Homozygous Delta-Thalassemia First Discovered in Japanese Family With Hereditary Persistence of Fetal Hemoglobin

By Yoshiro Ohta, Kotaro Yamaoka, Ikuo Sumida, Shigeru Fujita, Toshio Fujimura, and Toshiuki Yanase

A family with δ-thalassemia has been discovered, in which five members are thought to be δ-thalassemia homozygotes with complete deficiency of Hb A2, and three members are probably heterozygotes with low levels of Hb A2 (1.2–1.6%). In four members among these, persistence of fetal hemoglobin of Swiss type was observed. The formal genetics of these two entities was discussed. The proposita showed first thalassemia-like stigmata with iron deficiency anemia, but after iron therapy administered over 2 months, the stigmata disappeared. All the other members of the family were free from clinical symptoms with normal morphology, MCH, and osmotic fragility of the red cells.

The thalassemias are characterized by discordant rates of synthesis of structurally normal polypeptide chains resulting in a deficiency of hemoglobin, and, depending on the chain involved, four types are presently known: α-, β-, δ-, and δβ-thalassemia. Among these, in the δβ-thalassemia heterozygote (F-thalassemia) Hb A2 exists in normal quantity but is completely deficient in the homozygote with thalassemic stigmata. Hb Lepore disease with thalassemic stigmata can be easily differentiated from other hemoglobinopathies. In the δ-thalassemia heterozygote, Hb A2 values are approximately half normal without any clinical manifestation. The existence of homozygous δ-thalassemia has thus been anticipated for a long time, but no such case has been described thus far. In a systematic screening survey for hemoglobin variants, which was conducted by the authors' group in Western Japan from 1957 through 1969, a kindred with a δ-chain thalassemia homozygote was recently detected. The present paper is concerned with the clinical picture and chemical characterization of this synthetic variant.
Table 1.—Hematological Findings of the Proposita Before and After 2 Months of Iron Therapy

<table>
<thead>
<tr>
<th></th>
<th>Before</th>
<th>After</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dl)</td>
<td>12.5</td>
<td>15.4</td>
</tr>
<tr>
<td>RBC (× 10⁶/cu mm)</td>
<td>4.16</td>
<td>5.92</td>
</tr>
<tr>
<td>Ht (%)</td>
<td>32</td>
<td>46</td>
</tr>
<tr>
<td>MCH (μg)</td>
<td>30</td>
<td>26</td>
</tr>
<tr>
<td>MCV (μμ³)</td>
<td>77</td>
<td>78</td>
</tr>
<tr>
<td>Reticulocyte (%)</td>
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<td>—</td>
</tr>
<tr>
<td>Morphological findings target cell</td>
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</tr>
<tr>
<td>Osmotic fragility</td>
<td>Decreased</td>
<td>Normal</td>
</tr>
<tr>
<td>WBC (per cu mm)</td>
<td>7600</td>
<td>5200</td>
</tr>
<tr>
<td>Serum iron (μg/dl)</td>
<td>23</td>
<td>50</td>
</tr>
<tr>
<td>Hb A₂ (%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hb F (%)</td>
<td>1.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Materials and Methods

Hemoglobin analysis was performed by thin layer starch gel electrophoresis using Tris-EDTA-borate/sodium metaborate buffer in a discontinuous system at pH 8.6, according to a slight modification of the method of Smithies. Amido black 10 B was used for protein staining, and ortho-dianisidine reagent for the demonstration of different hemoglobin zones. Quantity of hemoglobin F was determined by the method of Singer et al. The normal value of Hb F proportion measured by this assay was below 1%. The distribution of fetal hemoglobin in red cells was examined by the Betke’s acid elution technique. Quantitative estimations of Hb A₂ were performed by DEAE-Sephadex column chromatography following the descriptions of Huisman and by starch block electrophoresis (Kunkel and Wallenius). The normal value for Hb A₂ determined by these assays averaged 2.5%.

Results

The proposita is a 26-yr-old female born to Japanese parents residing in Fukuoka City. She visited the outpatient clinic of Kyushu University School of Medicine on May 13, 1969, complaining of palpitation, and was diagnosed as having atrial septal defect. There was no history of jaundice or anemia. Physical examination showed a well-developed female with neither hepatomegaly nor splenomegaly.

Fig. 1.—Peripheral blood smears from four members of homozygous delta thalassemia: (1) IV-1 (proposita) before iron therapy, (2) III-9, (3) IV-3, and (4) IV-6. Numbers refer to designation of individuals in Fig. 5.
Hematological Examination: Table 1 shows a mild hypochromic anemia accompanied by anisocytosis and target cells on the peripheral blood smears (Fig. 1). The osmotic fragility curves were shifted to the left but, after iron therapy, returned to normal (Fig. 2). Serum haptoglobin (hemoglobin-binding capacity) was 109 mg/dl. The myelogram was nearly normal: nucleated cell count $36 \times 10^4$ cu mm, erythroblast 28.8%, neutrophile leukocyte 47.2%, eosinophilic leukocyte 2.4%, monocyte 3.2%, lymphocyte 16.4%, and plasma cell 0.8%. Other laboratory examinations showed no noteworthy findings except a positive seroreaction for syphilis (Table 2).

Characterization of the Proposita's Hemoglobin: It was first noted that Hb A$_2$ was completely absent on thin-layer starch gel electrophoresis (Fig. 3). Quantitation of the hemoglobin fractions on DEAE-Sephadex column chromatography showed that only Hb A was present (Fig. 4). The 1-min alkali denaturation test for fetal hemoglobin gave normal results.

Clinical Course: As a consequence of iron therapy administered over 2 months, morphological abnormality, reduced osmotic fragility of the red cells and hypochromic anemia as mentioned above returned to normal ranges (Table 1). On the other hand, antisyphilitic therapy with antibiotics of several kinds resulted in no change in the serum reaction. Hb A$_2$ was always absent on repeated chemical assays.

<table>
<thead>
<tr>
<th>Table 2.—Laboratory Findings of Proposita</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total serum protein $8.6$ g/dl</td>
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<tr>
<td>Kunkel's test</td>
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<tr>
<td>T.T.T.</td>
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<tr>
<td>G.O.T.</td>
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<tr>
<td>G.P.T.</td>
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<tr>
<td>L.D.H.</td>
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<td>Icterus index</td>
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<tr>
<td>Cholesterol</td>
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<tr>
<td>Total serum lipid</td>
</tr>
<tr>
<td>Lipid phosphate</td>
</tr>
<tr>
<td>Creatinine</td>
</tr>
<tr>
<td>Urea-N</td>
</tr>
<tr>
<td>Na</td>
</tr>
<tr>
<td>K</td>
</tr>
<tr>
<td>Ca</td>
</tr>
<tr>
<td>Cl</td>
</tr>
<tr>
<td>Haptoglobin-hemoglobin</td>
</tr>
<tr>
<td>binding capacity</td>
</tr>
<tr>
<td>Seroreaction for syphilis $\left( ^+^+ \right)$</td>
</tr>
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</table>

Fig. 2.—Osmotic fragility curves from three members of homozygous delta thalassemia; (solid line) normal adult, (line with long and short dashes) III-9, (dashed line) IV-6, (dotted line 1) proposita before and (dotted line 2) after iron therapy.
(4) **Family Study:** The parents of the proposita are first cousins (Fig. 5). Father (III-13) was in good health, and hematological examinations for him are shown in Table 3: Serum iron was 95 μg/dl, on peripheral blood smears no morphological abnormality was seen, and the osmotic fragility curve was within normal limits. Electrophoresis of the hemoglobin showed low levels of the Hb A₂ fraction, comprising 1.5% of the total hemoglobin (Fig. 6). Fetal hemoglobin was estimated to be 4.6%, distributed heterogeneously in the red cells.

Sister (IV-5) is in good health, and hematological findings are given in Table 3. Serum iron is rather low, and Hb A₂ comprises 1.2% of the total; the fetal hemoglobin was 2.4%, being distributed heterogeneously in the red cells.

Mother (III-9) and uncle (III-2) are both in good health, and the hematological findings, including serum iron level, red cell morphology, and osmotic fragility of red cells, were within normal limits (Table 3). On electrophoresis and DEAE-Sephadex column chromatography of their hemoglobins, Hb A₂...
was completely absent. The levels of Hb F estimated by the method of 1-min alkali denaturation were 0.9%.

Two brothers (IV-3 and IV-6) had hematological findings within normal limits, but Hb A2 was absent and Hb F was 3.3 and 4.7%, respectively (Table 3).

Another uncle (III-5) is in good health, and hematological findings were also normal. Hb A2 level was approximately half normal with a normal Hb F level.

**DISCUSSION**

Delta thalassemia heterozygotes with levels of Hb A2 ranging below 1.5-2.5% have been found in several family members, and Fessas and Stamatoyannopoulos (1962) and Thompson et al. (1965) demonstrated two cases with thalassemic stigmata thought to be a type of δ-thalassemia interacting with other conditions (F-thalassemia), in which Hb A2 was completely absent. However, no δ-thalassemia homozygote in pure form has been reported thus far.

In the present cases diagnosis of δ-thalassemia homozygote was made on the basis of the following criteria: (1) complete absence of Hb A2 component on starch block, starch gel electrophoresis, and DEAE-Sephadex column chromatography, (2) normal serum iron levels, and (3) familial occurrence. Hematological findings in the proposita (hypochromic anemia, morphological abnormalities and reduced osmotic fragility of the red cells) appear to involve "thalassemic stigmata." Most of these stigmata, however, disappeared after iron therapy. Family studies showed that five members had no
<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Hb (g/dl)</th>
<th>RBC (10^6/cu mm)</th>
<th>Ht (per cent)</th>
<th>MCH (µg)</th>
<th>RBC Morphology</th>
<th>Osmotic Fraility</th>
<th>Serum Iron (µg/dl)</th>
<th>Hb F* (per cent)</th>
<th>Hb A:+ (per cent)</th>
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<tbody>
<tr>
<td>(1) Homozygote:</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV-1</td>
<td>26</td>
<td>F</td>
<td>12.5</td>
<td>4.16</td>
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<td>15.4</td>
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<td>14.6</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>IV-3</td>
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<td>16.8</td>
<td>4.60</td>
<td>43</td>
<td>36</td>
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<td>—</td>
<td>125</td>
<td>3.3</td>
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<td>IV-6</td>
<td>16</td>
<td>M</td>
<td>15.0</td>
<td>4.50</td>
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<td></td>
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<td>III-5</td>
<td>60</td>
<td>M</td>
<td>13.4</td>
<td>4.07</td>
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<td>33</td>
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<td></td>
<td>Normal</td>
<td>Normal</td>
<td>23</td>
<td>2.4</td>
</tr>
</tbody>
</table>

* Hb F was determined by the method of Singer et al.9
† Hb A₂ was determined by starch block electrophoresis.12
trace of Hb A2 and three members had reduced levels of Hb A2. The former five may thus be regarded as homozygous for δ-thalassemia gene, and the latter three as heterozygous. Morphological change and reduced osmotic fragility of the red cells seen in the proposita, though characteristic of thalassemias, may be attributable to iron deficiency anemia, since the other four homozygotes showed no specific abnormal findings.

These findings appear to be in good agreement with Motulsky's suggestion\(^1\) that the suppression of delta-chain synthesis does not affect the red cell morphology and does not result in any clinical symptoms.

Complete deficiency of Hb A2 has been demonstrated in other conditions; the homozygous state for high fetal hemoglobin,\(^1^8,1^9\) Hb Lepore,\(^2^6\) and δβ-thalassemia (F-thalassemia).\(^2^1\) These conditions are obviously ruled out in the present cases in which thalassemic stigmata are not significantly manifested. Hb F is not homogeneously distributed in the red cells, and no abnormal hemoglobin fraction is present. In individuals III-13, IV-3, IV-5, and IV-6, fetal hemoglobin levels are slightly elevated, ranging from 2.4 to 4.7%.

Another situation leading to an increase of Hb F is seen in certain kindreds where there is probably a different genetic mechanism specifically controlling Hb F synthesis at high levels. Aside from the association of high F with other hematologic disorders, a few types of hereditary persistence of Hb F are known (Table 4).\(^2^2\)

The condition associated with the persistence of hemoglobin F synthesis into adult life in the absence of any hematological abnormalities was first described by Edington and Lehmann.\(^2^3\)

According to an extensive study of an American black community (Conley

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**Fig. 6.** Thin layer starch gel electrophoresis in Tris-EDTA-borate system, pH 8.6; amido-black 10 B stain. (1) and (8) normal adult; (2) III-13; (3) III-9; (4) proposita (IV-1); (5) IV-3; (6) IV-6; (7) IV-5; and (9) normal infant. Numbers refer to designation of individuals in Fig. 5.
HOMOZYGOUS DELTA-THALASSEMIA

Table 4.—Comparison of the Different Forms of Hereditary Persistence of Hb F

<table>
<thead>
<tr>
<th>% Hb F in heterozygotes</th>
<th>Black Type</th>
<th>Greek Type</th>
<th>Swiss Type</th>
<th>Our Cases (Japanese)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18–30 (26)*</td>
<td>10–20 (15)</td>
<td>1–3 (2)</td>
<td>2.4–4.7 (3.7)</td>
<td></td>
</tr>
<tr>
<td>% Hb A2 in heterozygotes</td>
<td>1.0–2.1 (1.6)</td>
<td>1.2–3.0 (2.1)</td>
<td>Normal</td>
<td>Normal (?)</td>
</tr>
<tr>
<td>Distribution of Hb F in RBC</td>
<td>Homogeneous</td>
<td>Homogeneous</td>
<td>Heterogeneous</td>
<td>Heterogeneous</td>
</tr>
<tr>
<td>% Hb A2 in association with delta thalassemia</td>
<td>Absent</td>
<td>Absent (?)</td>
<td>(?)</td>
<td>1.2–1.5 (1.4)</td>
</tr>
</tbody>
</table>

* Mean value in parentheses.

e et al. (1963), the levels of Hb F and Hb A2 in the heterozygous condition are 19–30% and 1.0–2.1%, respectively, and in all cases Hb F is homogeneously distributed in red cells on Betke staining.

In a study in Greece (Fessas and Stamatoyannopoulos, 1964), on the other hand, the finding differs from that in the black population; the levels of Hb F and Hb A2 in heterozygotes are 10–12% and 1.2–3.0%, respectively, and the Hb F is homogeneously distributed in the red cells. From this it may be said that the genes responsible for the both Greek and black types of hereditary persistence of Hb F are associated with the suppression of β- and δ-chain synthesis.

Another form of this condition, demonstrated in Switzerland, was characterized by the persistence of low levels of Hb F into adult life (Marti, 1963). Hb F levels are 1–3%, and Hb A2 levels are normal in the heterozygote, and Hb F was heterogeneously distributed in the red cells. Hereditary persistence of Hb F of this type, called Swiss type, is in good agreement with those figures of the present cases (Table 4). As seen in Table 3, fetal hemoglobin is slightly elevated and heterogeneously distributed in the red cells.

The δ-thalassemia gene carried by individuals III-9 and III-13 is conceivably derived from their common ancestor(s), but the genetic mechanism of formation of the homozygous state in individuals III-2 and III-9 is difficult to interpret. However, it is possible that there is also consanguinity in the parents (II-3 and II-4), since the rate of consanguinity in Japan is of the highest order in the world, particularly in kindreds of rural origin where more than two common ancestors are not infrequently encountered.

Among the six children born to individuals III-9 and III-13, all of the four children who were examined (individuals IV-2 and IV-4 could not be examined) received one or two δ-thalassemia genes. So far as this finding is concerned, there is no contradiction in the parent-child relationship. However, some problems arise, when the δ-thalassemia locus is considered together with that of the persistence of Hb F and the phenotypes of the parents are contrasted with those of their children; that is, individual IV-1 is δ-thalassemia homozygote without persistence of Hb F, whereas individuals IV-3, IV-5, and IV-6 δ-thalassemia heterozygote or homozygotes with persistence of Hb F. These findings may possibly be explained by the following mechanisms:

The first interpretation is based on the assumption that the locus responsible for the Hb F persistence of this type is closely linked to the δ-β structural locus. On this assumption, let us now denote the δ-thalassemia gene and its normal allele as δN and δN, and the mutant gene responsible for the Hb F
persistence of this type and its normal allele as $\gamma^P$ and $\gamma^N$, respectively. Then, the genotype of individual III-9 will be $\delta^N \gamma^P / \delta^N \gamma^N$, and that of individual III-13 $\delta^N \gamma^P / \delta^N \gamma^N$ or $\delta^N \gamma^P / \delta^N \gamma^P$. If the genotype of individual III-13 is $\delta^N \gamma^P / \delta^N \gamma^N$, the genotypes of children born to the parents respectively possessing $\delta^N \gamma^P / \delta^N \gamma^N$ and $\delta^N \gamma^P / \delta^N \gamma^N$ should be only $\delta^N \gamma^P / \delta^N \gamma^P$ ($\delta$-thalassemia heterozygote with persistence of Hb F) and $\delta^N \gamma^P / \delta^N \gamma^N$ ($\delta$-thalassemia homozygote without persistence of Hb F). The phenotypes of individuals IV-1 and IV-5 correspond well to this assumption, but those of individuals IV-3 and IV-6 do not. However, appearance of the phenotype in the latter two may be not inconsistently explained when it is assumed that crossover occurred between the $\delta^{Th} \gamma^N$ and $\gamma^P \gamma^N$ loci of a paternal chromosome.

The second interpretation involves the existence of genetically heterogeneous types in the persistence of Hb F. In the present kindred the $\delta$-thalassemia is transmitted together with the persistence of Hb F in three individuals and segregated in one. This indicates that the persistence of Hb F may be due to a mutant allele appearing at an autosomal locus involving Hb F synthesis (high F locus), which is presumably independent of $\delta$-chain synthesis. Individuals III-13 and IV-5 may thus be double heterozygotes for the $\delta$-thalassemia and high Hb F genes. In fact, as mentioned above, the persistence of Hb F in the present cases is much different from the Greek and black types in the following points: (1) slight elevation of Hb F, (2) heterogeneous distribution of Hb F in the erythrocytes, and (3) existence of Hb A2 in the double heterozygotes for the $\delta$-thalassemia and Hb F persistence genes.

The third interpretation involves the possibility that Hb F persistence of this type is secondary to another unrecognized defect in the erythrocytes. Any of these interpretations may not be tenable, but the second one appears to be more likely than the others. Obviously, more informative matings are necessary to establish which, if any, is correct.

ACKNOWLEDGMENT

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REFERENCES

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YOSHIRO OHTA, KOTARO YAMAOKA, IKUO SUMIDA, SHIGERU FUJITA, TOSHIHIRO FUJIMURA and TOSHIYUKI YANASE