The Molecular Basis of α Thalassemia in India. Its Interaction With the Sickle Cell Gene

By Andreas E. Kulozik, Bimal C. Kar, Graham R. Serjeant, Beryl E. Serjeant, and David J. Weatherall

The α globin genotype of a total of 282 Indians from Orissa state has been analyzed. The overall α thalassemia gene frequency is 0.29, most frequently caused by the −α42 and −α23 deletions. In one family a novel −α3’ deletion removing the α1 globin gene with some of its flanking sequences has been found, suggesting further sequence homology of the α globin gene cluster to the α1 globin gene. Patients with sickle cell disease and α thalassemia had higher hemoglobin (Hb) levels, RBC counts, and Hb A2 levels, and lower reticulocyte counts, MCV, MCH, and Hb F levels than those with a normal α genotype. The frequency of splenomegaly was not influenced by the α globin genotype. A higher prevalence of α thalassemia was found in patients >10 years of age than in the younger group, suggesting a possible advantageous effect of α thalassemia on the survival of patients with sickle cell disease.

T HE a THALASSEMIA syndromes are characterized by reduced or absent α globin chain synthesis resulting in globin chain imbalance. The relative excess of γ- or β-chains form the tetramers, hemoglobin (Hb) Bart’s or Hb H, respectively, causing a predominantly hemolytic anemia. In normal persons there are four α globin genes arranged as a pair of highly homologous linked genes on the short arm of each chromosome 16. The molecular pathology inactivating these genes is heterogeneous including point mutations and deletions of different sizes affecting either one or both genes of the pair. The clinical picture of α thalassemia is determined by the number of functioning genes remaining and includes intrauterine death (Hydrops fetalis), thalassemia intermedia (Hb H disease), and absence of symptoms with or without hematologic abnormalities. Another clinically important feature of α thalassemia is its interaction with other hemoglobinopathies like α-thalassemia 2. The molecular pathology inactivating these genes is heterogeneous including point mutations and deletions of different sizes affecting either one or both genes of the pair. The clinical picture of α thalassemia is determined by the number of functioning genes remaining and includes intrauterine death (Hydrops fetalis), thalassemia intermedia (Hb H disease), and absence of symptoms with or without hematologic abnormalities.

RESULTS

Prevalence of deletional α thalassemia. The α globin genotype was determined by gene mapping in a total of 126 patients with SS disease. Four patients had five α globin genes (aaαα/aaα), 55 the normal complement of four α globin

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467
and it is now possible to determine directly its frequency in different populations, to distinguish different types of deletions, and to study the influence of α thalassemia on other hemoglobinopathies such as sickle cell disease. Most commonly α thalassemia results from deletions originating from unequal crossover events in the α globin gene cluster (−3.7 kb and 4.2 kb deletions). In one family a novel 3.5 kb deletion removed the α globin gene with some of its flanking sequences. Deletion of both α globin genes was not observed although the presence of total α globin gene cluster deletions was not excluded in all cases. The occurrence of nondeletional forms of α thalassemia was suggested by low Hb S levels and RBC indices in persons with the sickle cell trait.

The clinical significance of the interaction of alpha thalassemia with sickle cell disease has been controversial. There is general agreement that SS patients with α thalassemia have higher total hemoglobin levels and changes compatible with less rapid hemolysis. The ascertainment biases in the Indian patient group are complex and impossible to quantify. Furthermore, as almost all cases were seen only once it is unclear whether the measured hematologic indices represented the steady state for the individual. However, the present study confirms that differences of hematologic parameters previously observed among SS patients with an α thalassemia group. Significant differences were also found for PCV in both groups, for Hb F levels. It was not possible to study the influence of a thalassemia on other hematologic traits.

### Table 4. Hematologic Findings of Indian Patients With SS Disease According to Their α Globin Genotype. The Numbers Are Mean Values ± 1 SD With Ranges in Brackets.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Hemoglobin (g/dL)</th>
<th>RBC (x10^12/L)</th>
<th>MCV (fL)</th>
<th>MCH (pg)</th>
<th>HbF (%)</th>
<th>Reticulocytes (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aa/aa</td>
<td>4</td>
<td>8.3 ± 2.16</td>
<td>2.91 ± 0.93</td>
<td>29.2 ± 2.6</td>
<td>(27-33)</td>
<td>5.25 ± 2.63</td>
<td>(3-9)</td>
</tr>
<tr>
<td>aa/α</td>
<td>54</td>
<td>8.3 ± 1.55</td>
<td>3.01 ± 0.66</td>
<td>27.9 ± 3.30</td>
<td>(22-38)</td>
<td>7.28 ± 5.66</td>
<td>(2-29)</td>
</tr>
<tr>
<td>−α/α</td>
<td>54</td>
<td>9.0 ± 1.77</td>
<td>3.53 ± 0.72</td>
<td>25.9 ± 2.72</td>
<td>(17-34)</td>
<td>5.52 ± 3.76</td>
<td>(1-19)</td>
</tr>
<tr>
<td>−α/−α</td>
<td>13</td>
<td>9.2 ± 1.59</td>
<td>4.19 ± 0.73</td>
<td>22.1 ± 2.6</td>
<td>(19-28)</td>
<td>5.08 ± 2.5</td>
<td>(2-11)</td>
</tr>
</tbody>
</table>

Statistical analysis of differences (t test) between groups aa/aa; −α/α; −α/−α gave highly significant values (P < .005) for RBC, MCV, MCH, Hb F and between the −aa and the aa/α groups for Hb F levels. Significant differences were also found for PCV (P < .005), for Hb and reticulocyte counts (P < .025) between the aa/aa and the −aa/−aa groups. Reticulocyte counts and Hb F levels were transformed logarithmically before statistical analysis was performed.
The cross sectional nature and poor retrospective documentation of the Indian group complicated the interpretation of the effects of \( \alpha \) thalassemia on survival. The significantly greater \(-\alpha\) gene frequency among older patients found here may be due to a notional effect of \( \alpha \) thalassemia delaying the onset of symptoms but it may also suggest that \( \alpha \) thalassemia has a positive effect on the survival of SS patients. This is of interest in view of the conflicting data on \( \alpha \) thalassemia and survival of SS patients in other communities. A study of small West African, Equatorial African, and American populations showed that \( \alpha \) thalassemia diagnosed by gene mapping was more common in SS patients than in carriers (AS) or normal individuals (AA) and that the frequency of \( \alpha \) thalassemia in SS patients increased with age.\(^{25} \) This trend was confirmed in a similar study performed in Benin and the Central African Republic\(^{27} \) but not in other studies in the Central African Republic,\(^{27} \) in Senegal,\(^{26} \) in Nigeria,\(^{21} \) or in Jamaica.\(^{4} \)

The reduction of the Hb S level in the sickle cell trait associated with \( \alpha \) thalassemia can be explained by a greater affinity of \( \beta^A \) than \( \beta^A \) chains for \( \alpha \) chains in limited supply.\(^{29} \) As the \( \alpha 2 \) globin gene is normally expressed at approximately two to three times the rate of the \( \alpha 1 \) globin gene,\(^{30,31} \) the lesions deleting the \( \alpha 1 \) or the \( \alpha 2 \) globin gene might have been expected to have different phenotypic effects, a notion that was supported by an umbilical cord blood analysis in Melanesians in whom homozygotes for the \(-\alpha 2 \) deletion had significantly higher Hb Bart's levels than homozygotes for the \(-\alpha 3^7 \) deletion.\(^{32} \) However, Hb S levels did not differ in Indian AS persons heterozygous for the \(-\alpha 2 \) deletion removing the entire \( \alpha 2 \) globin gene, the \(-\alpha 3^7 \) deletion removing part of the \( \alpha 1 \) globin gene or the \(-\alpha 3^3 \) deletion removing the entire \( \alpha 1 \) globin gene.

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