The Molecular Basis of \( \alpha \) 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 \( \alpha \) globin genotype of a total of 282 Indians from Orissa state has been analyzed. The overall \( \alpha \) thalassemia gene frequency is 0.29, most frequently caused by the \( -\alpha^4 \) and \( -\alpha^3 \) deletions. In one family a novel \( -\alpha^{42} \) deletion removing the \( \alpha \) globin gene with some of its flanking sequences has been found, suggesting further sequence homology of the \( \alpha \) globin gene cluster to the \( \alpha1 \) globin gene. Patients with sickle cell disease and \( \alpha \) 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 \( \alpha \) genotype. The frequency of splenomegaly was not influenced by the \( \alpha \) globin genotype. A higher prevalence of \( \alpha \) thalassemia was found in patients >10 years of age than in the younger group, suggesting a possible advantageous effect of \( \alpha \) thalassemia on the survival of patients with sickle cell disease.

RESULTS

Prevalence of deletional \( \alpha \) thalassemia. The \( \alpha \) globin genotype was determined by gene mapping in a total of 126 patients with SS disease. Four patients had five \( \alpha \) globin genes (\( \alpha \alpha \alpha / \alpha \alpha \)), 55 the normal complement of four \( \alpha \) 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 (−α/α; −α/−α); large deletions, probably resulting from illegitimate recombination, and nondeletional forms are geographically more localized and less common.2

In India, there have been sporadic reports of α thalassemia. Some cases of Hb H disease have been found in West-Bengal and screening of newborns in West-Bengal and Bombay showed that 2% and 4% of cord bloods contained Hb Bart's. Furthermore, 9.4% of a tribal population from East Godaveri District in Andhra Pradesh were found to have the variant Hb Koya Dora, which is caused by an α globin chain termination mutant. Using gene mapping analysis α thalassemia has been diagnosed in a high proportion of a tribal population in south and east India, the latter study indicating an estimated gene frequency of 0.32 among patients with SS disease.12

The exact molecular basis of α thalassemia in India has not yet been described. The most common molecular basis for α thalassemia in this population are the genomically widespread 3.7 kb and 4.2 kb deletions. In one family a novel 3.5 kb deletion removed the α1 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.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 or without α thalassemia of predominantly West African ancestry also occur in Indian patients. There is less agreement on the clinical effects of α thalassemia. Higgs et al9 studying Jamaican patients reported that homozygotes had significantly less chronic leg ulceration and acute chest syndrome and a significantly greater persistence of splenomegaly. One might have expected that the hematologic effect of α thalassemia in Indians would also be reflected by a greater prevalence of splenomegaly in the α thalassemia group. However, while in Indians splenomegaly was common and occurred in later ages than in Jamaicans this was probably related to the raised Hb F levels found in Indians and an additional effect of α thalassemia on the rate of splenomegaly was not observed. This might suggest that the effect of α thalassemia is not large enough to be noticeable in a population with another strong genetic ameliorating factor like raised Hb F levels. It was not possible to study the influence of α thalassemia on symptoms of SS disease in Indians as most of these were rare in the whole group.12 However, as virtually all patients, regardless of their α globin genotype, had a history of painful crises, and dactylitis was also common the prevalence of these symptoms did not appear to be affected by the co-inheritance of α thalassemia.

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></th>
<th>αα/αα</th>
<th>α/αα</th>
<th>−α/αα</th>
<th>−α/−α</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>4</td>
<td>54</td>
<td>54</td>
<td>13</td>
</tr>
<tr>
<td>Hb (g/dL)</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>2.91 ± 0.93</td>
<td>3.01 ± 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>(g/dL)</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>
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</table>

Statistical analysis of differences (t test) between groups αα/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.

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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\(^{26}\) or in Senegal,\(^{26}\) Nigeria,\(^{26}\) or in Jamaica.\(^{8}\)

The reduction of the Hb S level in the sickle cell trait associated with \( \alpha \) thalassemia can be explained by a greater affinity of \( \beta^\alpha \) than \( \beta^\beta \) 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 \(-\alpha^3\) 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 \(-\alpha^3\) deletion removing part of the \( \alpha_1 \) globin gene or the \(-\alpha^3\) deletion removing the entire \( \alpha_1 \) globin gene. Preferential transcription of the \( \alpha_2 \) globin gene might thus not be maintained on the deleted chromosome although additional nondeletion mutations in the structurally intact \( \alpha \) 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 \( \alpha \) 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 \( \alpha \) globin genes.\(^{16,17,33-35}\) The 5’ breakpoint of the 3.5 kb deletion lies within the Z box 5’ of the \( \alpha_1 \) globin gene (Fig 2). A further \( \alpha \) globin like sequence (\( \theta_1 \)) has been shown to be located about 3 kb downstream of the \( \alpha_1 \) gene and the 3’ breakpoint of the 3.5 kb deletion lies within DNA sequences 3’ of the \( \alpha_1 \) globin gene, which have not yet been sequenced, but would be located on the 5’ side of the not yet fully defined \( \theta_1 \) gene. It is possible that the 3.5 kb deletion results from unequal crossover between the Z box 5’ of the \( \alpha_1 \) globin gene and notional homologous sequences 5’ of the \( \theta_1 \) gene, which would be consistent with DNA sequence homology further extending to the 3’ side of the \( \alpha \) 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 \( \alpha_1 \) globin gene.

ACKNOWLEDGMENT

We thank Dr Doug Higgs for the \( \alpha \) globin gene probes and helpful advice, Dr Bill Wood for discussions on the manuscript, and Linda Roberts for the secretarial assistance.

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