CONCISE REPORT

Hydrops Fetalis due to an Unusual Form of Hb H Disease

By Vivian Chan, T.K. Chan, S.T. Liang, A. Ghosh, Y.W. Kan, and D. Todd

The occurrence of Hb H hydrops fetalis is reported for the first time. The mother has \( \text{a}^{\text{a}} \text{thalassemia 1} \) (\( \text{a}^{\text{a}} \text{a} \text{a} / \text{a}^{\text{a}} \text{a} \text{a} \)) and the father has non-deletion \( \text{a} \) thalassemia \( \text{a}^{\text{a}} \text{a} \text{a} / \text{a}^{\text{a}} \text{a} \text{a} \)). The complete deletion of the \( \text{a} \) cluster on one chromosome was confirmed by quantitation of \( \text{a} \) and \( \text{g} \) gene numbers, the normal \( \text{a} \) and \( \text{g} \) gene patterns arising from the remaining normal chromosome, and the decreased \( \text{a} / \text{g} \) globin chain ratio of 0.57. The non-deletion \( \text{a} \) thalassemia defect could only be identified by the imbalanced \( \text{a} / \text{g} \) globin chain ratio of 0.65 in the presence of normal gene numbers and patterns. The newborn was markedly anemic, unlike those with classical Hb H disease, because the non-deletion \( \text{a} \) thalassemia defect is more severe than \( \text{a} \) thalassemia 2. The decreased \( \text{g} \) genes during fetal life might have additional deleterious effects. In this family, the distinct \( \text{BamHI} \) restriction fragment length polymorphism in the hypervariable region of the \( \text{g} \) genes may be used for future prenatal diagnosis.

The hybridized filters were then washed under stringent conditions and autoradiographed for two to four days.

To quantitate the numbers of \( \text{a} \) and \( \text{g} \) genes, two filters containing \( \text{XhoI} \) digests were hybridized with either equal amounts of \( \text{32P} \)-labeled \( \text{a} \) and \( \text{g} \) gene probes or \( \text{32P} \)-labeled \( \text{g} \) and \( \beta \) gene probes. The \( \beta \) gene probe was a 0.9-kb \( \text{EcoRI-BamHI} \)-digested fragment of the cloned \( \text{pBR} \) \( \beta \) gene. The autoradiographs were scanned on a laser densitometer (Ultrascan, LKB, Bromma, Sweden). The intensity of the \( \beta \) gene fragment served as an internal control for comparison of the \( \text{a} \) and \( \text{g} \) gene numbers in each sample. The relative intensities of the \( \text{a} \) and \( \text{g} \) genes in the \( \text{HindIII} \) digest and that of \( \text{g} \) and \( \beta \) genes in the \( \text{KpnI} \) digest were also quantitated by laser densitometry. In addition, DNA from two normal, non-thalassemic subjects was digested and hybridized as controls.

To exclude the hemoglobin variant \( \text{a} \text{a} \text{a} / \text{a} \text{a} \text{a} \), DNA was digested with \( \text{MspI} \) and hybridized to an \( \text{a} \) gene probe. \( \text{a} \text{a} \text{a} / \text{a} \text{a} \text{a} \) has an extra restriction site for \( \text{MspI} \), generating two fragments (216 base pairs [bp] and 121 bp), as compared to a single 337-bp fragment in the normal control.

Hematologic studies were performed by standard methods, and red cell indices were measured with a Coulter (Hialeah, Fla) electronic cell counter. For demonstration of Hb H-like inclusion in red cells, whole blood was incubated with brilliant cresyl blue as described previously.

Hemoglobin electrophoresis on cellulose acetate (pH 8.6; Helena Laboratories, Tex) as well as Hb isoelectrofocusing ([IEF] LKB) (pH 6 to 9) were performed. Globin chain synthesis was studied by incubating reticulocyte-rich blood with \( \text{14C} \)-labeled amino acids for two hours followed by chain analysis on a carboxymethyl cellulose (CM 52) column.

RESULTS

Case Presentation

A pregnant Chinese woman who previously delivered an infant with hydrops fetalis was referred for prenatal diagnosis. Fetal DNA extracted from amniotic fluid cells and digested with the restriction enzyme \( \text{Bgl II} \) showed the presence of 13.4- and 7.5-kb \( \text{a} \) genes and a 5.3-kb \( \beta \) gene. DNA of both parents was studied at the same time. Hybridization with \( \text{32P} \)-labeled \( \text{a} \) and \( \text{g} \) globin probes showed normal patterns. The pregnancy was allowed to proceed to term, with fortnightly ultrasound monitoring of fetal well-being. No abnormality was detected until 37 weeks, when the placenta was noted to be enlarged with mild fetal ascites. At 39 weeks the mother delivered a female infant who was found to be anemic, with ascites and hepatomegaly. The baby died a few minutes after birth from cardiac and respiratory failures. No gross congenital abnormality was noted at...
necropsy. Both parents and the baby had group O, rhesus D-positive blood.

**Hematologic Findings**

The propositus had a Hb concentration of 9.2 g/dL and Hb electrophoresis showed Hb F (28%), Hb A (38.5%), in addition to Hb H (2.5%) and Hb Barts (31%). Isoelectrofocusing showed the presence of an additional band in the position of Hb Portland II (\(\text{Hb} \beta\beta\)), which amounts to 6.2%. The father had Hb concentration (14.4 g/dL) and mean corpuscular volume (MCV) (80.7 fL; normal range, 80 to 94 fL) within normal limits; his abnormalities were the presence of occasional red cells with Hb H-like inclusions and an \(\alpha/\beta\) globin chain ratio of 0.65. The mother had a Hb concentration of 12.7 g/dL. MCV of 74.7 fL, occasional red cells with inclusions, and imbalance of globin chain synthesis (\(\alpha/\beta\) globin chain ratio, 0.57).

**DNA Analyses**

\(\alpha\) Genes. Normal \(\alpha\) gene fragments were obtained when DNA from the propositus and her parents were digested with each of seven restriction enzymes and hybridized to \(\alpha\) globin probe (Table 1). These were comparable to that found in the two non-thalassemic controls.

\(\xi\) Genes. \(\xi\) Restriction fragments generated by the same enzymes are also listed in Table 1. These were similar to that of the normal controls, except for small variations in fragment lengths for restriction sites within the hypervariable region (HVR), between the \(\xi_2\) and \(\xi_1\) genes\(^9\) (Fig 1). No additional \(\xi\) gene fragments, which may be characteristic of \(\alpha\) thalassemia 1 genotype, were observed in either parents or the propositus.

**Quantitation of Gene Numbers**

Quantitation of the number of \(\alpha\) and \(\xi\) genes in each sample is given in Table 2. 

<table>
<thead>
<tr>
<th>Probes</th>
<th>Enzyme</th>
<th>(N_1)</th>
<th>(N_2)</th>
<th>Father</th>
<th>Propositus</th>
<th>Mother</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\alpha)</td>
<td>EcoRI</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>BamHI</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
<td>14.5</td>
</tr>
<tr>
<td></td>
<td>BgIII</td>
<td>13.4, 7.5</td>
<td>13.4, 7.5</td>
<td>13.4, 7.5</td>
<td>13.4, 7.5</td>
<td>13.4, 7.5</td>
</tr>
<tr>
<td></td>
<td>HindIII</td>
<td>17.5, 4.6, 3.8</td>
<td>17.5, 16.5, 4.6, 3.8</td>
<td>19, 17.5, 4.6, 3.8</td>
<td>19, 4.6, 3.8</td>
<td>17.5, 4.6, 3.8</td>
</tr>
<tr>
<td></td>
<td>HpaI</td>
<td>14, 4.1</td>
<td>14, 4.1</td>
<td>14, 4.1</td>
<td>14, 4.1</td>
<td>14, 4.1</td>
</tr>
<tr>
<td></td>
<td>KpnI</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>XbaI</td>
<td>19.7</td>
<td>19.7</td>
<td>19.7</td>
<td>19.7</td>
<td>19.7</td>
</tr>
</tbody>
</table>

\(\xi\) Genes. \(\xi\) Restriction fragments generated by the same enzymes were also listed in Table 2. Similar to that of the normal controls, except for small variations in fragment lengths for restriction sites within the hypervariable region (HVR), between the \(\xi_2\) and \(\xi_1\) genes with \(\xi_3\) genes\(^9\) (Fig 1). No additional \(\xi\) gene fragments, which may be characteristic of \(\alpha\) thalassemia 1 genotype, were observed in either parents or the propositus.

The presence of 2.5% Hb H and 31% Hb Barts in the cord blood of this infant is compatible with a diagnosis of Hb H disease. However, her degree of anemia (Hb 9.2 g/dL) was greater than that found in newborns with the classical Hb H disease (Hb 12.4 to 14.2 g/dL).\(^{10}\) This probably was the cause of neonatal death. ABO and rhesus incompatibilities have been excluded by the parents' blood group and negative Coombs' test. Since the record of the previous hydropic sibling also showed similar amounts of Hb H and Hb Barts, it is likely that both infants suffered from the same syndrome. Gobelin chain synthesis and DNA analyses in this family in both mother and daughter were about half that of the normal controls and the father.

**Identification of Hemoglobin Quong Sze**

MspI digestion of DNA from the father and propositus showed no additional 216- and 121-bp fragments when hybridized with \(^32\)P-labeled \(\alpha\) gene probe. Only normal size fragments were observed in this family as well as in the two non-thalassemic control subjects.

**Restriction Fragment Length Polymorphism**

The BamHI digestion pattern of this family indicated distinct restriction fragment length polymorphism (RFLP) in the HVR. Apart from the presence of the 5.7-kb \(\xi_3\) gene fragment in all three members, the father had two \(\xi\) restriction fragments of 12.1 and 10.5 kb, respectively, which arise from his two \(\xi_1\) genes. The mother possessed only one 10.5-kb \(\xi_1\) fragment and the propositus had inherited the 12.1-kb \(\xi_1\) fragment from her father but none from her mother (Fig 2).

**DISCUSSION**

The presence of 2.5% Hb H and 31% Hb Barts in the cord blood of this infant is compatible with a diagnosis of Hb H disease. However, her degree of anemia (Hb 9.2 g/dL) was greater than that found in newborns with the classical Hb H disease (Hb 12.4 to 14.2 g/dL).\(^{10}\) This probably was the cause of neonatal death. ABO and rhesus incompatibilities have been excluded by the parents' blood group and negative Coombs' test. Since the record of the previous hydropic sibling also showed similar amounts of Hb H and Hb Barts, it is likely that both infants suffered from the same syndrome. Gobelin chain synthesis and DNA analyses in this family

*Table 1. Size of Different Fragments of DNA From the Family Members and Two Non-thalassemic Controls (N1 and N2) Obtained After Restriction With Seven Different Enzymes and Hybridization With an \(\alpha\) or \(\xi\) Gene Probe*

All values are expressed in kilobases.
Fig 1. (A) Schematic diagram of the $\beta$-\(\alpha\) gene cluster showing the restriction endonuclease sites used in this study: B, BamHI; Bg, BglII; E, EcoRI; H, HpaI; H, HindIII; K, KpnI; X, XbaI. (B) Autoradiographs of $\beta$ gene fragments generated by digestion of DNA with different restriction enzymes, BamHI, EcoRI, BglII, HindIII, and hybridized to a $^{32}$P-labeled $\beta$ gene probe. The gene patterns of the father (F), propositus (P), and mother (M) are compared with that of two non-thalassemic controls (N1 and N2). Numbers at the side indicate the fragment lengths in kb, and brackets indicate fragments in the hypervariable region. The weak 14.5-kb fragment in the BamHI digests and 7.5-kb fragment in the BglII digests are cross-reacting $\alpha$ genes.
define a new syndrome of severe Hb H disease resulting in hydrops fetalis.

The mother gave normal α and θ gene patterns with multiple enzyme restrictions. In the quantitation of gene numbers, an XbaI digest was chosen because the resultant restricted gene fragments (α and θ; 19.7 kb; β; 12.4 kb) had similar lengths and could thus be transferred onto nitrocellulose filters with the same efficiency. The additional use of β gene probe in the hybridization served as an internal control for each sample. Since the ratios of α/α2 and θ/θ1 genes were normal, the decreased α/β and θ/β ratios to half that of normal subjects was compatible with the conclusion that the complete θ-α cluster is deleted in one chromosome. Phenotypically, the imbalanced α/β globin chain synthetic ratio of 0.57 confirms this conclusion of severe α thalassemia, that is, θα-thalassemia 1. This defect had recently been described in an American black and was also thought to be associated with Hb H disease in a mentally retarded patient described by Weatherall and coworkers.

The father had normal α, θ gene patterns as well as normal numbers of these genes. The reduced α/β globin chain synthetic ratio of 0.65 suggests a moderately severe α thalassemia trait of the non-deletion type (αα7).

Various causes of non-deletion α thalassemia have been described in different ethnic groups: in an Italian patient, a Saudi Arabian, and a Chinese patient. The latter patient had mutation of amino acid 125 from leucine to proline in the α2 gene, resulting in a markedly unstable chain and an α thalassemia phenotype. The patient with this structural α mutant originated from Quong Sze province in China. However, the history of three generations of the present family showed no members from this province. Furthermore, MspI digest of the father's DNA did not show any characteristic fragments associated with the αQuongSze gene. It would appear that the father has another type of non-deletion defect, and further work will be needed to elucidate its exact nature.

Thus, the Hb H disease in this propositus arises from inheritance of 7α thalassemia 1 from the mother and non-deletion α thalassemia (αα7) from the father. The non-deletion α thalassemia (αα7) results in a lower α/β globin chain ratio than the usual type of α thalassemia 2 (α−). This would account for the more severe anemia in the propositus compared to other newborns with Hb H disease. Her Hb Barts level (31%) was also slightly higher than observed in these latter infants (Hb Barts range, 24% to 29%). Furthermore, she had 6.2% Hb Portland II. It is known that the θ chain is important in early embryonic life and in homozygous α thalassemia 1, with no α chains, θ production is maintained up to the time of delivery, when both Hb Portland I (α2γ2) and Hb Portland II are found. It is possible that the complete deletion of the θ-α gene cluster on one chromosome in our patient resulted in reduced quantities of embryonic hemoglobin as well as the ability to compensate for α chain deficiency and these led to the fatal outcome.

In this family, RFLP in the BamHI θ restriction map can be applied for future prenatal diagnosis. This was not recognized before the birth of this unusual hydrops fetalis. Since the mother has only one 10.5-kb θ1 fragment arising from her

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**Fig 2.** The possible application of θ-BamHI fragments for prenatal diagnosis in this family. The top panel shows the autoradiograph of the θ gene pattern after DNA digestion with BamHI. The family members (father, F; propositus, P; mother, M) are compared to two non-thalassemic controls (N1 and N2). The specific genotype of the propositus is indicated below. The bottom left and right panels are lined diagrams of the possible gene patterns of future fetal DNA samples; the genotypes distinctive of those patterns are indicated below each panel.
only \( \zeta \) gene, while the father possesses both 12.1- and 10.5-kb fragments from his two \( \zeta \) genes, the inheritance of only 12.1-kb fragment by the propositus is distinctive of a \( \xi\alpha T/\zeta\zeta\alpha \) genotype (Fig 2). This linkage of 12.1-kb BamHI \( \xi \) fragment with the non-deletion \( \alpha \) thalassemia gene can be applied specifically to this family. In future fetal DNA samples, the detection of only a 10.5-kb \( \xi \) fragment would be due to the inheritance of two normal chromosomes from the parents, ie, \( \xi\alpha\alpha/\zeta\zeta\alpha \). Alternately, the detection of 12.1- and 10.5-kb fragments, similar to that of the father, would indicate a \( \xi\alpha\zeta T/\zeta\zeta\alpha \) genotype. This use of RFLP would thus allow accurate prenatal diagnosis and prevention of another Hb H hydrops fetalis in this family.

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