target area for genetic rearrangement affecting the α-globin genes is not interrupted by extensive non-homologous intervening sequences and is expanded from the ~0.5 kb of the structural gene to 3 kb, an expansion which presumably conveys a commensurately increased likelihood of recombinant events. These extended areas of sequence homology contain not only both α-thal-2 deletions but also two recently reported deletions that are indistinguishable from the α-thal-2 genotypes, but which occur during the cloning of normal human α-globin DNA (27). The similarity of all of these various deletions suggests that special sites that determine DNA recombination (30) play a role in α-globin DNA recombination.

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