Hemoglobin Nigeria (α-81 Ser—Cys): A New Variant Associated With α-Thalassemia

By George R. Honig, Mir Shamsuddin, R. George Mason, Loyda N. Vida, Larry M. Tremaine, George E. Tarr, and Nasrollah T. Shahidi

Hematologic evaluation of a Nigerian obstetrical patient disclosed the presence of sickle-cell trait as well as evidence of a hemoglobin α-chain abnormality. Hemoglobins containing the variant α-chain were isolated by DEAE-cellulose column chromatography, and analysis of the purified α-chain demonstrated a ser—cys substitution at α-81. The abnormal α-chain represented approximately 45% of the total, and hemoglobins containing this α-chain appeared to have normal stability and functional properties. In addition to the abnormal hemoglobins that were identified in this patient, she also was found to have persistent microcytosis in the absence of iron deficiency, and the percentage of HbS in her erythrocytes was less than that usually present in individuals with sickle cell trait. These findings, together with a reduced α/β globin synthesis ratio from her peripheral blood reticulocytes, indicated the presence of α-thalassemia trait. Hematologic findings from members of the patient’s family suggest that an α-thalassemia gene may be linked to that of the structurally abnormal α-chain.

In the course of routine hematologic studies of an obstetrical patient, a positive sickle-cell test was found, and hemoglobin electrophoresis demonstrated the presence of HbS as well as a structurally abnormal α-chain variant. In this report we describe the characterization and some of the hematologic properties of this newly identified hemoglobin variant.

MATERIALS AND METHODS

Hematologic and Hemoglobin Procedures

Hematologic measurements were made with a model S Coulter counter that was standardized daily. Serum ferritin determinations were performed by a radioimmunoassay method using a commercial assay kit (Ramco Laboratories, Inc., Houston, Texas). Other determinations were by routine laboratory methods. Electrophoresis of hemoglobins from stroma-free lysates was carried out in agar gel at pH 6.2 and in starch gel and cellulose acetate with Tris-EDTA-borate buffer, pH 8.6, or Tris-EDTA-glycine buffer, pH 9.2, to which sucrose was added. Relative concentrations of the various hemoglobins were estimated by DEAE-cellulose chromatography.

Isolation of the Variant Hemoglobins and Globin Chains

The hemoglobins were fractionated by DEAE-cellulose chromatography with glycine-containing buffers according to Abraham et al. The buffer gradients were modified as described in the legend to Fig. 2. Effluent fractions corresponding to individual hemoglobins were pooled after the initial chromatographic fractionation, or after repeated chromatography when required, and the hemoglobins were concentrated by vacuum dialysis. Globin was prepared by precipitation in aceticone-HCl at –20°C, and the globin chains were separated by carboxymethylcellulose chromatography with buffers containing 8 M urea. The globin-containing effluent fractions were desalted by gel filtration through Bio-gel P-2, which was equilibrated with 0.5 M formic acid. The proteins were recovered by lyophilization.

Peptide Isolation and Purification

The variant α-chain was digested with trypsin for 2 hr at 37°C in NH4HCO3 buffer, pH 8.5. The trypsin-resistant insoluble core that remained was washed thoroughly with water, oxidized with performic acid, and digested for 8 hr with chymotrypsin in NH4HCO3 at 25°C. The peptides were fractionated by PA-35 column chromatography using a linear pyridine-acetic acid gradient as described by Jones. Peptides requiring further purification were subjected to an additional chromatographic step using a Dowex 50-X2 column. For some of the structural studies, samples of the purified α-chain were cleaved with cyanogen bromide. The cleavage products were fractionated by gel filtration through a 2.5 × 250 cm column of Sephadex G-50, which was equilibrated with 20% acetic acid.

Amino Acid Analyses

Globin and peptide samples for amino acid analysis were hydrolyzed for 24 hr in 6 M HCl under reduced pressure at 110°C. The analyses were performed with a Beckman-Spinco amino acid analyzer model 121-M equipped with a System AA integrator. Cysteine was measured as cystic acid following performic acid oxidation.

Sequence analysis of the segment α77–91 of the variant α-chain was by a manual Edman procedure. For this analysis, a preparation of cyanogen bromide fragment CB-III was reduced with β-mercaptoethanol and alkylated by treatment with 4-vinylpyridine to convert cysteine to the more stable S-(4-pyridyldethyl) cysteine derivative.

For comparison of the number of reactive sulfhydryl groups in the variant α-chain with that of normal, the two forms of HbA2 were prepared from a fresh blood sample from the patient. Iodoacetamide, 0.01 M, was added to equal volumes of the carbon monoxide-saturated hemoglobins in phosphate buffer, pH 7.15. After incubation for 1 hr at room temperature, the samples were dialyzed thoroughly against water. The α-chains were isolated and treated with performic acid for oxidation of unreacted sulfhydryl groups.

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Cysteic acid and S-carboxymethylcysteine were determined by amino acid analysis of acid hydrolysates of the normal and variant \( \alpha \)-chains.

**Oxygen Equilibrium and Hemoglobin Gelation Studies**

Oxygen equilibrium curves of whole blood samples were determined with an Instrumentation Laboratories blood gas analyzer, Model 217, as previously described.\(^1\) Briefly, the blood samples were equilibrated at 37°C with varying mixtures of CO\(_2\) (2%-9%) in air, and CO\(_2\) in nitrogen, to achieve a range of pH and pO\(_2\) values. After equilibration with each gas mixture, aliquots were taken for measurement of pO\(_2\), pH, and percent oxygen saturation. Curves were constructed from the data obtained at each pH by plotting the points on rectilinear graph paper and connecting them by visual inspection to obtain the best apparent fit. At least six points were obtained at each pH, and in each determination that was used for calculation, all of the points lay on a clearly defined sigmoid curve. The p50 values were estimated from these curves, and were used for calculation of the Bohr effect. For determination of the Hill coefficient, data from the oxygen equilibrium curve at pH 7.4 were used. For this calculation the log \( S/1 - S \) from each point was plotted as a function of the log pO\(_2\). The points fit closely to a straight line that was drawn by visual inspection; the Hill coefficient was estimated by calculation of the slope of this line. For each of the measurements, comparable determinations were made using fresh blood samples obtained from an individual with sickle-cell trait who served as a control. Determinations of 2,3-diphosphoglycerate were by the method of Keitt,\(^4\) using trichloroacetic acid extracts of whole blood.

The autooxidation study followed the procedures described by Mansouri and Winterhalter.\(^5\) For this study, the normal and variant forms of HbA\(_2\) were isolated by DEAE-cellulose chromatography\(^5\) using cyanide-free buffers. The hemoglobins, in 0.1 M phosphate buffer, pH 7.2, were incubated under sterile conditions at 37°C.

Gelation studies of sickle hemoglobin mixtures were carried out by the method of Singer and Singer,\(^6\) as modified by Bookchin et al.\(^7\)

**Globin Synthesis Studies**

Synthesis studies were done in vitro with peripheral blood reticulocytes. \(^1\)-Leucine\(^{\text{14C}}\) was used as the labeled precursor, with the incubation procedure as previously described.\(^8\)

**RESULTS**

**Hematologic Findings**

Initial hematologic testing of the patient demonstrated a positive sickling test. Electrophoresis of a stroma-free lysate of her erythrocytes in starch gel, or cellulose acetate, with Tris-EDTA-borate buffer demonstrated a typical sickle-cell trait pattern except for a lower than usual percentage of HbS. When the electrophoresis was carried out on cellulose acetate with Tris-EDTA-glycine-sucrose buffer, however, a duplication of each of the hemoglobin bands became apparent (Fig. 1). The patient was not anemic, but she demonstrated persistent microcytosis in spite of a normal serum ferritin level (Table I). Her erythrocyte morphology was mildly abnormal, with microcytosis, hypochromia, anisopoikilocytosis, and occasional target forms.

**Isolation of the Variant Hemoglobin**

DEAE-Sephadex chromatography\(^9\) of the patient’s hemoglobin yielded single peaks of hemoglobins A\(_2\), S, and A. Because of the increased resolution that was achieved by electrophoresis with glycine-containing buffer, we subsequently employed a chromatographic procedure using DEAE-cellulose with glycine-containing buffers.\(^5\) With this method we achieved complete resolution of two forms of HbA\(_2\), and partial resolution of the HbS and HbA components (Fig. 2). In each case, the hemoglobin containing the variant \( \alpha \)-chain

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**Table 1. Hematologic and Hemoglobin Findings From the Patient and Her Family**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Hb (g/dl)</th>
<th>RBC (x 10(^{12})/liter)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>Reticulocytes (%)</th>
<th>Serum Ferritin (ng/ml)</th>
<th>HbA(_2) (%)</th>
<th>HbF (%)</th>
<th>HbS (%)</th>
<th>L-leu(^{\text{14C}}) Incorporation (aM/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proposita*</td>
<td>12.2</td>
<td>5.51</td>
<td>69</td>
<td>21.6</td>
<td>2.8</td>
<td>88</td>
<td>3.2</td>
<td>2.0</td>
<td>28.6</td>
<td>0.76</td>
</tr>
<tr>
<td>Husband</td>
<td>13.7</td>
<td>4.59</td>
<td>91</td>
<td>30.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.01</td>
</tr>
<tr>
<td>Child</td>
<td>11.7</td>
<td>4.78</td>
<td>79</td>
<td>24.4</td>
<td>—</td>
<td>28</td>
<td>3.9</td>
<td>4.3</td>
<td>33.9</td>
<td>—</td>
</tr>
</tbody>
</table>

*All values indicated for the proposita were obtained at least 6 mo postpartum.*

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Fig. 1. Hemoglobin electrophoresis with Tris-EDTA-glycine-sucrose buffer, pH 9.2 (A) Sickle-cell trait. (B) The patient, showing duplication of each of the hemoglobin bands.
emerged from the column following elution of the normal \( \alpha \)-chain form. The partially purified HbS and HbA components were concentrated and subjected to a second chromatography step, which allowed sufficient quantities of the variant hemoglobins to be isolated for structural analysis of the variant \( \alpha \)-chain. During the rechromatography, the hemoglobins containing the abnormal \( \alpha \)-chain appeared to resolve more readily than during the initial fractionation; the variant \( \alpha \)-chain hemoglobins also became noticeably darker in color than those with \( \alpha^A \), suggesting that oxidative changes had taken place in a relatively selective manner.

Quantitation of the two forms of HbA\(_2\) that were recovered from the chromatographic fractionation appeared to represent the most suitable means for estimation of the percentage of hemoglobin containing the variant \( \alpha \)-chain. The percentage determined in this manner ranged from 41\% to 48\%, with a mean of 45\%.

**Structural Analysis of the Variant \( \alpha \)-Chain**

The amino acid composition of the purified \( \alpha \)-chain suggested the presence of an additional cysteine residue replacing a residue of serine (Table 2). The peptide isolation procedures initially failed to demonstrate tryptic peptide T-9, but all of the other peptides were recovered in good yield and had normal amino acid compositions (Table 3). A trypsin digest of the cyanogen bromide fragment CB-II yielded peptide T-9a, but the remainder of the peptide was not recovered from a similarly prepared digest of CB-III. When the trypsin-resistant core material from a CB-III preparation was subsequently oxidized with performic acid, the T-9b peptide fragment was recovered in good yield; this peptide contained the serine \( \rightarrow \) cysteine substitution (Table 3), and the Edman sequence analysis showed the site of the substitution to be \( \alpha-81 \).

### Hemoglobin Function Studies

The oxygen equilibrium curve of whole blood from the patient was of normal position and configuration. The \( P_{O_2} \) at 50\% saturation (37°C, pH 7.4) was 25.6 mm Hg (normal 27.2 ± 0.6 [SD]) and the Bohr effect and Hill’s coefficient were normal (\( \Delta \log p_{O_2}/\Delta \) pH = −0.49, \( n = 2.7 \)). The level of 2,3-diphosphoglycerate was 20.2–21.3 \( \mu \)mole/g Hb (normal 15.0 ± 2.4).

**Absorption Spectrum and Autooxidation Study**

Because of the observation that the hemoglobins containing the variant \( \alpha \)-chain appeared to become darker in color during the rechromatography step, we compared the absorption spectra, in the range of 450–660 nm, of HbA and Hb Nigeria following the rechromatography procedure. The spectra of both hemoglobins appeared to be identical and neither showed evidence of significant methemoglobin formation. An autooxidation study of the HbA\(_2\) variant, using the normal HbA\(_2\) that was eluted from the same column as a control, showed the variant hemoglobin to be more resistant than the normal to methemoglobin formation (Fig. 3).

**Determination of Reactive Sulfhydryl Groups**

Purified samples of HbA\(_2\) containing normal and variant \( \alpha \)-chains were treated with iodoacetamide. The \( \alpha \)-chains of each were isolated by carboxymethylcellulose chromatography, hydrolyzed in 6 \( M \) HC\(_1\), and the S-carboxymethylcysteine content of each determined by amino acid analysis. Consistent with previous observations,\(^\text{12}\) no reactive cysteine was detectable in the \( \alpha \)-chain derived from the normal form of HbA\(_2\). In the variant \( \alpha \)-chain, 0.89 residues (uncorrected) of

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**Table 2. Amino Acid Composition of the Variant \( \alpha \)-Chain**

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>Normal Composition</th>
<th>( \alpha^A )</th>
<th>( \alpha^{A*} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>11</td>
<td>10.9</td>
<td>10.7</td>
</tr>
<tr>
<td>Histidine</td>
<td>10</td>
<td>10.0</td>
<td>9.8</td>
</tr>
<tr>
<td>Arginine</td>
<td>3</td>
<td>2.8</td>
<td>2.8</td>
</tr>
<tr>
<td>Cysteine</td>
<td>1</td>
<td>1.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>12</td>
<td>12.1</td>
<td>12.1</td>
</tr>
<tr>
<td>Threonine</td>
<td>9</td>
<td>8.7</td>
<td>8.6</td>
</tr>
<tr>
<td>Serine</td>
<td>11</td>
<td>9.7</td>
<td>8.8</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>5</td>
<td>5.2</td>
<td>5.3</td>
</tr>
<tr>
<td>Proline</td>
<td>7</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>7</td>
<td>7.3</td>
<td>7.5</td>
</tr>
<tr>
<td>Alanine</td>
<td>21</td>
<td>21.0</td>
<td>21.2</td>
</tr>
<tr>
<td>Valine</td>
<td>13</td>
<td>13.3</td>
<td>13.0</td>
</tr>
<tr>
<td>Leucine</td>
<td>18</td>
<td>18.1</td>
<td>18.0</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>3</td>
<td>2.5</td>
<td>2.8</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>7</td>
<td>6.6</td>
<td>7.0</td>
</tr>
</tbody>
</table>

\(^\text{*Indicated values represent the number of residues of each amino acid per \( \alpha \)-chain.}\)
Table 3. Amino Acid Composition of Tryptic and Chymotryptic Peptides of the Variant α-Chain*

| Amino Acid | T-1 | T-2 | T-3 | T-4 | T-5 | T-6 | T-7 | T-8 | T-9a | T-9b | T-10 | T-11 | C-1 | C-2 | C-3 | C-4 | C-5 | C-6 | C-7 | T-14 |
|------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Lysine     | 1.09(1) | 1.10(1) | 1.00(1) | — | 1.16(1) | 1.14(1) | 1.06(1) | 1.00(1) | — | 0.85(1) | — | 1.17(1) | — | — | — | — | — | — | 1.00(1) | — | 1.06(1) | — |
| Histidine  | — | — | — | 0.97(1) | — | 1.9(2) | 1.00(1) | — | 1.00(1) | 1.6(2) | — | — | 1.00(1) | — | 0.89(1) | 0.93(1) | — | — | — | — |
| Arginine   | — | — | — | — | 0.97(1) | — | — | — | — | — | 0.86(1) | — | — | — | — | — | — | 0.96(1) | — | — | — |
| Cysteine   | — | — | — | — | — | — | — | — | — | 0.89(1) | — | — | 0.88(1) | — | — | — | — | — | — | — |
| Aspartic acid | 1.20(1) | 0.95(1) | — | — | — | 1.00(1) | — | — | 4.00(4) | 1.95(2) | — | 1.96(2) | — | — | — | — | — | — | 1.00(1) | — | — |
| Threonine  | 0.67(1) | — | — | 1.73(2) | 0.81(1) | — | — | 1.00(1) | — | — | — | 1.00(1) | — | 0.97(1) | — | 0.66(1) | 0.89(1) | — | — |
| Serine     | 0.79(1) | — | — | 0.79(1) | 1.58(2) | — | — | 1.12(2) | — | — | 1.09(1) | — | — | — | 0.88(1) | 1.97(2) | 0.88(1) | — | — |
| Glutamic acid | — | — | 3.02(3) | — | 0.97(1) | — | — | — | — | — | — | 0.94(1) | — | — | — | — | — | — |
| Proline    | 1.01(1) | — | — | — | — | — | — | 1.19(1) | — | 1.08(1) | — | — | 1.35(1) | 1.00(1) | — | — | — | — |
| Glycine    | — | — | — | 1.00(1) | 3.00(3) | — | — | 1.09(1) | 1.95(2) | — | — | — | — | — | — | — | — | — |
| Alanine    | 0.87(1) | — | 1.98(2) | 4.10(4) | — | 1.10(1) | — | 3.88(4) | 3.11(3) | — | — | 2.88(3) | 1.10(1) | 0.95(1) | 1.07(1) | — | — | — |
| Valine     | 1.17(1) | 0.98(1) | — | 0.98(1) | — | 0.94(1) | — | 2.81(3) | — | 1.84(2) | — | 1.10(1) | — | 0.98(1) | — | 2.20(2) | — | — | — |
| Methionine | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Leucine    | 0.89(1) | — | 1.23(1) | 1.00(1) | — | — | — | 1.06(1) | 3.00(3) | 1.12(1) | — | 2.88(3) | 1.68(2) | 1.00(1) | — | 1.08(1) | 1.49(2) | — | — |
| Tyrosine   | — | — | — | 0.80(1) | — | 1.21(1) | — | — | — | — | — | — | — | — | — | — | — | — | 1.04(1) |
| Phenylalanine | — | — | — | 2.10(2) | 1.91(2) | — | — | — | — | — | 0.96(1) | — | 1.30(1) | — | 1.08(1) | — | — | — |
| Tryptophan | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — | — |

*Indicated values represent the number of amino acid residues per peptide; values for the corresponding normal peptides are shown in parentheses.
S-carboxymethylcysteine were recovered per chain, suggesting that the sulfhydryl group of the cysteinyl residue at α-81 is fully reactive in the tetramer hemoglobin molecule.

Effects of the Variant α-Chain On Sickle Hemoglobin Gelation

The coexistence of HbS with the structurally abnormal α-chain in the patient's erythrocytes allowed us to assess the influence of the variant α-chain on the gelation of HbS. The minimum gelling concentration (MGC) of freshly prepared stroma-free lysates of the patient's erythrocytes, based on 6 determinations, was 35.4 ± 1.5 g/dl. With a control consisting of an equal percentage of HbS as that of the patient, prepared as a mixture with HbA, the MGC was 37.7 ± 0.5 g/dl, suggesting that the α-chain of Hb Nigeria has no ameliorative effect on the gelation of sickle hemoglobin.

Hemoglobin Synthesis Studies

L-leucine-14C incorporation into hemoglobins A and S by reticulocytes from the patient proceeded to a similar degree in the hemoglobins containing structurally normal α-chains as in those containing the variant α-chain (Table 4). In both of the forms of HbS, α-chain radioactivity was slightly greater than that of βS; whereas in the βA-containing hemoglobins, which constituted the major hemoglobin components, α-chain radioactivity was less than that of β (Table 4). A study of incorporation of L-leucine-14C into globin chains of an unfractionated sample demonstrated a lesser degree of incorporation into the βS chain than that usually seen in sickle-cell trait (Fig. 4); the total α/β synthesis ratio (αA + αNigeria / βA + βS) was 0.76.
Hematologic Findings in Other Family Members

The husband of the patient had a normal hemoglobin pattern and normal hematologic findings (Table I), and the $\alpha/\beta$ globin synthesis ratio of his peripheral blood reticulocytes was 1.01. Their child, from a study done at 1 yr of age, also had normal hematologic findings, with erythrocyte indices appropriate for her age.20 Her erythrocytes contained HbS at a level toward the lower limit of the usual range seen in individuals with sickle-cell trait, but Hb Nigeria was not detectable either by electrophoresis or by chromatography. All other members of this family live in Africa and were not available for study.

DISCUSSION

The normal functional properties of Hb Nigeria are consistent with the location of residue $\alpha$-81 at the surface of the hemoglobin molecule, at a position that does not involve any of the interchain contacts.21 The finding that the cysteinyl sulfhydryl group was fully reactive in the intact hemoglobin molecule is a further indication that this group does not participate in intramolecular bond formation.

Several lines of evidence point to the association of $\alpha$-thalassemia in the patient in whom the structurally abnormal $\alpha$-chain was identified: (1) she exhibited persistent microcytosis, hypochromia, and abnormal erythrocyte morphology, in spite of adequate iron stores; (2) the variant $\alpha$-chain represented approximately 45% of the total, a higher level than that typical of abnormal $\alpha$-chain heterozygotes;22 (3) the percentage of HbS in her erythrocytes was substantially lower than that usually present in individuals with sickle cell trait;23,24 and (4) the $\alpha/\beta$ globin synthesis ratio from her reticulocytes was decreased from normal.24

The findings in the patient are those of the $\alpha$-thalassemia trait phenotype, corresponding to the presence of two $\alpha$-thalassemia genes.25,26 Her presumed genotype, therefore, is $\alpha^{Thal} \alpha^{Thal} \alpha^{Nigeria} \alpha^A$. This genotype may occur in two possible linkage patterns, one in which both of the $\alpha$-thalassemia genes are linked on a single chromosome, or a trans configuration in which they exist on separate chromosomes. In the latter arrangement, the gene for the Hb Nigeria $\alpha$-chain would be in linkage with an $\alpha$-thalassemia gene.

The findings from the child of this woman and her phenotypically normal husband provide an opportunity to determine which of these possibilities may exist in this family. The child does not have Hb Nigeria, and therefore, in the absence of an improbable crossover,27 she inherited from her mother a chromosome that is representative of the maternal haplotype with either one or two $\alpha$-thalassemia genes.

Fig. 5. The presumed genotypes of the patient and her husband and child.

Individuals with sickle-cell trait who also have $\alpha$-thalassemia trait characteristically demonstrate decreased erythrocyte indices and a lower percentage of HbS compared to that of the usual form of sickle-cell trait.23,24 In contrast, sickle-cell trait accompanied by the silent-carrier $\alpha$-thalassemia phenotype, representing the presence of a single $\alpha$-thalassemia gene, is expressed by normal erythrocyte indices and a percentage of HbS that may be somewhat decreased but nevertheless is within the usual range of values seen in individuals with sickle-cell trait.25,26 The latter group of findings in this child (Table I) suggests that she inherited only a single $\alpha$-thalassemia gene.

The presumed genotypes of the family members based on these findings (Fig. 5) imply that an $\alpha$-thalassemia gene may be linked to the gene for the structurally abnormal $\alpha$-chain of Hb Nigeria. Other $\alpha$-chain structural variants that appear to be linked similarly to an $\alpha$-thalassemia gene include hemoglobins Mahidol (Q),28,29 J Tongariki,30 and J Cape Town.31

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REFERENCES

Hemoglobin Nigeria (alpha-81 Ser replaced by Cys): a new variant associated with alpha-thalassemia

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