Alpha-Thalassemia Is Related to Prolonged Survival in Sickle Cell Anemia

By J. Gregory Mears, Herbert M. Lachman, Dominique Labie, and Ronald L. Nagel

We have determined the frequency of deletional α-thalassemia in black populations in the USA and Africa that harbor sickle cell anemia. In normals, the frequency of the chromosome bearing a deletion of one of the two normal α gene loci, designated (−α), ranged from 0.12 to 0.16, and in sickle trait subjects, the frequency ranged from 0.18 to 0.20. By contrast, in sickle cell anemia subjects, the frequency was significantly greater and ranged from 0.22 to 0.33. Analysis demonstrated that the greater frequency in the last group was primarily a result of an increased number of subjects with α-thalassemia trait (also called homozygous α-thalassemia-2). In addition, the frequency of the (−α) chromosome was found to increase progressively with age, supporting the hypothesis that α-thalassemia is favorable to the survival of subjects with sickle cell anemia. Thus, individuals who inherit α-thalassemia and sickle cell anemia may represent a subgroup of patients with a longer life expectancy.

SICKLE CELL ANEMIA is the most thoroughly studied disease in which the derangement is identifiable at the molecular level.1 Despite an impressive array of facts concerning the molecular and cellular events of sickling, an understanding of the variable clinical course observed in sickle cell anemia remains elusive.2 One factor postulated to favorably modify the course of sickle cell anemia is the concurrent inheritance of α-thalassemia.3-6 Other observers, however, dispute this claim.7,8 In past studies, a major difficulty masking the possible interrelationship of α-thalassemia and sickle cell anemia has been the reliable identification of the milder forms of α-thalassemia.9 Recently, restriction endonuclease mapping of the α gene loci in black populations has demonstrated that α-thalassemia is a result of α gene deletion and that chromosomes bearing the deletion of one of the two normal α gene loci are quite common in various black populations, with a frequency of approximately 16%.10-12 Nondeletion α-thalassemia genes have not been described. Thus, restriction endonuclease mapping provides an accurate assay for the silent carrier state (or heterozygous α-thalassemia-2), in which one of the four normal α gene loci is missing, and for α-thalassemia trait (or homozygous α-thalassemia-2), in which two α genes are deleted in trans.10-12 The more severe forms of α-thalassemia are very rare in black populations because chromosomes bearing two α gene deletions in cis are extremely uncommon.10,11

We now describe an analysis of α gene deletion by restriction endonuclease mapping of DNA from black Americans and Africans. The results demonstrate that subjects with sickle cell anemia possess chromosomes bearing α gene deletions in significantly greater numbers than normals or sickle cell trait subjects. In addition, we demonstrate a positive relationship between α gene deletions and prolonged survival in sickle cell anemia.

MATERIALS AND METHODS

Twenty milliliters of peripheral blood anticoagulated with EDTA was obtained following informed consent from 143 black subjects. Sixty-three were black Americans residing in New York City, 55 were West Africans, and 25 were Equatorial Africans. Normals and sickle trait subjects were random unrelated volunteers, whereas the sickle cell anemia subjects were unrelated volunteers followed in sickle cell anemia clinics in New York City and Africa. Patient accrual was done without knowledge of clinical severity or hematologic measurements.

Hemolysates from all subjects underwent routine cellulose acetate hemoglobin electrophoresis at alkaline pH, and hemoglobin phenotype was determined. Following the removal of plasma by centrifugation, the red blood cells in each sample were selectively lysed and the intact leukocytes harvested by centrifugation at 4,000 g for 10 min. Leukocyte pellets from the African subjects were frozen in dry ice and transported to New York City for further processing.

Leukocyte pellets were digested with 50 µg/ml Proteinase K in the presence of 0.1% sodium dodecyl sulfate at 55°C for 16 hr. High molecular weight DNA was purified by repeated phenol extraction and concentrated by ethanol precipitation. After extensive dialysis against 1.5 mM NaCl/0.15 mM EDTA, 15 µg of each DNA sample was digested with the restriction endonuclease Bam H1 (New England Biolabs, Beverly, MA) in the buffer recommended by the manufacturer and using twice the recommended amount to ensure complete digestion over 2 hr at 37°C. The DNA samples were then electrophoresed in 0.9% agarose and transferred to nitrocellulose filter (Schleicher and Schuell, Keene, NH), as described by E. Southern.13

Filter hybridization was performed as previously described, employing the plasmid JW 101 containing α-cDNA that had been nick-translated by the technique described by T. Maniatis et al.14 in the presence of 32P-deoxynucleotides (Amersham, Arlington Heights, IL) to generate a probe with a specific activity of 1–3 × 106 cpm/µg.

Following hybridization, the filters were washed in the presence of
a low salt concentration at 65°C, as previously outlined, and exposed to x-ray films at -70°C for 24-48 hr. The autoradiograms were then analyzed for \( \alpha \) gene content as follows: Bam HI has been shown to generate a single DNA fragment of 14 kilobases (kb) that carries both normal gene loci, whereas the deletion of one of the two \( \alpha \) gene loci results in a smaller fragment of 10.5 kb (Fig. 1). Thus, for this study, a normal \( \alpha \) gene complement, designated \((\alpha\alpha/\alpha\alpha)\), was assigned when a DNA sample was shown to possess only 14-kb fragments. Theoretically, individuals possessing a \((\alpha\alpha/-\alpha)\) genotype could be confused with normals. However, the \((\alpha\alpha/-\alpha)\) deletion is extremely rare in black populations and would therefore have no significant impact on our study. The silent carrier state, designated \((\alpha\alpha/-\alpha)\), was assigned if only 10.5-kb fragments were observed (Fig. 2). Chi-square analysis with Yates’ correction where applicable was employed in the statistical comparisons.

RESULTS

The West African population consisted of 29 normals (AA), 11 sickle traits (AS), and 15 subjects with sickle cell anemia (SS). Equatorial Africans consisted of 25 sickle cell anemia subjects, whereas the American population consisted of 25 normals, 10 sickle traits, and 28 sickle cell anemia patients.

The results of the restriction endonuclease mapping of the samples are presented in Table 1, along with a calculation of the frequency of the \((-\alpha)\) chromosome in the various samples. In West African normals, the \((-\alpha)\) chromosome frequency was 0.16, and in the 7 sickle trait subjects, it was 0.18. In 15 sickle cell anemia subjects it was 0.33. The difference in the \((-\alpha)\) chromosome frequency between the normals and the sickle cell anemia subjects was significant \((p < 0.025)\). In the 25 black Americans with normal adult hemoglobin, the frequency of the \((-\alpha)\) chromosome was 0.12; it was 0.20 in 10 sickle trait subjects. However, in the 28 sickle cell anemia subjects, the \((-\alpha)\) chromosome frequency was 0.30, significantly greater than in the normals \((p < 0.001)\) or the sickle trait subjects \((p < 0.05)\). In 25 sickle cell anemia subjects from Equatorial Africa, the \((-\alpha)\) chromosome frequency was 0.22.

When the black populations are collated, 54 normal blacks possess a \((-\alpha)\) chromosome frequency of 0.14, whereas 68 sickle cell anemia subjects possess a \((-\alpha)\) chromosome frequency of 0.28; a difference that is statistically significant \((p < 0.001)\).

Utilizing the combined sickle trait population from the USA and West Africa to calculate the frequency of the \((-\alpha)\) chromosome to be 0.19, the sickle cell anemia population from these two regions does not fit

![Fig. 1. Black rectangles represent exons, whereas the white rectangles represent introns within the \( \alpha \) gene. The upper strand represents the normal map of the \( \alpha \) gene loci, and the lower strand represents the deletion of one \( \alpha \) gene. The mechanism giving rise to the deletion in blacks is thought to be an unequal crossover between nonhomologous \( \alpha \) gene regions, although the exact point of crossover has not been determined (see ref. 18 for a review). The arrows represent recognition sites for the restriction endonuclease Bam HI, and the underlying brackets labeled “14 kb” and “10.5 kb” are the sizes of the fragments generated by Bam HI cleavage.](image)

![Fig. 2. Autoradiogram of DNA digested with Bam H1 and hybridized to \(^{32}\)P-\( \alpha \)-cDNA (JW 101). All channels contain DNA from sickle cell anemia subjects. Channels 1, 3, and 5 contain only 14-kb fragments and thus possess a full complement of \( \alpha \) genes (\( \alpha\alpha/\alpha\alpha \)). Channel 2 contains a 14-kb and a 10.5-kb fragment and thus has a deletion of one \( \alpha \) gene (\( \alpha\alpha/-\alpha \)), whereas channel 4 contains only 10.5-kb fragments and thus has a deletion of two \( \alpha \) genes in trans (\(-\alpha/-\alpha\)).](image)

![Table 1. Frequency of Alpha Gene Deletion and Alpha-Thalassemia Genotypes](table)
Hardy-Weinberg proportions for this chromosome primarily because of a significantly greater proportion of subjects homozygous for the (-a) chromosome (expected number = 2, observed number = 6, \( p < 0.025 \)).

Table 1 also demonstrates the frequencies of the silent carrier state and \( \alpha \)-thalassemia trait in the populations studied. An increase in frequency of the silent carrier state was seen in sickle trait subjects when compared to normals, in addition to the increased frequency of \( \alpha \)-thalassemia in the comparable sickle cell anemia populations.

The ages of 66 of 68 sickle cell anemia subjects were recorded and separated by decades, as shown in Table 2. Of 19 subjects between the ages of 1 and 10 yr, the (-a) chromosome frequency was 0.18, and the population fit Hardy-Weinberg equilibrium. However, for the 26 subjects in the second decade, the (-a) chromosome frequency was 0.27, and for the 21 subjects older than 20, the (-a) chromosome frequency was 0.36. The increase in (-a) chromosome frequency between the first and third decades was statistically significant \(( p < 0.01)\). The increase in (-a) chromosome frequency was seen in all three populations—USA: 1–10 = 0.13, >20 = 0.33; West Africa: 1–10 = 0.21, >20 = 0.50; Equatorial Africa: 1–10 = 0.19, >20 = 0.33. A similar analysis of AA subjects failed to identify any age-related trend in the (-a) chromosome frequency.

**DISCUSSION**

Our observations of the occurrence of deletional \( \alpha \)-thalassemia in frequencies ranging from 32% to 50% in sickle cell anemia populations have strengthened speculations that \( \alpha \)-thalassemia is at least one independent variable that may favorably modify the clinical course of sickle cell anemia.\(^\text{18}\) By a methodology allowing an accurate determination of \( \alpha \) gene deletions, we have examined the question of the impact of \( \alpha \)-thalassemia in three populations harboring sickle cell anemia and have found a positive relationship of \( \alpha \)-thalassemia to prolonged survival in sickle cell anemia in all three. This observation may be limited by the small sample sizes, although it is strengthened by being present in three separate populations.

One possible pitfall in our study is the ascertainment of sickle cell anemia subjects. By recruiting subjects from sickle cell anemia clinics, a possible bias for patients with clinically more severe disease arises, particularly if only a small fraction of the total sickle cell anemia population is cared for in the clinic. However, the weight of clinical evidence suggests that the impact of \( \alpha \)-thalassemia, if any, favors a more benign course for sickle cell anemia.\(^\text{3,8}\) Also, at least for the described New York City population, patient accrual to the clinic is comprehensive, such that very few undiagnosed sickle cell anemia subjects are found when community screening is performed (unpublished data). Thus, our sampling from sickle cell anemia clinics, blinded for clinical severity, is very unlikely to introduce a negative bias.

Another potential difficulty pursuing such an analysis in the USA is the heterogeneous nature of the black American population both in terms of its diverse ethnic origins in Africa, as well as the infusion of white genes, now estimated to be about 22%.\(^\text{20}\) The difference in (-a) chromosome frequency between normals and sickle cell anemia subjects in the USA may be partly explained by the possibility that subject selection for sickle genes results in a gene pool population biased toward genes whose ancestry migrated from Africa. However, gene pool dilution is absent in selected ethnic groups in Africa, where the difference between normals and sickle cell anemia subjects persists. Additionally, one would predict no (-a) chromosome frequency differences between sickle trait subjects and sickle cell anemia subjects from either the USA or Africa, regardless of gene admixture, if selection for the (-a) chromosome does not occur in offspring. Thus, gene pool dilution or sample bias does not resolve the discrepancy in (-a) chromosome frequency among normals, sickle trait subjects, and sickle cell anemia subjects in both the USA and West Africa.

Hardy-Weinberg equilibrium for the (-a) chromosome should be obtained in random matings of sickle trait subjects if the mutation rate remains constant, gene flow is insignificant, and selection for or against a particular genotype does not occur.\(^\text{21}\) The sickle cell anemia populations do not fit Hardy-Weinberg proportions for the (-a) chromosome, suggesting that subjects also inheriting \( \alpha \)-thalassemia are at an advantage for survival.

From the conclusion drawn thus far, that \( \alpha \)-thalassemia is favorable to the survival of subjects with sickle cell anemia, one would predict an increasing percentage of individuals with \( \alpha \)-thalassemia in older age groups. Such a correlation has been observed in our sickle cell anemia populations. It is interesting to note that the frequency of the (-a) chromosome in the first decade of life in subjects with sickle cell anemia is 0.18, which is similar to the (-a) chromosome frequency in

<table>
<thead>
<tr>
<th>Years</th>
<th>aa/aa</th>
<th>aa/–a</th>
<th>–a/–a</th>
<th>(-a) Chromosome Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1–10</td>
<td>13</td>
<td>5(0.26)</td>
<td>10(0.05)</td>
<td>0.18</td>
</tr>
<tr>
<td>11–20</td>
<td>16</td>
<td>6(0.23)</td>
<td>10(0.15)</td>
<td>0.27</td>
</tr>
<tr>
<td>&gt;20</td>
<td>9</td>
<td>9(0.43)</td>
<td>3(0.14)</td>
<td>0.36</td>
</tr>
</tbody>
</table>

Numbers in parentheses represent the frequency of the silent carrier state, designated (\( \alpha \alpha /-\alpha \)), and \( \alpha \)-thalassemia trait, designated (-a/–a).
our normal populations and a previously published calculation. In the second and third decades, a greater frequency is observed.

There appear to be two determinants influencing the increasing frequency of the (−α) chromosome. One is an approximately threefold rise in the frequency of the α-thalassemia trait genotype (−α/−α) by the second decade (Table 2). This determinant is the primary reason for the observed Hardy-Weinberg disequilibrium, which suggests that the major survival benefit is accrued by subjects with α-thalassemia trait. Since clinical experience has shown that most childhood deaths in sickle cell anemia occur by age 5, we would expect a rising frequency of the (−α) chromosome within the first decade. However, our sample size does not permit us to meaningfully address this issue. A second determinant is an approximately twofold rise in the frequency of the silent carrier genotype (aa/−α) by the third decade (Table 2). Further studies of older sickle cell anemia subjects are required to determine if this is a continuing trend and to evaluate its significance. Our data do not allow us to establish whether α-thalassemia has an effect on sickle trait subjects.

Our results differ somewhat from those reported by Higgs et al., in which no age-related trend was discerned until later in life. However, this study was not designed to directly address the question of a possible survival benefit. Subject selection based on hemoglobin A2 levels is satisfactory for the identification of a limited study population but may result in a biased sample. However, in view of our small sample sizes and the above noted difference in the studies, larger studies of black African populations and further Jamaican studies are necessary to resolve this issue.

Although it appears from our data that subjects inheriting sickle cell anemia and α-thalassemia trait (−α/−α) have an advantage for survival and thus are more likely to reach adult life, it is not clear whether such individuals display a milder clinical course characterized by fewer and less severe crises. Many factors may well interplay, including, but not limited to, α-thalassemia. A recent observation suggests that sickle cell anemia subjects who also inherit α-thalassemia have higher than usual concentrations of hemoglobin F, which is known to inhibit gelation, as well as decreased hemolysis, although others dispute this.

Genetic factors responsible for a favorable clinical course in sickle cell anemia are likely to be manyfold. The genetic component responsible for the elevated hemoglobin F levels and mild sickle cell anemia in Saudi Arabia is one example, whereas our observations of an increased frequency of α-thalassemia in subjects with sickle cell anemia is another independent variable. Clearly, others exist, but presently remain unidentified. An understanding of the molecular genetic mechanisms responsible for a favorable course in sickle cell anemia is important for several reasons. First, fetuses destined for a mild form of the disease may be diagnosed by fetal DNA sampling in utero and spared abortion. Second, an understanding of the genetics of mild sickle cell anemia may result in therapeutic endeavors at the gene level or at the red cell level capable of favorably modulating the disease.

ACKNOWLEDGMENT

We thank Drs. K. P. E. Amegnizin, R. Cabannes, L. Kaptue Noche, P. Patel, E. Roth, and M. Santorineou for patient referrals. We are indebted to Kim Schaefer and Beth Gross for excellent technical assistance and to Mary Ann O’Connell for manuscript preparation.

REFERENCES

3. Aksoy M: The first observation of homozygous hemoglobin S-alpha thalassemia disease and two types of sickle cell thalassemia disease: (a) sickle cell-alpha thalassemia disease, (b) sickle cell-beta thalassemia disease. Blood 22:757, 1963
7. Natta C: Failure of the alpha-thalassemia gene to decrease the severity of sickle cell anemia. Blood 51:1163, 1978
Alpha-thalassemia is related to prolonged survival in sickle cell anemia

JG Mears, HM Lachman, D Labie and RL Nagel