Homozygous Hemoglobin Knossos (α₂β₂7(B9) Ala → Ser): A New Variety of β⁺-Thalassemia Intermedia Associated With δ⁻-Thalassemia

By F. Baklouti, E. Doriáée, L. Morlé, P. Laselve, D. Peyramond, M. Aubry, J. Godet, and J. Delaunay

Hb Knossos (β²7 (B9) Ala → Ser) is a recently discovered hemoglobin variant endowed with β-thalassemic properties.1 We present the first homozygous cases. The propositus, a 19-year-old man is originally from northeast Algeria, but is unrelated to other Algerians who have hemoglobin Knossos. He has a β⁺-thalassemia intermedia syndrome, including microcytic, hypochromic anemia, enlargement of the spleen, and an increase in the number of reticulocytes. The reduction of β-chain synthesis is pronounced (α/non α:2.76). Whole cells containing Hb Knossos have a dramatically low oxygen affinity (P₅₀:38 mm Hg). The propositus also has homozygous δ⁻-thalassemia. The chromosome carrying these mutations is characterized by the DNA haplotype

Hb Knossos was previously described.5 Hematological abnormalities associated with δ-thalassemia trait or a Lepore fusion gene. Anisocytosis, anisochromia, anisocytosis, and target cells were present. The propositus (III, 1) is a 19-year-old Algerian who has lived in France since 1982. He was born in Rabta, 80-km west of Setif in northeast Algeria. His parents are not consanguineous, but both originate from Rabta. The present family is apparently unrelated to the two Algerian families in which Hb Knossos was previously described.1,3,4 Hematological abnormalities were discovered during evaluation for an unrelated orthopedic problem. Mild cutaneous pallor was noted. The spleen extended 1 cm below the costal margin. Radiological evaluation of the skeleton revealed abnormalities of the right humerus and the left femur, a CS widening of the right humerus and the left femur, and an increase in the number of reticulocytes. The reduction of β-chain synthesis is pronounced (α/non α:2.76). Whole cells containing Hb Knossos have a dramatically low oxygen affinity (P₅₀:38 mm Hg). The propositus also has homozygous δ⁻-thalassemia.

MATERIALS AND METHODS

Case report. The propositus (II, 1, Fig 1B), is a 19-year-old Algerian who has lived in France since 1982. He was born in Rabta, 80-km west of Setif in northeast Algeria. His parents are not consanguineous, but both originate from Rabta. The present family is apparently unrelated to the two Algerian families in which Hb Knossos was previously described.1,3,4 Hematological abnormalities were discovered during evaluation for an unrelated orthopedic problem. Mild cutaneous pallor was noted. The spleen extended 1 cm below the costal margin. Radiological evaluation of the skeleton revealed abnormalities of the right humerus and the left femur, a CS widening of the right humerus and the left femur, and an increase in the number of reticulocytes. The reduction of β-chain synthesis is pronounced (α/non α:2.76). Whole cells containing Hb Knossos have a dramatically low oxygen affinity (P₅₀:38 mm Hg). The propositus also has homozygous δ⁻-thalassemia.

Methods. Hemoglobin analysis was performed by isoelectric focusing (IEF) according to Basset et al., or on agarose gels provided by Sebia for Hb AIC determination. Globin chains were analyzed by polyacrylamide gel electrophoresis (PAGE) in the presence of Triton X-100 and 8 mol/L of urea. Gels were scanned at 570 nm. Hb A₂ was assayed using microchroomatofocusing and Hb F by the procedure of Bette et al.1,12 γ-Chains were studied by Triton-urea PAGE after alkali precipitation of Hb A and/or Knossos. α and β chains from homozygotes were separated by ion exchange chromatography.16 The β-chain was subjected to aminoethylation and to trypsin digestion. The peptides were analyzed by fingerprinting or reverse-phase high-performance liquid chromatography (HPLC) on a μ Bondapak C18 column (Waters, Milford, Mass) in a LKB system (Bromma, Sweden), using an increasing gradient of acetonitrile in 0.01 mol/L of ammonium acetate (pH 5.7), with a flow rate of 1.50 ml/min.12,13 Amino acid composition of abnormal peptide βT3 was performed in a Biotronic amino acid analyzer. In vitro globin chain synthesis was carried out essentially according to Lingrel and Borsook.14 Oxygen dissociation curves were performed in a Hemocor differential spectrophotometer.

DNA was obtained from leukocytes as described and was digested (10 to 15 μg) in the presence of restriction endonucleases, using conditions recommended by the manufacturers. DNA fragments were separated in 0.8% agarose gels, transferred to nitrocellulose filters, and hybridized with ³²P-labeled probes. Four probes were used to recognize the seven polymorphic sites described by Orkin et al.: (a) a 1.3-(kb) Bam H1/EcoRI gene fragment in pBR 328; (b) a 0.8-kb Pst I/EcoRI Aγ IVS II in pSP 65; (c) a 1.7-kb Pst I/EcoRI Bγg II/Xba I fragment in pBR 328; and (d) a 0.95-kb Bam H1/EcoRI βIVS II in pBR 322.

RESULTS

In the untransfused propositus (II, 1), Hb A₂ was absent (Table 1, Fig 2a and c). Hb A was replaced by a component focusing slightly more cathodically (Fig 2a). This change became more apparent when IEF was carried out in agarose...
Table 1. Red Cell Indices and Hemoglobin Parameters

<table>
<thead>
<tr>
<th>Index (Parameters)</th>
<th>I.1</th>
<th>I.2</th>
<th>I.3</th>
<th>I.4</th>
<th>II.1</th>
<th>II.3</th>
<th>II.4</th>
<th>II.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (× 10^12/L)</td>
<td>5.84</td>
<td>4.75</td>
<td>5.32</td>
<td>4.86</td>
<td>5.24</td>
<td>5.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>16.0</td>
<td>11.7</td>
<td>10.1</td>
<td>11.1</td>
<td>9.5</td>
<td>11.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>79.5</td>
<td>74.4</td>
<td>58.4</td>
<td>70.2</td>
<td>58.3</td>
<td>71.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>27.3</td>
<td>24.7</td>
<td>19.1</td>
<td>22.8</td>
<td>18.1</td>
<td>23.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>4.80</td>
<td>1.80</td>
<td>6.00</td>
<td>1.0</td>
<td>2.80</td>
<td>1.50</td>
<td></td>
<td></td>
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<tr>
<td>Serum iron (μmol/L)</td>
<td>18</td>
<td>6</td>
<td>15</td>
<td>16.5</td>
<td>20.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hb Knossos (%)</td>
<td>96.6</td>
<td>—</td>
<td>—</td>
<td>95.3</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>βα/α (β %)†</td>
<td>37.5</td>
<td>37.6</td>
<td>37.1</td>
<td>—</td>
<td>38.1</td>
<td></td>
<td></td>
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<tr>
<td>Hb F (%)</td>
<td>0.38</td>
<td>0.81</td>
<td>2.30</td>
<td>1.60</td>
<td>4.72</td>
<td>2.43</td>
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<tr>
<td>Gγ (%)†</td>
<td>41.1</td>
<td>20.0</td>
<td>21.1</td>
<td>36.5</td>
<td>26.5</td>
<td>39.7</td>
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</tr>
<tr>
<td>Hb A2 (%)‡</td>
<td>1.94</td>
<td>1.44</td>
<td>0.00</td>
<td>2.24</td>
<td>0.00</td>
<td>2.39</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α/α (α/α)‡</td>
<td>1.69</td>
<td>1.35</td>
<td>2.76</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>P50, mm Hg§</td>
<td>33.5</td>
<td>35.5</td>
<td>38.0</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>n Hb§</td>
<td>2.56</td>
<td>2.52</td>
<td>2.62</td>
<td>—</td>
<td>—</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>2,3-DPG (μmol/g Hb§)</td>
<td>14.12</td>
<td>13.88</td>
<td>13.04</td>
<td>—</td>
<td>—</td>
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</table>

II.1 and II.4 are homozygotes; 7 propositus. The reason for the elevation of the reticulocyte count in I.1 is unknown.

* Determined by chromatofocusing.
† Determined by scanning of polyacrylamide-urea-Triton gels.
‡ Normal values 2.69% ± 0.62% (n = 352).
§ Normal values (n = 15): P50 = 27.20 ± 1.57 mm Hg; n = 2.69 ± 0.11; 2,3-DPG = 12.83 ± 1.90 μmol/g Hb.

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normal chromosome 11 from their father. In the parents, the \( \alpha/\alpha \) ratios and the P were found to be moderately increased.

DISCUSSION

We described the third Algerian family with Hb Knossos.\(^{1,2}\) To our knowledge, the propositus and her sister are the first recorded homozygous cases. The similarity of haplotypes associated with the Greek\(^{18}\) and the Algerian mutations suggests that the same chromosome is involved among persons of northern and southern Mediterranean origin, in contrast to the mutation found in blacks and in Creole people that is not flanked by a \( \delta^+ \)-thalassemia gene and is associated with another haplotype.\(^{18}\) Although it is not known on which side of the Mediterranean basin hemoglobin Knossos first arose, it is worth noting that the three Algerian families carrying this hemoglobin originate from a 200-km\(^2\) region in northeastern Algeria. Given the apparently low frequency of the \( \delta^+ \)-thalassemia gene,\(^5\) the detection of homozygotes in independent families would probably require a large scale screening of \( \beta^+ \)-thalassemia intermedia patients in this part of Algeria.

The difficulty in detecting Hb Knossos must be emphasized. All families described to date that display Hb Knossos were discovered because of other \( \beta \)-chain abnormalities, \( \beta \)-thalassemia, Hb Lepore, or Hb 5\(^{16}\), facilitated demonstration of Hb Knossos. The present family was recognized by study of a homozygote. The association of a microcytosis with normal serum iron and an inconstantly decreased Hb A\(_2\) usually suggests a mild form of \( \alpha \)-thalassemia, but Hb Knossos should be considered and the appropriate screening technique should be used.

The present study of Hb Knossos homozygotes confirms the presence of a \( \delta^+ \)-thalassemia gene in cis to the \( \beta \) gene mutation previously suspected\(^{18,21}\) or demonstrated\(^7\) among persons of Mediterranean origin. \( \delta^+ \)-Thalassemia has been described in different populations.\(^{2,5,19,22-23}\) In homozygous cases,\(^{22}\) a distinct \( \delta^+ \)-thalassemia gene must be involved since the hematological data are not consistent with a homozygous Hb Knossos. No large deletion was found to be associated with the \( \delta^+ \)-thalassemia gene cis to the \( \delta^+ \) gene.\(^3\) Similarly, previous studies on \( \delta^+ \)-globin genes have shown nondeletion defects.\(^{22,24}\)

These two cases of homozygous Hb Knossos may be
compared to Hb E disease. Both Hb Knossos and Hb E reflect mutations that also contribute to abnormal mRNA splicing.\textsuperscript{18,22} The 8-year-old child (II.4) has significant anaemia but is asymptomatic. The propositus had comparable ages of foetal haemoglobin. Nature 184:1877, 1959.

Homozygous Hb Knossos may evolve into a more serious clinical syndrome as has been noted in patients with Hb Knossos and Hb Lepore whom we recently described.\textsuperscript{3} The 8-year-old child (II.4) has significant anaemia but is asymptomatic. Both Hb Knossos and I-lb E are silent deletions. \textit{Nouv Rev Fr Hématol} 15:527, 1975.

The present report describes the two first cases of homozygous Hb Knossos in an Algerian family. This genotype produces the phenotype of \(\beta^T\)-thalassemia intermedia. The most salient features found in the propositus are the marked imbalance in globin chain synthesis, the reduced affinity of Hb Knossos for oxygen, and the association of the \(\beta^{Knossos}\) gene with a \(\beta\)-thalassemia gene and DNA haplotype I.\textsuperscript{1}

### ACKNOWLEDGMENT

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### REFERENCES


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