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 −α3' 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 3' 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 α 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 β thalassemia or sickle cell disease. It has been shown that co-inheritance of α thalassemia improves the hematologic parameters of heterozygous β thalassemia and results in a relatively mild clinical picture of homozygous β thalassemia.

In black patients with homozygous sickle cell (SS) disease α thalassemia has been shown to reduce the rate of hemolysis and reported to be associated with a lower prevalence of leg ulcers and the acute chest syndrome. However, in Saudi Arabian and Indian patients in whom the clinical and hematologic picture is generally milder due to raised Hb F levels, the influence of α thalassemia on the features of SS disease has not been well characterized.

The aim of this study was to determine the molecular basis of α thalassemia and to study its interaction with the sickle cell gene in an Indian population from Orissa State.

PATIENTS AND METHODS

During a 3-week period in February/March 1986 a field study was performed of a population in Western Orissa, India, the β gene frequency of which has been shown to be high. The index patients attended the adult and pediatric sickle cell clinics of the VSS Medical College, Burla, Orissa, or were detected by screening of a predominantly tribal population (Pan and Kond) in a rural area in Phulbani District. Clinical and hematologic examinations were performed on 131 patients with homozygous SS disease, 143 persons with the sickle cell trait (AS), and 13 persons with a normal globin genotype (AA) who were parents or siblings of the index cases. An aliquot of EDTA blood from each individual was stored at −20°C and transported frozen to Oxford where DNA was extracted and α globin gene mapping performed by Southern blot analysis.

DNA was successfully isolated from 282 (126 SS, 143 AS, 13 AA) of the 287 individuals, digested with restriction endonucleases BamH1 and BglI and hybridized to 32P labeled genomic PstI α and BamH1/EcoRI α fragment probes, respectively. The normal α globin gene arrangement (αα) is contained on a 14 kilobase (kb) BamH1 fragment (Fig 1), triplicated a globin genes (ααα) on an 18 kb fragment, and a deleted gene (−α) on a 10 kb BamH1 fragment (Fig 1). The two common α thalassemia deletions (−α3') and (−α4') result from unequal crossover within areas of homology in the α globin gene cluster designated Z box and X box, respectively.

To distinguish these two deletions, BglII digested DNA when hybridized to the α probe produces a 16 kb (−α3') or a 8.4 kb (−α4') fragment instead of the normal 12.6 kb fragment (Fig 1). Most α thalassemia deletions can also be detected by probing BamH1 and BglII digests with the α and the γ globin gene probes.

Two rare forms previously found in Filipino and Thai individuals delete the entire α globin gene cluster and can therefore not be directly detected with the probes used. However, a complete α cluster deletion can be excluded in the presence of two inter γ HVR alleles. During these studies a novel form of α thalassemia was noted and further characterized by restriction endonuclease digestions (Table 1) and hybridization to the PstI α and a 3' PR1 α probe.

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 (ααααα), 55 the normal complement of four α globin
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 (−α1; −α2); large deletions, probably resulting from illegitimate recombination, and nondeletional forms are geographically more localized and less common.18

In India, there have been sporadic reports of α thalassemia. Some cases of Hb H disease have been found in West-Bengal20 and screening of newborns in West-Bengal and Bombay showed that 2% and 4% of cord bloods contained Hb Barts.20,21 Furthermore, 9.4% of a tribal population in south and east India, the latter study indicating an estimated gene frequency of 0.32 among an a globin chain termination mutant.22 Using gene mapping method 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.8,10,11 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.12 However, the present study confirms that differences of hematologic parameters previously observed among SS patients with α thalassemia is not large enough to be noticeable in a population.13

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>n</th>
<th>αα/αα</th>
<th>αa/αα</th>
<th>−α/αα</th>
<th>−α/−α</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>54</td>
<td>54</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Hb</td>
<td>8.3 ± 2.16</td>
<td>8.3 ± 1.55</td>
<td>9.0 ± 1.77</td>
<td>9.2 ± 1.59</td>
</tr>
<tr>
<td>(g/dL)</td>
<td>(5.4-10.1)</td>
<td>(3.9-11.4)</td>
<td>(5.7-13.5)</td>
<td>(6.0-11.8)</td>
</tr>
<tr>
<td>RBC</td>
<td>3.0 ± 0.93</td>
<td>3.0 ± 0.66</td>
<td>3.53 ± 0.72</td>
<td>4.19 ± 0.73</td>
</tr>
<tr>
<td>(x 10^12/μL)</td>
<td>(1.65-3.62)</td>
<td>(1.05-4.56)</td>
<td>(1.75-5.00)</td>
<td>(2.92-5.49)</td>
</tr>
<tr>
<td>MCH</td>
<td>29.2 ± 2.6</td>
<td>27.9 ± 3.30</td>
<td>25.9 ± 2.72</td>
<td>22.1 ± 2.6</td>
</tr>
<tr>
<td>(pg)</td>
<td>(27-33)</td>
<td>(22-38)</td>
<td>(17-34)</td>
<td>(19-28)</td>
</tr>
<tr>
<td>MCV</td>
<td>91.0 ± 9.06</td>
<td>88.3 ± 8.88</td>
<td>81.6 ± 8.63</td>
<td>70.6 ± 3.73</td>
</tr>
<tr>
<td>(fl)</td>
<td>(81-103)</td>
<td>(68-113)</td>
<td>(60-98)</td>
<td>(66-80)</td>
</tr>
<tr>
<td>PCV</td>
<td>25.9 ± 6.74</td>
<td>25.9 ± 3.84</td>
<td>28.4 ± 4.93</td>
<td>29.8 ± 4.62</td>
</tr>
<tr>
<td>(%))</td>
<td>(17.0-32.6)</td>
<td>(13.5-35.3)</td>
<td>(17.1-38.3)</td>
<td>(20.5-38.1)</td>
</tr>
<tr>
<td>Reticulocytes</td>
<td>5.25 ± 2.63</td>
<td>7.28 ± 5.66</td>
<td>5.52 ± 3.76</td>
<td>5.08 ± 2.5</td>
</tr>
<tr>
<td>(%)</td>
<td>(3-9)</td>
<td>(2-29)</td>
<td>(1-19)</td>
<td>(2-11)</td>
</tr>
<tr>
<td>log (% + 1)</td>
<td>0.77 ± 0.17</td>
<td>0.84 ± 0.25</td>
<td>0.76 ± 0.20</td>
<td>0.75 ± 0.17</td>
</tr>
<tr>
<td>Hb F</td>
<td>17.2 ± 6.41</td>
<td>17.3 ± 4.98</td>
<td>17.3 ± 5.42</td>
<td>11.1 ± 4.56</td>
</tr>
<tr>
<td>(%)</td>
<td>(9.8-23.8)</td>
<td>(7.6-31.5)</td>
<td>(6.5-28.2)</td>
<td>(4.6-22.0)</td>
</tr>
<tr>
<td>log (% + 1)</td>
<td>1.24 ± 0.16</td>
<td>1.24 ± 0.12</td>
<td>1.24 ± 0.15</td>
<td>1.06 ± 0.16</td>
</tr>
<tr>
<td>Hb A₂</td>
<td>1.53 ± 0.39</td>
<td>1.69 ± 0.42</td>
<td>1.93 ± 0.42</td>
<td>2.73 ± 0.51</td>
</tr>
<tr>
<td>(%)</td>
<td>(1.2-2.0)</td>
<td>(1.1-2.9)</td>
<td>(1.1-3.0)</td>
<td>(1.6-3.4)</td>
</tr>
</tbody>
</table>

Statistical analysis of differences (t test) between groups αα/αα; αa/αα; −α/αα; −α/−α gave highly significant values (P < .0005) for RBC, MCV, MCH, Hb A₂ and between the −α/αα and the αα/αα groups for Hb F levels. Significant differences were also found for PCV (P < .005), for Hb and reticulocyte counts (P < .025) between the αα/αα and the −α/αα 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 α thalassemia on survival. The significantly greater—α—gene frequency among older patients found here may be due to a notional effect of α thalassemia delaying the onset of symptoms but it may also suggest that α thalassemia has a positive effect on the survival of SS patients. This is of interest in view of the conflicting data on α thalassemia and survival of SS patients in other communities. A study of small West African, Equatorial African, and American populations showed that α thalassemia diagnosed by gene mapping was more common in SS patients than in carriers (AS) or normal individuals (AA) and that the frequency of α thalassemia in SS patients increased with age. This trend was confirmed in a similar study performed in Benin and the Central African Republic, in Senegal, in Nigeria, or in Senegal, or in Jamaica.4 The reduction of the Hb S level in the sickle cell trait associated with α thalassemia can be explained by a greater affinity of β+ than β chains for α chains in limited supply. As the 2α globin gene is normally expressed at approximately two to three times the rate of the α globin gene, the lesions deleting the α1 or the 2α 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 −α2 deletion had significantly higher Hb Bart’s levels than homozygotes for the −α3 deletion. However, Hb S levels did not differ in Indian AS persons heterozygous for the −α2 deletion removing the entire α2 globin gene, the −α3 deletion removing part of the α1 globin gene or the −α3 deletion removing the entire α1 globin gene. Preferential transcription of the α2 globin gene might thus not be maintained on the deleted chromosome although additional nondeletion mutations in the structurally intact α globin genes of the persons studied here have not been excluded. Alternatively, it is possible that a difference of the phenotypic effect between the various deletion types is not visible in the heterozygous state and would therefore have to be small.

The novel 3.5 kb deletion observed in one Indian family raises questions regarding the structure of the α globin gene cluster. The 3.7 kb and 4.2 kb deletions result from unequal crossover events within areas of DNA sequence homology, Z and X box, respectively, which have probably been preserved during the concerted evolution of the α globin genes. The 5' breakpoint of the 3.5 kb deletion lies within the Z box. A further α globin like sequence (θ1) has been shown to be located about 3 kb downstream of the α1 gene and the 3' breakpoint of the 3.5 kb deletion lies within DNA sequences 3' of the α1 globin gene, which have not yet been sequenced, but would be located on the 5' side of the not yet fully defined θ1 gene. It is possible that the 3.5 kb deletion results from unequal crossover between the Z box 5' of the α1 globin gene and notional homologous sequences 5' of the θ1 gene, which would be consistent with DNA sequence homology further extending to the 3' side of the α globin gene cluster. However, confirmation of these speculations must await sequencing of the 3.5 kb deletion breakpoints and of normal DNA 3' to the α1 globin gene.

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