CONCISE REPORT

Alpha-Gene Deletions in Black Newborn Infants With Hb Bart’s

By Kwaku Ohene-Frempong, Eric Rappaport, Jean Atwater, Elias Schwartz, and Saul Surrey

The presence of increased Hb Bart’s (γ4) in cord blood is believed to be an indication of α-thalassemia. We have used restriction endonuclease analyses of DNA to compare the number of α-genes with the percentage of Hb Bart’s in 6 older children who had Hb Bart’s at birth and 17 newborns. Four children with >2% Hb Bart’s had Eco R1 α-gene patterns and hematologic data consistent with the presence of two α-genes, one per chromosome. Of the remaining 19 children, all of whom had <2% Hb Bart’s, 8 had 3, while 11 had 4 α-genes. Three infants with Hb Bart’s between 1% and 2% had 3 α-genes, while the other 5 with 3 α-genes had 1% or less. All infants with 4 α-genes had 1% or less Hb Bart’s. Infants with 3 α-genes may therefore have elevated or normal levels of Hb Bart’s at birth. DNA analysis is the definitive method for the determination of heterozygous α-thalassemia syndromes in newborns.

Hemoglobin Bart’s is a tetramer of γ-globin chains found in many newborn infants of several ethnic groups. It is usually no longer present after several months of life. The most striking occurrence of this hemoglobin is in Asian newborns with hydrops fetalis due to homozygous alpha-thalassemia, where greater than 80% of the total hemoglobin is Hb Bart’s1 and there is deletion of all four α-genes.2,3 Lesser amounts of Hb Bart’s, approximately 25%, are found in infants with HbH disease,4 who have only one functioning α-gene.5 The finding of smaller amounts of Hb Bart’s in a newborn has generally been taken to denote the presence of one of the heterozygous forms of α-thalassemia, either α-thalassemia trait or the silent carrier.6,7 Recent family and population studies have shown that α-thalassemia trait in Chinese is due to deletion of two α-genes on one chromosome, or to homozygosity for a single α-gene per chromosome.8 In African Americans α-thalassemia trait is due to a single α-gene per chromosome, the abnormal arrangement due to a nonhomologous crossover.9,10 The silent carrier has three of the four normal α-genes. In Thailand, the presence of different amounts of Hb Bart’s has been related to the various α-thalassemia syndromes,7 while in people of African origin, the interpretation has not been as clear. Some African-American children with 2%–9.3% Hb Bart’s have α-thalassemia trait,6,11 but in others the presence of this hemoglobin has been attributed to a developmental abnormality.12 The significance of less than 2% Hb Bart’s has not been clearly determined. In this study, we demonstrate the relationship of varying numbers of α-genes to the levels of Hb Bart’s in African-American newborns.

MATERIALS AND METHODS

Subjects

The subjects included 17 unselected African-American children screened at birth for abnormal hemoglobins. In addition, 6 older children (age 2–8) who had Hb Bart’s in the newborn period and 3 of their mothers were studied. Three of these children were described previously.11 Informed consent was obtained from the mother in each case, and the study was approved by the Committee for Protection of Human Subjects of The Children’s Hospital of Philadelphia.

Hematologic Studies

Hematologic studies were done by standard methods. Globin synthesis studies of peripheral blood were performed on three of the older children and one mother according to previously described methods.11 The normal mean α/β ratio in our laboratory is 1.00 ± 0.05 (1 SD). The percentage of Hb Bart’s in cord blood was determined by chromatography on carboxymethyl cellulose at pH 6.6.13 Iron deficiency was ruled out in the older children and mothers by normal free erythrocyte protoporphyrin and serum iron levels.

Analysis of α-Genes

DNA was isolated from heparinized peripheral or cord blood, digested with Eco R1, separated by electrophoresis on a vertical 0.7% agarose gel, and transferred to nitrocellulose filters.14,15 Hybridization with 32P-labeled α + β globin cDNA, washing, and autoradiography were done as previously described.14,15

RESULTS

Eco R1 digestion of normal human DNA yields a single 23 kb fragment containing the duplicated α-genes.8,13 A shorter fragment, approximately 19 kb in length, is found when only one α-gene is present. Individuals with both 23 and 19 kb fragments have three α-genes (αα/-α), while those with only the 19 kb fragment have either two (–α/-α) or one α-gene.
Table 1. Hematologic Findings and α-Gene Analysis in Six Older Children and Three Mothers

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Age (yr)</th>
<th>Hb (g/dL)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>Globin Synthesis (α/β)</th>
<th>Hb Bart's at Birth (%)</th>
<th>Eco R1 α Bands (kb)</th>
<th>Number of α Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>8</td>
<td>11.5</td>
<td>65.6</td>
<td>22.1</td>
<td>0.83</td>
<td>6.3*</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>C2</td>
<td>8</td>
<td>10.8</td>
<td>64.3</td>
<td>21.2</td>
<td>0.83</td>
<td>6.0*</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>C3</td>
<td>8</td>
<td>11.9</td>
<td>69.1</td>
<td>22.6</td>
<td>—</td>
<td>3.6*</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>M4</td>
<td>24</td>
<td>11.5</td>
<td>86.1</td>
<td>28.8</td>
<td>0.89</td>
<td>—</td>
<td>23/19</td>
<td>3</td>
</tr>
<tr>
<td>C4</td>
<td>3</td>
<td>10.9</td>
<td>76.5</td>
<td>26.5</td>
<td>0.95</td>
<td>1-2†</td>
<td>23/19</td>
<td>3</td>
</tr>
<tr>
<td>M5</td>
<td>22</td>
<td>11.3</td>
<td>67.5</td>
<td>22.9</td>
<td>—</td>
<td>—</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>C5</td>
<td>3</td>
<td>10.9</td>
<td>66.1</td>
<td>22.2</td>
<td>0.86</td>
<td>&gt;2†</td>
<td>19</td>
<td>2</td>
</tr>
<tr>
<td>M6</td>
<td>27</td>
<td>13.4</td>
<td>89.9</td>
<td>29.8</td>
<td>—</td>
<td>—</td>
<td>23</td>
<td>4</td>
</tr>
<tr>
<td>C6</td>
<td>2</td>
<td>12.0</td>
<td>73.5</td>
<td>24.8</td>
<td>—</td>
<td>1-2†</td>
<td>23/19</td>
<td>3</td>
</tr>
</tbody>
</table>

M4-6 are the mothers of children C4-6, respectively.

*Measured by chromatography.13
†Determined by starch gel electrophoresis.11

DISCUSSION

The results presented in this article support the findings of earlier investigations that individuals with α-thalassemia have increased levels of Hb Bart's at birth. The DNA analyses specifically confirm the speculation that African-American newborns with 2%-9.3% Hb Bart's have α-thalassemia trait.11 All children with greater than 2% Hb Bart's indeed have 2 α-genes and the lowest red cell indices of the group studied. The suspicion that the silent carrier has 1%-2% Hb Bart's at birth is partly confirmed by this study. The 3 children with 1%-2% Hb Bart's have 3 α-genes. On the other hand, 5 infants with 3 α-genes had 0%-1.0% Hb Bart's. Although as a group the children with 3 α-genes had a slightly higher mean Hb Bart's level at birth than those with 4 α-genes (0.52% versus 0.36%), individuals with 3 or 4 α-genes could not be identified by the levels of Hb Bart's. The results...
also show that normal individuals with 4 a-genes may have small amounts (up to 1%) of Hb Bart's at birth. It is also clear that the usual techniques, such as measurement of red cell indices, globin synthesis, or Hb Bart's at birth, cannot identify all individuals with 3 a-genes. Estimations of the incidence of a-thalassemia in the African-American population based on any of these techniques have yielded inconsistent results. The nature of a-thalassemia in people of African descent has been clarified by recent DNA studies and now more accurate population data can be determined using these new methods. In their analysis of the a-genes in 211 African Americans, Dozy et al. estimated the frequency of the chromosome with a single a-locus (−α) to be 16%. The expected frequency of the homozygous state (−α/−α), alpha-thalassemia trait, would be 0.16, or 2.56%. This calculation agrees with the findings of Friedman et al. that 3% of African-American neonates had 2%–9.3% Hb Bart's and α-thalassemia trait. The expected frequency of the heterozygous state (−α/αα), or silent carrier, is 27%. In the study by Friedman et al., only 12% of newborns had 1%–2% Hb Bart's. As shown in the present study, many infants with lesser amounts of Hb Bart's are also silent carriers. It would be of interest to compare the levels of Hb Bart's in Asian newborns with the a-gene number and arrangement, in a manner similar to the studies reported here.

ACKNOWLEDGMENT

We wish to thank Dr. Shlomo Friedman for his important role in earlier studies of some of these families and Jill Chambers, Diane Muni, and Frank Butler for their excellent technical assistance. We are grateful to Carol Way for expert help in photography and in the preparation of this manuscript.

REFERENCES


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**Table 2. Cord Blood Hb Bart's Levels and a-Gene Analysis in 17 Newborn Infants**

<table>
<thead>
<tr>
<th>Eco RI a Bands (kb)</th>
<th>Number of a Genes</th>
<th>Number of Newborns</th>
<th>Hb Bart's (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>23/19</td>
<td>3</td>
<td>6</td>
<td>1 5</td>
</tr>
<tr>
<td>23</td>
<td>4</td>
<td>11</td>
<td>0 0 11</td>
</tr>
<tr>
<td>Total</td>
<td>17</td>
<td>0 1 16</td>
<td></td>
</tr>
</tbody>
</table>

*Measured by chromatography.*
Alpha-gene deletions in black newborn infants with Hb Bart's

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