

**REVIEW ARTICLE****Hydrops Fetalis Caused by  $\alpha$ -Thalassemia: An Emerging Health Care Problem**

By David H.K. Chui and John S. Wayne

**H**YDROPS FETALIS is a serious disorder, usually indicative of an ominous prognosis for the affected fetus. There are many causes, including both hereditary and acquired diseases.<sup>1-3</sup> In southeast Asia,  $\alpha$ -thalassemia is the most common cause of fetal hydrops, accounting for 60% to 90% of the cases.<sup>4-7</sup> With population migrations during the past decades, this syndrome is now seen in increasing numbers in other parts of the world.

$\alpha$ -Thalassemia is caused by mutations of the  $\alpha$ -globin genes, leading to decreased or absent  $\alpha$ -globin chain production from the affected genes.  $\alpha$ -Globin chains are the subunits for both fetal hemoglobin ( $\alpha_2\gamma_2$ ) and adult hemoglobin ( $\alpha_2\beta_2$ ). Therefore, severe  $\alpha$ -thalassemias can cause anemia in fetuses and in adults. Together with  $\beta$ -thalassemias which are caused by mutations of the  $\beta$ -globin genes, the thalassemias are among the most common single gene mutations in humans.<sup>8</sup> They are found mostly in areas where malaria was and may still be endemic.

Each person normally has a total of four  $\alpha$ -globin genes, two of which are encoded in tandem (in cis) on each chromosome 16 (Fig 1). There are  $\alpha$ -thalassemia deletions that remove either one or two  $\alpha$ -globin genes on each chromosome 16.<sup>9-11,11a</sup> If both parents are carriers of a deletion removing two  $\alpha$ -globin genes in cis on one chromosome 16, there is a one in four risk that in each pregnancy, the fetus might inherit both parental deletional mutations, and lack all  $\alpha$ -globin genes.

Because  $\alpha$ -globin chains normally are produced throughout gestation, fetuses without  $\alpha$ -globin genes would suffer from severe anemia, and thus hypoxia, heart failure, and hydrops fetalis. They would usually survive in utero until the third trimester of gestation when they would succumb to their genetic defects. In contrast to a normal fetus whose major hemoglobin is Hb F ( $\alpha_2\gamma_2$ ), these fetuses have primarily Hb Bart's ( $\gamma_4$ ). This disorder, first described in 1960, is known as homozygous  $\alpha$ -thalassemia or Hb Bart's hydrops fetalis syndrome.<sup>12,13</sup>

**MOLECULAR AND POPULATION GENETICS***The (--SEA) Deletion in Southeast Asia*

The  $\alpha$ -globin gene cluster is made up of one embryonic  $\zeta$ -globin gene ( $\zeta 2$ ), two  $\alpha$ -globin genes ( $\alpha 2$  and  $\alpha 1$ ), and four pseudogenes. It spans 30 kb and is located on the short arm of chromosome 16 near the telomere (Fig 1). A major regulatory region, HS-40, is found 40 kb upstream to the  $\zeta$ -globin gene and is indispensable for the expression of  $\alpha$ -globin genes *in cis*.<sup>14,15</sup>

In southeast Asia, a common  $\alpha$ -thalassemia mutation is the (--SEA) deletion. It is 20.5 kb in length, deleting both  $\alpha$ -globin genes, but sparing the embryonic  $\zeta$ -globin gene (Fig 1).<sup>9-11</sup> Homozygosity for this deletion, (--SEA/--SEA), is the most common cause of the Hb Bart's hydrops fetalis syndrome. The molecular basis of this genetic disorder was deciphered in 1974, the first ever for human diseases.<sup>16,17</sup>

The carrier rates of the (--SEA) deletion in southeast Asia are high (Table 1), and there are considerable variations in different regions.<sup>7,18-22</sup> However, detailed surveys are lacking. Among people of southeast Asian origin living in the United States, 5.4% of them were found to be carriers of the (--SEA) deletion, whereas 1% were found to be carriers of the (--FIL) deletion (see the following section).<sup>23,24</sup>

*Large Deletions Which in Combination With (--SEA) Can Cause Hydrops Fetalis*

In southeast Asia, there are three known, large  $\alpha$ -thalassemia deletions which remove all the  $\zeta$ - and  $\alpha$ -globin genes in cis, ie, the (--FIL) deletion in the Philippines, the (--THAI) deletion in Thailand, and the (--HW) deletion measuring over 300 kb found in a Chinese family (Fig 1).<sup>25,26</sup> Diagnostic strategies designed for the (--SEA) deletion would not recognize these large deletions. For Southern analysis, probes which are distal to the deletion breakpoints, such as the LO, 3' $\alpha$ HVR (hypervariable region), and 5' $\alpha$ HVR, have to be used.<sup>24-26</sup> Family studies often are helpful.<sup>26</sup> Recently, PCR methods with primers designed to detect either the (--FIL) or the (--THAI) deletions have been developed.<sup>26a,26b</sup>

*From the Provincial Hemoglobinopathy DNA Diagnostic Laboratory and Department of Pathology, Faculty of Health Sciences, McMaster University, Hamilton, Ontario, Canada.*

*Submitted September 23, 1997; accepted October 22, 1997.*

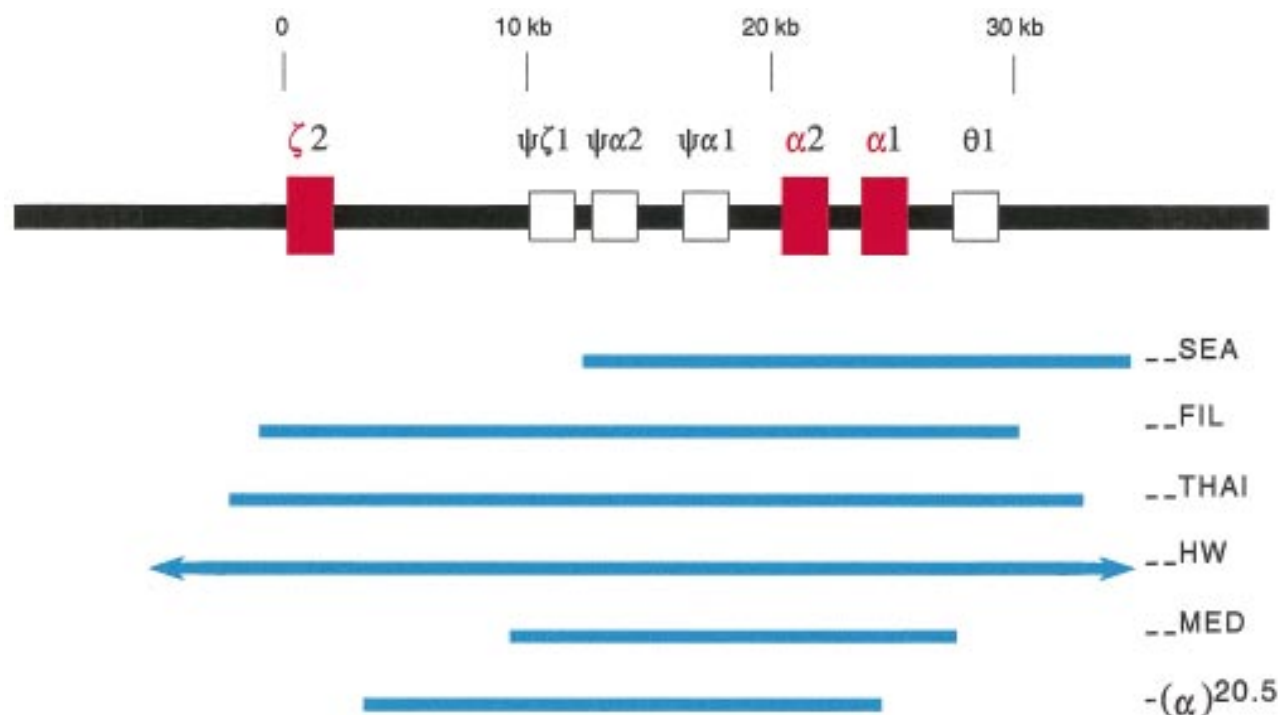
*Supported in part by the Medical Research Council of Canada, Cooley's Anemia Foundation of New York, the US National Institutes of Health (D.H.K.C.), the Ontario Thalassemia Foundation (J.S.W.), and the Ontario Ministry of Health.*

*Address reprint requests to David H.K. Chui, MD, Department of Pathology, Room 2N31, McMaster University Medical Centre, 1200 Main St W, Hamilton, Ontario, Canada L8N 3Z5.*

*The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.*

© 1998 by The American Society of Hematology.

0006-4971/98/9107-0041\$3.00/0



**Fig 1.** Deletions in the  $\alpha$ -globin gene cluster. The  $\alpha$ -globin gene cluster is located on the short arm of chromosome 16 near the telomere. The three active globin genes,  $\zeta 2$ ,  $\alpha 2$ , and  $\alpha 1$ , are represented by red boxes, whereas the four pseudogenes are represented by open boxes. The extent of the deletions are shown by the solid blue lines. The  $(-\text{HW})$  deletion encompasses more than 300 kb, and is represented with arrows in the figure.<sup>26</sup> The  $-(\alpha)^{20.5}$  deletion spares the  $\zeta 2$  gene, and removes the  $\alpha 2$  gene and the 5' region of the  $\alpha 1$  gene.<sup>29</sup>

In a survey of 1,500 people of Filipino ancestry living in Hawaii, the carrier rate of the two  $\alpha$ -globin gene deletion is 10%, and approximately 66% of these are of the  $(-\text{FIL})$  deletion.<sup>27</sup> On the other hand, these large deletions are uncommon in Hong Kong and Taiwan, accounting for less than 3% of those who are carriers of the two  $\alpha$ -globin gene deletion.<sup>7,18</sup>

It is generally accepted that fetuses homozygous for these large deletions do not present with the Hb Bart's hydrops fetalis syndrome because of their demise early in gestation (see the following section on Pathophysiology).<sup>25</sup> However, fetuses who are compound heterozygous for the  $(-\text{SEA})$  deletion and one of these large deletions, such as  $(-\text{SEA}/-\text{FIL})$ , develop the Hb Bart's hydrops fetalis syndrome.<sup>25,26</sup>

#### *Deletions Causing Hydrops Fetalis in the Mediterranean Region*

Hb Bart's hydrops fetalis has been reported in Cyprus,<sup>28,29</sup> Greece,<sup>30-33</sup> Sardinia,<sup>34</sup> and Turkey.<sup>35</sup> It is caused by either of two deletional mutations,  $(-\text{MED})$  and  $-(\alpha)^{20.5}$ .<sup>29,32</sup> These deletions are similar to the  $(-\text{SEA})$  deletion in that they remove or disrupt both  $\alpha$ -globin genes, but not the  $\zeta$ -globin gene (Fig 1). Because they are quite rare, Hb Bart's hydrops fetalis syndrome is not common in the Mediterranean populations.<sup>36</sup> In some cases, consanguinous marriages are involved.<sup>34</sup>

#### *Other $\alpha$ -thalassemia Mutations*

There are many other deletions which remove either two  $\alpha$ -globin genes in cis, the entire  $\zeta$ - $\alpha$ -globin cluster which is

sometimes designated as  $(-\text{Tot})$ , or the HS-40 sequences, thus leading to the silencing of the  $\alpha$ -globin gene expression.<sup>9-11,11a</sup> They have been described in almost every population, and might contribute to causing the hydrops fetalis syndrome. However, no such cases have been recorded, mainly because these deletions are rare.

Deletions which remove only one single  $\alpha$ -globin gene on chromosome 16 are common.<sup>9-11</sup> A person who is homozygous for these deletions would have a hematologic profile similar to one who is a carrier of the  $(-\text{SEA})$  deletion because functionally both individuals possess two  $\alpha$ -globin genes. However, those who are either heterozygous or homozygous carriers of the single  $\alpha$ -globin gene deletions are not at risk for conceiving fetuses who will lack all  $\alpha$ -globin genes. For example, even though single  $\alpha$ -globin gene deletions are frequently found in people of African ancestry and in the Indian subcontinent, Hb Bart's hydrops fetalis has not been reported in these populations.<sup>37</sup>

**Table 1.** Prevalence of  $(-\text{SEA})$   $\alpha$ -Thalassemia Deletion in Southeast Asia

Regions	Proportions of Population who are Heterozygous Carriers (%)	References
Hong Kong	4.5	18
Northern Taiwan	3.5	7, 19
Southern China	5.0-8.8	20
Northern Thailand	14.0	21
Central Thailand	3.7	22

### Unusual $\alpha$ -thalassemia Mutations Causing Hydrops Fetalis

There are rare reports in the literature describing fetuses with the Hb Bart's hydrops fetalis syndrome who also had one apparently normal  $\alpha$ -globin gene.<sup>7,38-41</sup> Recently, the genotypes of two such fetuses were described, one with ( $\alpha$ -Tot/ $\alpha$ -deletion of GAG in codon 30  $\alpha$ ) and the other one with ( $\alpha$ -SEA/ $\alpha$ -codon 59 GGC  $\rightarrow$  GAC or Gly  $\rightarrow$  Asp  $\alpha$ ).<sup>41</sup>

A person with a single normal  $\alpha$ -globin gene, as a result of either the deletion of three  $\alpha$ -globin genes, or a combination of two  $\alpha$ -globin gene deletion and a point mutation of the third  $\alpha$ -globin gene, ordinarily has the so-called Hb H disease. This person has moderate anemia in adult life but usually without need for transfusion, and no hydropic changes during fetal development.<sup>9-11</sup> The mechanisms and pathophysiology to account for the severe anemia and hydropic changes of the two aforementioned fetuses remain to be clarified.

### PATHOPHYSIOLOGY AND CLINICAL FINDINGS

#### Hemoglobins in Normal and Hydropic Fetuses

During embryogenesis, there are three embryonic hemoglobins, Hb Gower 1 ( $\zeta_2\epsilon_2$ ), Hb Gower 2 ( $\alpha_2\epsilon_2$ ), and Hb Portland 1 ( $\zeta_2\gamma_2$ ). By 6 to 7 weeks postconception, the production of embryonic  $\zeta$ - and  $\epsilon$ -globin chains becomes almost undetectable.<sup>42</sup> Thereafter, the major hemoglobin in the fetus is Hb F ( $\alpha_2\gamma_2$ ), and later supplemented by less than 10% of Hb A ( $\alpha_2\beta_2$ ) until birth.<sup>43,44</sup>

In an embryo with a genotype of ( $\alpha$ -FIL/ $\alpha$ -FIL), lacking the entire  $\zeta$ - $\alpha$ -globin gene clusters, the hemoglobins present would be homotetrameric  $\epsilon_4$  and Hb Bart's ( $\gamma_4$ ). These hemoglobins do not undergo heme-heme interaction and Bohr effect, and have very high oxygen affinity.<sup>45</sup> They are incapable of oxygen delivery to tissues in the rapidly growing embryo. Therefore, it is generally accepted that the affected embryo would succumb to severe hypoxia very early in gestation, and miscarriage should ensue, thus precluding the detection of these conceptions.<sup>25</sup>

Fetuses with a genotype of ( $\alpha$ -SEA/ $\alpha$ -SEA) usually survive into the third trimester of gestation.<sup>9-11</sup> This is generally attributed to the fact that the embryonic  $\zeta$ -globin genes continue to be operative in these fetuses, leading to the formation of about 10% to 20% embryonic Hb Portland 1 ( $\zeta_2\gamma_2$ ), and to a much lesser extent, Hb Portland 2 ( $\zeta_2\beta_2$ ).<sup>46-49</sup> Hb Portland 1 possesses physiological oxygen dissociation capability and can deliver oxygen to fetal tissues.<sup>50</sup> Nevertheless, the amounts of these hemoglobins are insufficient to keep pace with the remarkable growth and development of the fetus, especially during the third trimester of gestation. Ultimately the fetus would succumb to hypoxia and heart failure either in utero or shortly after birth.<sup>9-11</sup>

#### Fetal Abnormalities

The affected fetuses have decreased hemoglobin synthesis because of the absence of  $\alpha$ -globin genes (Fig 2). Most of the hemoglobin present is Hb Bart's ( $\gamma_4$ ) which is unable to deliver oxygen to tissues, is relatively unstable, and can precipitate and cause shortened red cell survival and possibly ineffective erythropoiesis.<sup>45,51</sup> These fetuses are usually severely anemic, although in some, the hemoglobin level can be as high as 100 g/L.<sup>10</sup> The circulating erythrocytes are markedly hypochromic

and anisopoikilocytic (Fig 3). There are many nucleated erythroblasts in the peripheral blood, indicative of erythropoietic stress. Extensive extramedullary erythropoiesis is found in many organs and sites. This is the cause for the massive hepatomegaly. The spleen can also be enlarged.

The change from  $\zeta$ - to  $\alpha$ -globin synthesis in a fetus normally is completed by the sixth to seventh week postconception.<sup>42</sup> Thereafter, in fetuses lacking  $\alpha$ -globin genes, severe anemia and fetal hypoxia would occur, which can adversely affect subsequent organogenesis and fetal development. One effect of anemia and hypoxia, ie, placentomegaly, can be detected by ultrasonography in some fetuses as early as the 10th week of gestation (see section on Prenatal Diagnosis by Ultrasonography).<sup>52</sup> In one series, 17% of affected newborns were found to have congenital anomalies.<sup>5</sup> They included hydrocephaly and microcephaly, as well as cardiopulmonary, skeletal, and genitourinary malformations. In another survey of 65 affected newborns in Thailand, severe reduction in brain weight was noted in some.<sup>10</sup> Hypoplasia of lung, thymus, adrenals, and kidneys has been observed.<sup>53</sup> Anomalous genitalia were present in other infants.<sup>53-55</sup> Recently, limb reduction defects such as absence of hand and forefoot were reported.<sup>55-57,57a</sup>

At birth, these newborns are usually pale, large and edematous, sometimes with anasarca. However, not all affected newborns are grossly hydropic (Fig 4A), illustrating the wide spectrum of clinical presentations of this disorder. Often, there are signs of high output heart failure, such as cardiomegaly, pericardial effusion (Fig 4B), pleural effusion, and ascites. The placenta is massively enlarged.<sup>5</sup>

#### Maternal Complications

There is an increased incidence of serious maternal complications in these pregnancies. It is likely that the placentomegaly is one important causative factor. It was estimated that half of these women could die from complications resulting from these pregnancies if there was no medical care.<sup>10</sup> In a study of 46 women who were pregnant with affected fetuses, 61% developed hypertension during pregnancy, of whom half developed severe pre-eclampsia.<sup>5</sup> Polyhydramnios was present in 59% of the cases. Eleven percent suffered antepartum hemorrhage as a result of either unknown cause or placenta previa. Other less common complications included disseminated intravascular coagulation, renal failure, and pleural effusion. Oligohydramnios, abruptio placenta, premature labor, and congestive heart failure have also been reported.<sup>53,54,58</sup>

The mean gestation at delivery was 31 weeks with a range of 24 to 38 weeks.<sup>5</sup> Malpresentation of the infant during births occurred in 37% of the cases. Thirty-eight women delivered vaginally, 10 of whom experienced difficulty including 3 necessitating paracenteses to decrease fetal ascites to facilitate delivery. Caesarean section was performed in 8 women. Postpartum complications include retained placenta, hemorrhage, life-threatening hypertension, puerperal pyrexia, and anemia.<sup>6,54,59</sup>

### THE ONTARIO EXPERIENCE

The Province of Ontario has a population of eleven million people. There are 600,000 people of southeast Asian origins, such as China, Hong Kong, Laos, the Philippines, Taiwan, and

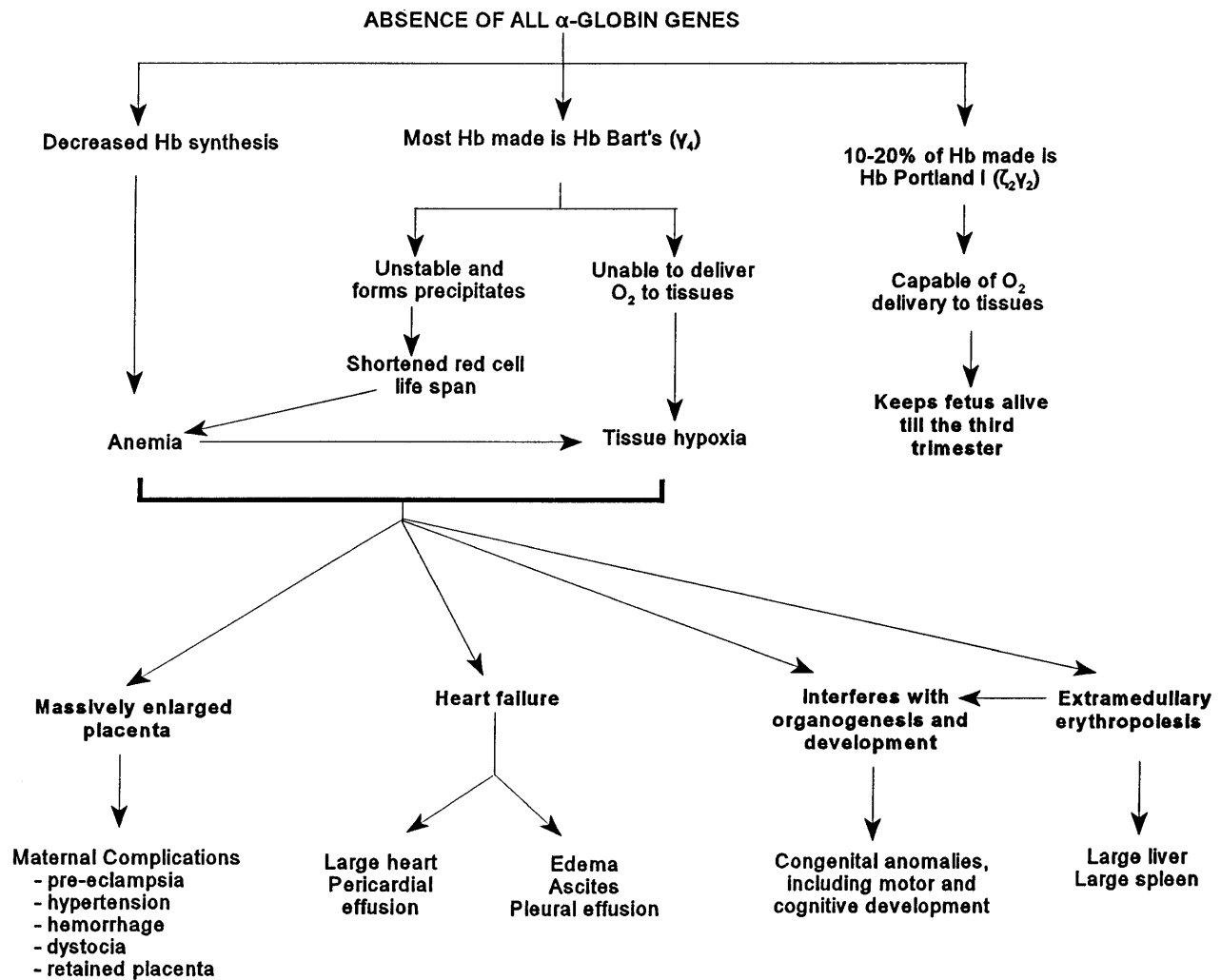


Fig 2. Figure depicting the pathophysiology caused by the absence of the  $\alpha$ -globin genes.

Vietnam. It is estimated that annually in Ontario there are approximately 20 to 60 pregnancies at risk for having fetuses with the Hb Bart's hydrops fetalis syndrome.

From 1989 until 1997, the DNA Diagnostic Laboratory in Ontario has performed prenatal tests for 50 pregnancies at risk

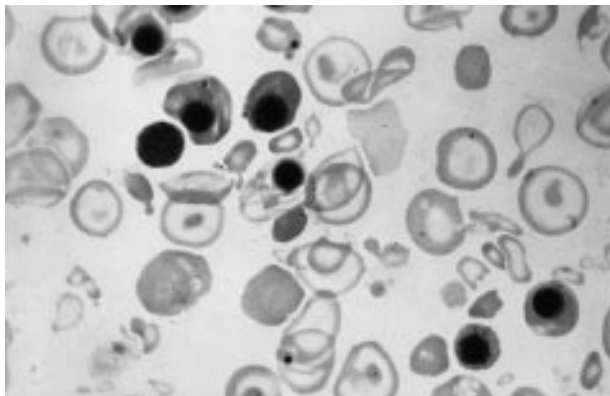
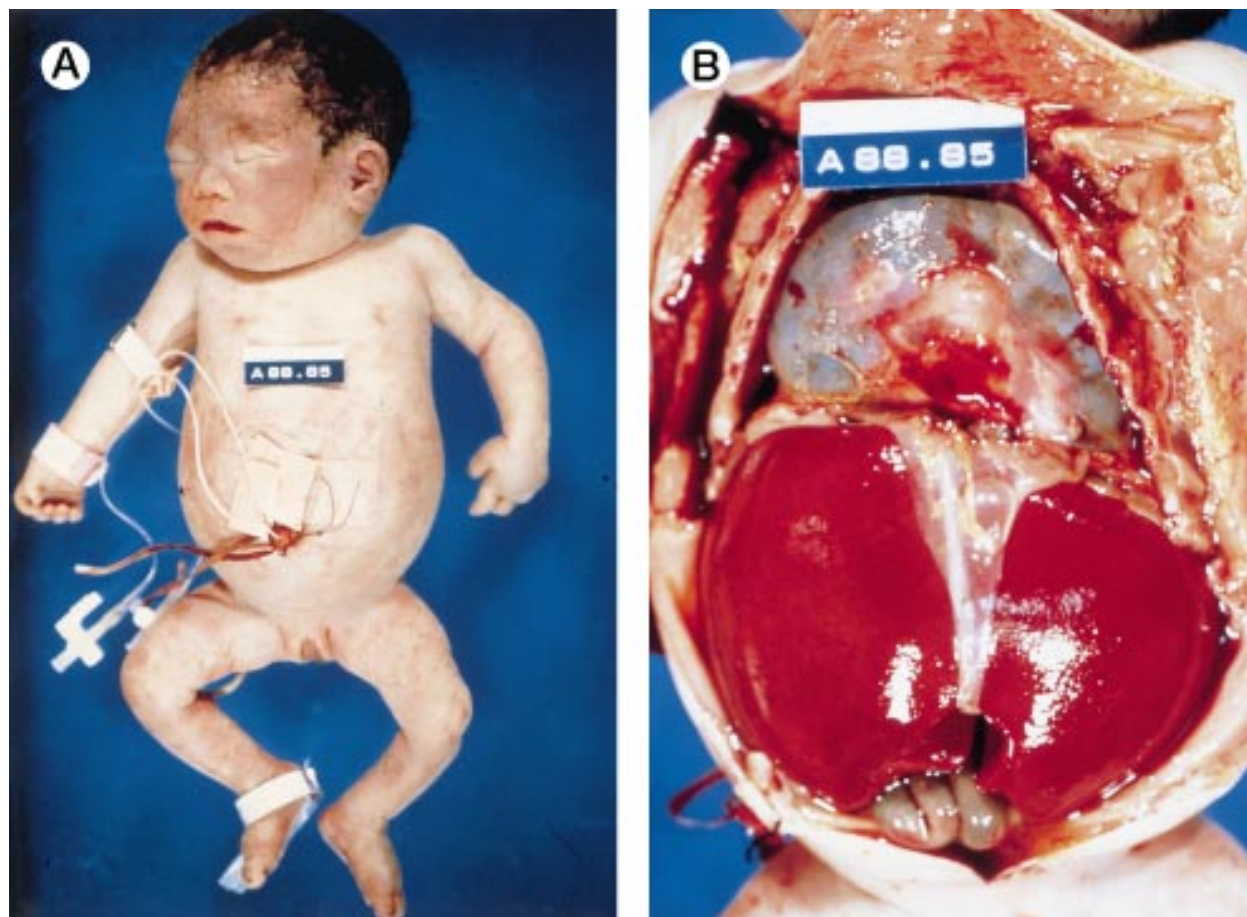


Fig 3. Peripheral blood smear of the newborn shown in Fig 4. Note the hypochromia, anisopoikilocytic changes, and erythroblastosis.

of the syndrome.<sup>60</sup> Three of these pregnancies were at risk for both hydrops fetalis and Hb H disease. More than half (30/50) were conducted on chorionic villus samples obtained during the first trimester of pregnancy, while the remainder were on amniotic fluid or amniocyte cultures obtained during the second trimester. Eleven fetuses with homozygous  $\alpha^0$ -thalassemia were diagnosed, whereas 26 were carriers of  $\alpha^0$ -thalassemia, 2 were carriers of  $\alpha^+$ -thalassemia, and 11 were found to have the normal complement of four  $\alpha$ -globin genes. These 50 pregnancies involved 37 couples. Only 4 couples at risk had been identified and counselled before their first pregnancy. Fifteen couples were found to be at risk during their pregnancies. As many as half of the couples referred were found to be at risk after they had one or more pregnancies ended with hydropic newborns. These observations indicate the grossly inadequate preconception carrier screening for  $\alpha$ -thalassemia in Ontario.

For the same period (1989-1997), there should have been several hundred pregnancies at risk in Ontario. These figures indicate that despite the availability of hospital-based genetic services, only 10% to 30% of the at risk pregnancies in Ontario are identified and provided with genetic counseling and diagnostic services. In the same period, the Laboratory was called on to



**Fig 4.** Autopsy photographs of a newborn with hemoglobin Bart's hydrops fetalis syndrome, delivered by caesarean section at 31 week of gestation.<sup>59</sup> Despite active resuscitation, the infant died within an hour after birth. Note edema of face and abdominal distension (A). The thoracic cavity is filled by the pericardial sac distended by effusion and cardiomegaly; there is massive hepatomegaly (B). Pictures courtesy of Prof H.A. Heggveit.

confirm 16 cases of hydrops fetalis caused by  $\alpha$ -thalassemia. Three cases were diagnosed by ultrasound examination, and later confirmed by DNA testing of fetal blood obtained by cordocentesis. The remaining 13 cases were diagnosed by DNA testing of cord blood at birth or fetal tissue obtained at autopsy.

The correct diagnosis of homozygous  $\alpha$ -thalassemia was sometimes missed at autopsy of affected fetuses. In one case, the final autopsy diagnosis was incorrectly ascribed to congenital heart disease. In another case, the woman later became pregnant again and was found to have an affected fetus again in the 24th week of the second pregnancy. These observations suggest that the numbers of pregnancies with this syndrome reported are likely to have been underestimated. Furthermore, incorrect diagnosis can adversely affect the maternal health care provided for subsequent pregnancies.

#### TREATMENT AND PROGNOSIS OF SURVIVING INFANTS

##### *Counseling, Prevention, and Care for the Pregnant Woman*

With very rare exceptions (see section below), all fetuses with the Hb Bart's hydrops fetalis syndrome succumb to severe fetal hypoxia in utero during the third trimester of gestation or within hours after birth.<sup>9-11</sup> The health care measures to combat this inevitably fatal disorder should be aimed at identifying

couples at risk in order to provide them with timely counseling, and prenatal diagnosis during early pregnancy. The screening tests to detect couples at risk are simple blood counts and hemoglobin electrophoresis that are widely available (see section on Carrier Detection and Prenatal Diagnosis).

When such pregnancy is not diagnosed until the second trimester or later, the emphasis should be to ensure the well-being of the pregnant woman before and after delivery. When the diagnosis of an affected fetus is confirmed, termination of the pregnancy should be possible when requested by the pregnant woman, regardless of the stage of the pregnancy.<sup>61</sup>

##### *Postdelivery or Intrauterine Transfusions*

There are now at least six surviving children born with homozygous  $\alpha$ -thalassemia.<sup>55,62-67</sup> These newborns either were transfused immediately postdelivery or received intrauterine transfusions. Their clinical courses are summarized in Table 2. All six children have been maintained on a regular transfusion program, and iron chelation when appropriate, similar to that for children with  $\beta$ -thalassemia major.<sup>68-70</sup> Bone marrow transplantation is a possible future treatment modality for some. With proper medical therapy and patients' compliance, significant progress has been made in the survival of  $\beta$ -thalassemia

Table 2. Summary of the Six Surviving Children Born With Homozygous  $\alpha$ -Thalassemia

Previous Birth of Hydropic Infant	Birth	Transfusion Treatment	Duration in Hospital After Birth	Follow-Up	References
No	Caesarean section at 32 wk	Postdelivery transfusion	48 d	At 6 yr, delay in speech and hearing	62, 64
Yes	Caesarean section at 28 wk	Postdelivery transfusion	73 d	At 5 yr, normal development	63, 64
Not recorded	Vaginal delivery with breech presentation at 28 wk	Postdelivery transfusion	64 d	At 10 mo, mild gross motor delay	64
No	Caesarean section at 31 wk, as part of triplet delivery	Postdelivery transfusion	2½ mo	At 2 yr, spastic quadriplegia and profound developmental delay	65
Yes	Caesarean section at 34 wk	Intrauterine transfusion beginning at 25 wk	11 d	Several congenital anomalies. At 2 yr, developmental delay in cognitive and motor functions	55, 66
No	Caesarean section at 37 wk	Intrauterine transfusion beginning at 32 wk	30 d	At 3 yr, normal neurological assessment	67

major patients, and similar prognosis is anticipated for these six children with homozygous  $\alpha$ -thalassemia.<sup>68-70</sup> There are three other reports of intrauterine transfusion to treat hydropic fetuses caused by  $\alpha$ -thalassemia.<sup>41,71,74</sup>

These newborns often had serious neonatal complications. Some also had congenital anomalies and delays in cognitive and motor functions. There are ethical issues with regards to aggressive medical intervention in fetuses who might harbor undiagnosed and possibly irreparable developmental abnormalities caused by severe fetal hypoxia in utero early during embryogenesis, and who would normally succumb to their serious genetic defects.<sup>66</sup> The high costs of such a treatment program in light of the other societal needs and demands for health care also deserve careful thought and discussion.<sup>66</sup>

#### *In Utero Hemopoietic Stem Cell Transplantation*

Intrauterine hemopoietic stem cell transplantation has been proposed as another treatment alternative.<sup>72</sup> Such a procedure was performed in at least three pregnancies, all without success.<sup>73-75</sup> The ethical and societal issues raised in the previous paragraph also apply to this form of medical intervention.

#### CARRIER DETECTION AND PRENATAL DIAGNOSIS

##### *Screening for Couples at Risk*

**Ethnic origins.** This syndrome is found almost always in couples of southeast Asian ancestry. Nevertheless, it has also been reported in the Mediterranean populations.<sup>28-35</sup> In practice, any person found to have low erythrocyte mean corpuscular volume (MCV; see following section) without iron deficiency should be considered a carrier of either  $\alpha$ - or  $\beta$ -thalassemia mutation.<sup>76-78</sup> History of previous births of hydropic infants in a southeast Asian family should alert the physician to the likely possibility of  $\alpha$ -thalassemia being present in the family.

**Low erythrocyte MCV.** The most important diagnostic criteria to detect thalassemia carriers are microcytosis (MCV <80 fL) or hypochromia (mean corpuscular hemoglobin [MCH] <27 pg).<sup>9,77,78</sup> In our laboratory, the mean and SD of MCV and MCH for ( $--^{SEA}/\alpha\alpha$ ) carriers are  $67.8 \pm 3.3$  fL and  $21.8 \pm 1.2$  pg, respectively. These laboratory results should be routinely reported and included as part of the prenatal screening records of all pregnant women.

On the other hand, adult carriers of two  $\alpha$ -globin gene deletion such as ( $--^{SEA}/\alpha\alpha$ ) usually do not have significant anemia.<sup>9</sup> Our laboratory has found that the hemoglobin levels for these men are  $135 \pm 10$  g/L, and for women  $121 \pm 10$  g/L. Therefore, it behooves all physicians caring for people during their adolescence or reproductive years to pay attention not only to the hemoglobin values, but also to the MCV or MCH results. If one partner of a couple is found to have microcytosis, the other partner should be tested to determine if he/she also has microcytosis. If both have microcytosis without iron deficiency, they should be investigated further to determine if they are at risk for conceiving fetuses with either Hb Bart's hydrops fetalis syndrome or  $\beta$ -thalassemia major.<sup>77,78</sup>

**Hemoglobin electrophoresis.** Hemoglobin electrophoresis and Hb A<sub>2</sub> levels are often performed as part of the laboratory investigations to diagnose thalassemia carriers. If the Hb A<sub>2</sub> level is elevated (>3.5%), the individual is considered to be a carrier of  $\beta$ -thalassemia mutation. If the Hb A<sub>2</sub> level is normal or low, the person is considered to be a carrier of  $\alpha$ -thalassemia mutation.<sup>77,78</sup> However, among people with microcytosis and high Hb A<sub>2</sub> levels, some are carriers of both  $\alpha$ - and  $\beta$ -thalassemia mutations.<sup>8,18,78a</sup> Of the 10 people diagnosed by our laboratory to be heterozygous carriers of both the ( $--^{SEA}$ )  $\alpha$ -thalassemia deletion and a  $\beta$ -thalassemia mutation, their mean MCV is 72.3 fL (range, 66.9 to 78.4), and Hb A<sub>2</sub> level is 5.4% (range, 4.5 to 7.1). Therefore, the  $\alpha$ -globin genotypes should be determined in all individuals who are referred for investigation as possible thalassemia carriers.

**Hb H inclusion bodies.** In adults carriers of  $\alpha$ -thalassemia mutations, there are excess  $\beta$ -globin chains within their erythroid cells. These  $\beta$ -globin chains can form homotetrameric Hb H ( $\beta_4$ ) precipitates (inclusion bodies) on incubation with a redox agent such as brilliant cresyl blue dye.<sup>79</sup> The search for these inclusions is laborious and the results are highly observer-dependent. Recent studies have suggested that an immunocytological assay for embryonic  $\zeta$ -globin chains (see below) or a polymerase chain reaction (PCR)-based DNA diagnostic technique can serve as alternative screening procedures.<sup>80,81</sup>

**Embryonic  $\zeta$ -globin chains.** Adult carriers of the ( $--^{SEA}$ ) deletion have a very minute amount of the embryonic  $\zeta$ -globin chains in their erythrocytes, demonstrable by immunologic techniques with anti-human-embryonic- $\zeta$ -globin-chain antibody.

ies.<sup>82-87</sup> In particular, the immunocytological staining of peripheral blood smears with the antibody has been shown to be highly sensitive and specific in detecting adult carriers of the ( $\alpha$ -SEA) deletion.<sup>60,84,87</sup>

Adult carriers of the large deletions such as the ( $\alpha$ -FIL) deletion do not have detectable  $\zeta$ -globin chains in their erythrocytes.<sup>87</sup> If both partners are found to be negative for the  $\zeta$ -globin assay, it is unlikely that they will conceive a fetus with the Hb Bart's hydrops fetalis syndrome. Even if the embryo did inherit both parental deletions, it should result in an early miscarriage caused by the demise of the affected embryo.<sup>25</sup> If both partners have microcytosis and only one is positive for the  $\zeta$ -globin chain assay, both individuals then need to have definitive DNA analysis to determine whether they are at risk of conceiving fetuses with the Hb Bart's hydrops fetalis syndrome.<sup>87</sup>

#### *Definitive Diagnosis by DNA Analysis for Couples at Risk*

For genetic counseling and prenatal diagnosis, it is essential to document the parental mutations at the DNA level either by Southern blot analysis or by PCR-based strategies.<sup>60</sup> There are several PCR-based diagnostic protocols designed to identify the ( $\alpha$ -SEA) and other  $\alpha$ -thalassemia deletions.<sup>88-93</sup> The diagnostic specificity of the PCR techniques has to be rigorously maintained in view of recent reports of misdiagnoses based on this strategy.<sup>94,95</sup>

#### *Prenatal Diagnosis by DNA or Hemoglobin Analysis*

Fetal tissue samples for DNA-based prenatal diagnosis are usually obtained by chorionic villus biopsy (CVS) during 10 to 11 weeks of pregnancy, or by amniocentesis during 16 to 20 weeks of pregnancy. The risk for miscarriage in CVS is estimated to be 1.0%, and that for amniocentesis to be 0.5%. Recently, a noninvasive method to obtain fetal nucleated erythroblasts from maternal blood early in pregnancy was reported.<sup>96,97</sup> In all prenatal diagnoses, the possibility of maternal tissue/blood contamination has to be guarded against. In some instances, nonpaternity can also be a confounding problem.

Fetal blood samples can be obtained by cordocentesis during the second trimester of gestation. Both the DNA-based analysis and hemoglobin electrophoresis can be performed on these blood samples. The major hemoglobin found in a normal fetus during the second trimester of gestation is Hb F ( $\alpha_2\gamma_2$ ), with 10% or less of Hb A ( $\alpha_2\beta_2$ ). In a fetus with homozygous  $\alpha$ -thalassemia, the major hemoglobin is Hb Barts ( $\gamma_4$ ), with 10% to 20% of Hb Portland 1 ( $\zeta_2\gamma_2$ ), and possibly some Hb H ( $\beta_4$ ). Hb F or Hb A are not present in these fetuses. These results can be obtained rapidly by using simple cellulose acetate electrophoresis. Similarly, cord blood samples obtained at delivery and uncontaminated by maternal blood can be used to genotype the newborn.

#### *Prenatal Diagnosis by Ultrasonography*

After the 20th week of gestation, ultrasound examination can readily detect the many hydropic changes found in fetuses with the Hb Bart's hydrops fetalis syndrome.<sup>54,98-101</sup> A recent study reported that hydropic changes in some fetuses could be detected as early as the 12th week of gestation.<sup>102</sup>

In a large study it was reported that increases in placental

thickness in some affected fetuses occurred as early as the 10th week of gestation.<sup>52</sup> By the 12th week, these measurements could identify most of the affected pregnancies. By the 18th week, the measurement could identify all affected pregnancies with a high specificity. These results were recently independently confirmed.<sup>103</sup>

Fetal cardiothoracic ratio measurements were also useful to diagnose affected fetuses.<sup>104</sup> Taken together, ultrasound findings of both placentomegaly and cardiomegaly at 12 to 14 weeks of gestation can be highly specific for affected fetuses in pregnancies at risk.<sup>105</sup> These measurements merit consideration as the next best alternative to diagnose hydropic fetuses, particularly in places where Hb Bart's hydrops fetalis syndrome is common and where DNA-based prenatal diagnosis is not readily available.

#### SUMMARY

Recent advances in molecular genetics have provided insights into the mutations and pathophysiology causing  $\alpha$ -thalassemias, as well as definitive clinical diagnostic tests for adult carrier detection and prenatal diagnosis. Hydrops fetalis caused by  $\alpha$ -thalassemia is found primarily though not exclusively among couples of southeast Asian origin, and is encountered in increasing numbers in North America and elsewhere.<sup>61,71,106-109,109a</sup> These pregnancies inevitably result in fetal death during the third trimester of gestation or shortly after birth, and frequently are associated with serious maternal morbidity and even mortality.

Presently, most couples at risk for conceiving fetuses with this serious genetic disorder are not identified. Other at risk couples are recognized only after the birth of one or more hydropic newborns, or hydrops fetalis is detected by ultrasonography during the second trimester of a pregnancy. The correct diagnosis of these hydropic fetuses is sometimes missed at autopsy. After experiencing the horrendous obstetrical, medical, and psychosocial problems associated with such pregnancies, many couples would decide against becoming pregnant again, and some would even request sterilization.<sup>61</sup>

A recurring theme of the many case reports of this syndrome is that the correct diagnosis could have been made early in pregnancy, should the physicians have been alert to this diagnostic possibility.<sup>26,53,54,57-59,61-67,71,106-117</sup> They reaffirm the need for public education, both for the medical community and for the population at large. Blood counts are often performed as part of the medical examinations, particularly on all pregnant women during their first visits to physicians. A low MCV result is the screening test for carriers of the thalassemias.

With carrier detection, timely genetic counseling, and the availability of prenatal diagnosis during early pregnancy, many couples at risk will be spared of serious medical and psychological ordeals in their quest for having families with children. It is therefore essential that this genetic disorder is recognized, so that the appropriate maternal health care and preventive measures can be provided to the affected couples and communities.

#### ACKNOWLEDGMENT

We wish to dedicate this review to the memory of the late Professor W.H.C. Walker. His foresight, encouragement, and support were pivotal in the establishment of the Provincial Hemoglobinopathy DNA Diagnos-

tic Laboratory. We are also indebted to our many clinical and laboratory colleagues in Ontario and elsewhere for their advice and collaboration, to Barry Eng, Margaret Patterson, and Shi-Ping Cai for their excellent technical assistance, and to Lynne Lacey for her help in the preparation of this manuscript.

## REFERENCES

- Holzgreve W, Curry CJR, Golbus MS, Callen PW, Filly RA, Smith JC: Investigation of nonimmune hydrops fetalis. *Am J Obstet Gynecol* 150:805, 1984
- Jauniaux E, Van Maldergem L, De Munter C, Moscoso G, Gillerot Y: Nonimmune hydrops fetalis associated with genetic abnormalities. *Obstet Gynecol* 75:568, 1990
- Arcasoy MO, Gallagher PG: Hematologic disorders and nonimmune hydrops fetalis. *Semin Perinatol* 19:502, 1995
- Thumasathit B, Nondasuta A, Silpisornkosol S, Lousuebsakul B, Unchalipongse P, Mangkornkanok M: Hydrops fetalis associated with Bart's hemoglobin in northern Thailand. *J Pediatr* 73:132, 1968
- Liang ST, Wong VCW, So WWK, Ma HK, Chan V, Todd D: Homozygous  $\alpha$ -thalassaemia: Clinical presentation, diagnosis and management. A review of 46 cases. *Br J Obstet Gynaecol* 92:680, 1985
- Tan SL, Tseng AMP, Thong P-W: Bart's hydrops fetalis—Clinical presentation and management—An analysis of 25 cases. *Aust NZ J Obstet Gynaecol* 3:233, 1989
- Ko T-M, Hsieh F-J, Hsu P-M, Lee T-Y: Molecular characterization of severe  $\alpha$ -thalassemias causing hydrops fetalis in Taiwan. *Am J Med Genet* 39:317, 1991
- Weatherall DJ: The thalassaemias. *BMJ* 314:1675, 1997
- Higgs DR, Vickers MA, Wilkie AOM, Pretorius I-M, Jarman AP, Weatherall DJ: A review of the molecular genetics of the human  $\alpha$ -globin gene cluster. *Blood* 73:1081, 1989
- Higgs DR:  $\alpha$ -Thalassaemia. *Baillieres Clin Haematol* 6:117, 1993
- Weatherall DJ: The thalassaemias, in Beutler E, Lichtman MA, Coller BS, Kipps TJ (eds): *Williams Hematology*, (ed 5). New York, NY, McGraw-Hill, 1987, p 581
- Huisman THJ, Carver MFH, Baysal E: A syllabus of thalassaemia mutations. Augusta, GA, The Sickle Cell Foundation, 1997
- Lie-Injo LE, Hie JB: Hydrops foetalis with a fast-moving haemoglobin. *Br Med J* 2:1649, 1960
- Lie-Injo LE: Alpha-chain thalassaemia and hydrops fetalis in Malaya: Report of five cases. *Blood* 20:581, 1962
- Higgs DR, Wood WG, Jarman AP, Shapre J, Lida J, Pretorius IM, Ayyub H: A major positive regulatory region located far upstream of the human  $\alpha$ -globin gene locus. *Genes Dev* 4:1588, 1990
- Vyas P, Vickers MA, Simmons DL, Ayyub H, Craddock CF, Higgs DR: Cis-acting sequences regulating expression of the human  $\alpha$ -globin cluster lie within constitutively open chromatin. *Cell* 69:781, 1992
- Ottolenghi S, Lanyon WG, Paul J, Williamson R, Weatherall DJ, Clegg JB, Pritchard J, Pootrakul S, Boon WH: Gene deletion as the cause of  $\alpha$  thalassaemia. *Nature* 251:389, 1974
- Taylor JM, Dozy A, Kan YW, Varmus HE, Lie-Injo LE, Ganesan J, Todd D: Genetic lesion in homozygous  $\alpha$  thalassaemia (hydrops fetalis). *Nature* 251:392, 1974
- Lau Y-L, Chan L-C, Chan Y-Y A, Ha S-Y, Yeung C-Y, Waye JS, Chui DHK: Prevalence and genotypes of  $\alpha$ - and  $\beta$ -thalassaemia carriers in Hong Kong—Implications for population screening. *N Engl J Med* 336:1298, 1997
- Hsieh FJ, Ko TM, Chen HY: Hydrops fetalis caused by severe  $\alpha$ -thalassaemia. *Early Hum Dev* 29:233, 1992
- Modell B (ed): *Guidelines for the control of haemoglobin disorders*. World Health Organization Hereditary Diseases Programme, 1994
- Lemmens-Zyugulska M, Eigel A, Helbig B, Sanguanserm Sri T, Horst J, Flatz G: Prevalence of  $\alpha$ -thalassemias in northern Thailand. *Hum Genet* 98:345, 1996
- Fucharoen S, Winichagoon P: Hemoglobinopathies in Southeast Asia. *Hemoglobin* 11:65, 1987
- Monzon CM, Fairbanks VF, Burgert EO Jr, Sutherland JE, Elliot SC: Hematologic genetic disorders among Southeast Asian refugees. *Am J Hematol* 19:27, 1985
- Hofgärtner WT, Keefe SFW, Tait JF: Frequency of deletion  $\alpha$ -thalassaemia genotypes in a predominantly Asian-American population. *Am J Clin Pathol* 107:576, 1997
- Fischel-Ghodsian N, Vickers MA, Seip M, Winichagoon P, Higgs DR: Characterization of two deletions that remove the entire human  $\zeta$ - $\alpha$  globin gene complex ( $--^{THAI}$  and  $--^{FIL}$ ). *Br J Haematol* 70:233, 1988
- Waye JS, Eng B, Chui DHK: Identification of an extensive  $\zeta$ - $\alpha$  globin gene deletion in a Chinese individual. *Br J Haematol* 80:378, 1992
- Lee L, Hsia YE, Donlon TA, Hunt JA: Determination of the breakpoints of the common  $\alpha$ -thalassaemia deletion in Filipinos in Hawaii. In preparation, 1998
- Eng B, Chui DHK, Waye JS: Unpublished observation, January 1998
- Hsia YE: Personal communication, October 1997
- Sophocleous T, Higgs DR, Aldridge B, Trent RJ, Pressley L, Clegg JB, Weatherall DJ: The molecular basis for the haemoglobin Bart's hydrops fetalis syndrome in Cyprus. *Br J Haematol* 47:153, 1981
- Nicholls RD, Higgs DR, Clegg JB, Weatherall DJ:  $\alpha^0$ -thalassaemia due to recombination between the  $\alpha 1$ -globin gene and an *AluI* repeat. *Blood* 65:1434, 1985
- Diamond MP, Cotgrove I, Parker A: Case of intrauterine death due to  $\alpha$ -thalassaemia. *Br Med J* 2:278, 1965
- Sharma RS, Yu V, Walters WAW: Haemoglobin Bart's hydrops fetalis syndrome in an infant of Greek origin and prenatal diagnosis of alpha-thalassaemia. *Med J Aust* 2:404, 1979
- Pressley L, Higgs DR, Clegg JB, Weatherall DJ: Gene deletions in  $\alpha$  thalassaemia prove that 5'  $\zeta$  locus is functional. *Proc Natl Acad Sci USA* 77:3586, 1980
- Kattamis C, Metaxotou-Mavromati A, Tsiarta E, Metaxatou C, Wasi P, Wood WG, Pressley L, Higgs DR, Clegg JB, Weatherall DJ: Haemoglobin Bart's hydrops syndrome in Greece. *Br Med J* 281:268, 1980
- Galanello R, Sanna MA, Maccioni L, Gasperini D, Melis MA, Rosatelli C, Monni G, Cao A: Fetal hydrops in Sardinia: Implications for genetic counselling. *Clin Genet* 38:327, 1990
- Gurgey A, Altay Ç, Beksaç MS, Bhattacharya R, Kutlar F, Huisman THJ: Hydrops fetalis due to homozygosity for  $\alpha$ -thalassaemia-1,-( $\alpha$ )-20.5 kb: The first observation in a Turkish family. *Acta Haematol* 81:169, 1989
- Pirastu M, Lee KY, Dozy AM, Kan YW, Stamatoyannopoulos G, Hadjiminis MG, Zachariades Z, Angius A, Furbetta M, Rosatelli C, Cao A: Alpha-thalassaemia in two Mediterranean populations. *Blood* 60:509, 1982
- Dozy AM, Kan YW, Embury SH, Mentzer WC, Wang WC, Lubin B, Davis JR Jr, Koenig HM:  $\alpha$ -Globin gene organisation in blacks precludes the severe form of  $\alpha$ -thalassaemia. *Nature* 280:605, 1979
- Halbrecht I, Shabtai F: An unusual case of hemoglobin Bart's hydrops fetalis. *Acta Genet Med Gemellol (Roma)* 24:97, 1975
- Chan V, Chan TK, Liang ST, Ghosh A, Kan YW, Todd D: Hydrops fetalis due to an unusual form of Hb H disease. *Blood* 66:224, 1985
- Trent RJ, Wilkinson T, Yakas J, Carter J, Lammi A, Kronenberg H: Molecular defects in 2 examples of severe Hb H disease. *Scand J Haematol* 36:272, 1986



41. Chan V, Chan V W-Y, Tang M, Lau K, Todd D, Chan TK: Molecular defects in Hb H hydrops fetalis. *Br J Haematol* 96:224, 1997
42. Peschle C, Mavilio F, Carè A, Migliaccio G, Migliaccio AR, Salvo G, Samoggia P, Petti S, Guerriero R, Marinucci M, Lazzaro D, Russo G, Mastroberardino G: Haemoglobin switching in human embryos: Asynchrony of  $\zeta \rightarrow \alpha$  and  $\epsilon \rightarrow \gamma$ -globin switches in primitive and definitive erythropoietic lineage. *Nature* 313:235, 1985
43. Hollenberg MD, Kaback MM, Kazazian HH Jr: Adult hemoglobin synthesis by reticulocytes from the human fetus at midtrimester. *Science* 174:698, 1971
44. Kazazian HH Jr, Woodhead AP: Hemoglobin A synthesis in the developing fetus. *N Engl J Med* 289:58, 1973
45. Bunn HF: Human hemoglobins: Normal and abnormal; methemoglobinemia, in Nathan DG, Oski FA (eds): *Hematology of Infancy and Childhood*, (ed 4). Toronto, Canada, Saunders, 1993, p 711
46. Weatherall DJ, Clegg JB, Boon WH: The haemoglobin constitution of infants with the haemoglobin Bart's hydrops foetalis syndrome. *Br J Haematol* 18:357, 1970
47. Todd D, Lai MCS, Beaven GH, Huehns ER: The abnormal haemoglobins in homozygous  $\alpha$ -thalassaemia. *Br J Haematol* 19:27, 1970
48. Randhawa ZI, Jones RT, Lie-Injo LE: Human hemoglobin Portland II ( $\zeta_2\beta_2$ ): Isolation and characterization of Portland hemoglobin components and their constituent globin chains. *J Biol Chem* 259:7325, 1984
49. Kutlar F, Reese AL, Hsia YE, Kleman KM, Huisman THJ: The types of hemoglobins and globin chains in hydrops fetalis. *Hemoglobin* 13:671, 1989
50. Tuchinda S, Nagai K, Lehmann H: Oxygen dissociation curve of haemoglobin Portland. *FEBS Lett* 49:390, 1975
51. Weatherall DJ: The thalassaemias, in Stamatoyannopoulos G, Nienhuis AW, Majerus PW, Varmus H (eds): *The Molecular Basis of Blood Disease*, (ed 2). Toronto, Canada, Saunders, 1994, p 167
52. Ghosh A, Tang MHY, Lam YH, Fung E, Chan V: Ultrasound measurement of placental thickness to detect pregnancies affected by homozygous  $\alpha$ -thalassaemia-1. *Lancet* 344:988, 1994
53. Nakayama R, Yamada D, Steinmiller V, Hsia E, Hale RW: Hydrops fetalis secondary to Bart hemoglobinopathy. *Obstet Gynecol* 67:176, 1986
54. Guy G, Coady DJ, Jansen V, Snyder J, Zinberg S:  $\alpha$ -thalassaemia hydrops fetalis: Clinical and ultrasonographic considerations. *Am J Obstet Gynecol* 153:500, 1985
55. Abuelo DN, Forman EN, Rubin LP: Limb defects and congenital anomalies of the genitalia in an infant with homozygous  $\alpha$ -thalassaemia. *Am J Med Genet* 68:158, 1997
56. Harmon JV Jr, Osathanondh R, Holmes LB: Symmetrical terminal transverse limb defects: Report of a twenty-week fetus. *Teratology* 51:237, 1995
57. Chitayat D, Silver MM, O'Brien K, Wyatt P, Wayne JS, Chui DHK, Babul R, Thomas M: Limb defects in homozygous  $\alpha$ -thalassaemia: Report of three cases. *Am J Med Genet* 68:162, 1997
- 57a. Lam YH, Tang MHY, Sin SY, Ghosh A, Lee CP: Limb reduction defects in fetuses with homozygous  $\alpha$ -thalassaemia. *Prenat Diagn* 17:1143, 1997
58. Mehr DS, Rector JT, Ngo K-Y: Pathological case of the month. Hydrops fetalis secondary to homozygous  $\alpha$ -thalassaemia-1 (Bart's hemoglobinopathy). *Arch Pediatr Adolesc Med* 148:1313, 1994
59. Bowman E, Watts J, Burrows R, Chui DHK: Hemoglobin Barts hydrops fetalis syndrome. *Haematologia (Budap)* 20:125, 1987
60. Wayne JS, Eng B, Cai S-P, Patterson M, Smith J, Tang W, Chui DHK: Carrier detection and prenatal diagnosis of hemoglobinopathies in Ontario. *Clin Invest Med* 16:358, 1993
61. Petrou M, Brugiattelli M, Old J, Hurley P, Ward RHT, Wong KP, Rodeck C, Modell B: Alpha thalassaemia hydrops fetalis in the UK: The importance of screening pregnant women of Chinese, other South East Asian and Mediterranean extraction for alpha thalassaemia trait. *Br J Obstet Gynaecol* 99:985, 1992
62. Beaudry MA, Ferguson DJ, Pearse K, Yonofsky RA, Rubin EM, Kan YW: Survival of a hydropic infant with homozygous  $\alpha$ -thalassaemia-1. *J Pediatr* 108:713, 1986
63. Bianchi DW, Beyer EC, Stark AR, Saffan D, Sachs BP, Wolfe L: Normal long-term survival with  $\alpha$ -thalassaemia. *J Pediatr* 108:716, 1986
64. Jackson DN, Strauss AA, Groncy PK, Bianchi DW, Akabutu J: Outcome of neonatal survivors with homozygous  $\alpha$ -thalassaemia. *Pediatr Res* 27:266A, 1990 (abstr)
65. Lam T-K, Chan V, Fok T-F, Li C-K, Feng C-S: Long-term survival of a baby with homozygous alpha-thalassaemia-1. *Acta Haematol* 88:198, 1992
66. Carr S, Rubin L, Dixon D, Star J, Dailey J: Intrauterine therapy for homozygous  $\alpha$ -thalassaemia. *Obstet Gynecol* 85:876, 1995
67. Naqvi A, Wayne JS, Morrow R, Nisbet-Brown E, Olivieri NF: Normal development of an infant with homozygous  $\alpha$ -thalassaemia. *Blood* 90:132a, 1997 (abstr)
68. Brittenham GM, Griffith PM, Nienhuis AW, McLaren CE, Young NS, Tucker EE, Allen CJ, Farrell DE, Harris JW: Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassaemia major. *N Engl J Med* 331:567, 1994
69. Olivieri NF, Nathan DG, MacMillian JH, Wayne AS, Liu PP, McGee A, Martin M, Koren G, Cohen AR: Survival in medically treated patients with homozygous  $\beta$ -thalassaemia. *N Engl J Med* 331:574, 1994
70. Dover GJ, Valle D: Therapy for  $\beta$ -thalassaemia—A paradigm for the treatment of genetic disorders. *N Engl J Med* 331:609, 1994
71. Corral E, Pérez N, Gutiérrez C, Marzouka E: Hidrops fetal no immune asociado a talasemia familiar: Transfusion intravascular. *Rev Chil Obstet Ginecol* 59:224, 1994
72. Jones DRE, Bui T-H, Anderson EM, Ek S, Liu DTY, Ringden O, Westgren M: *In utero* haematopoietic stem cell transplantation: Current perspectives and future potential. *Bone Marrow Transplant* 18:83, 1996
73. Diukman R, Golbus MS: In utero stem cell therapy. *J Reprod Med* 37:515, 1992
74. Westgren M, Ringden O, Eik-Nes S, Ek S, Anvret M, Brubakk A-M, Bui T-H, Giambona A, Kiserud T, Kjeldgaard A, Maggio A, Markling L, Seiger A, Orlandi F: Lack of evidence of permanent engraftment after in utero fetal stem cell transplantation in congenital hemoglobinopathies. *Transplantation* 61:1176, 1996
75. Eddleman K: In utero transfusion and transplantation in  $\alpha$ -thalassaemia, in Migliaccio AR (ed). *Stem Cell Therapy of Inherited Disorders*. Rome, Italy, 1996
76. Modell M, Modell B: Genetic screening for ethnic minorities. *BMJ* 300:1702, 1990
77. Chui DHK, Wayne JS, Chitayat D, Hutton EM: Screening for thalassaemia and sickle hemoglobin. *Can J Ob/Gyn & Women's Health Care* 5:453, 1993
78. Dumars KW, Boehm C, Eckman JR, Giardina PJ, Lane PA, Shafer FE for the Council of Regional Networks for Genetic Services (CORN): Practical guide to the diagnosis of thalassaemia. *Am J Med Genet* 62:29, 1996
- 78a. Lam YH, Ghosh A, Tang MHY, Chan V: The risk of  $\alpha$ -thalassaemia in offspring of  $\beta$ -thalassaemia carriers in Hong Kong. *Prenat Diagn* 17:733, 1997
79. Dacie JV, Lewis SM: *Practical Haematology* (ed 5). New York, NY, Churchill Livingstone, 1975, p 141
80. Chan LC, So JCC, Chui DHK: Comparison of haemoglobin H inclusion bodies with embryonic  $\zeta$  globin in screening for  $\alpha$  thalassaemia. *J Clin Pathol* 48:861, 1995
81. Chan AYY, So CKC, Chan LC: Comparison of the HbH inclusion test and a PCR test in routine screening for  $\alpha$  thalassaemia in Hong Kong. *J Clin Pathol* 49:411, 1996
82. Chui DHK, Wong SC, Chung S-W, Patterson M, Bhargava S, Poon M-C: Embryonic  $\zeta$ -globin chains in adults: A marker for

$\alpha$ -thalassaemia-1 haplotype due to a >17.5-kb deletion. *N Engl J Med* 314:76, 1986

83. Luo H-Y, Clarke BJ, Gaudie J, Patterson M, Liao S-K, Chui DHK: A novel monoclonal antibody based diagnostic test for  $\alpha$ -thalassaemia-1 carriers due to the ( $^{-SEA}$ ) deletion. *Blood* 72:1589, 1988

84. Tang W, Luo H-Y, Albitar M, Patterson M, Eng B, Waye JS, Liebhaber SA, Higgs DR, Chui DHK: Human embryonic  $\zeta$ -globin chain expression in deletion  $\alpha$ -thalassemias. *Blood* 80:517, 1992

85. Harada F, Ireland JH, Hsia YE, Chui DHK: Anti- $\zeta$  antibody screening for  $\alpha$ -thalassaemia using dried filter paper blood. *Biochem Med Metab Biol* 51:80, 1994

86. Luo H-Y, Deisseroth AB, Chui DHK: Human embryonic  $\zeta$ -globin gene expression in mouse-human hybrid erythroid cell lines. *Blood* 86:1212, 1995

87. Tang W, Luo H-Y, Eng B, Waye JS, Chui DHK: Immunocytological test to detect adult carriers of ( $^{-SEA}$ ) deletion  $\alpha$ -thalassaemia. *Lancet* 342:1145, 1993

88. Lebo RV, Saiki RK, Swanson K, Montano MA, Erlich HA, Golbus MS: Prenatal diagnosis of  $\alpha$ -thalassaemia by polymerase chain reaction and dual restriction enzyme analysis. *Hum Genet* 85:293, 1990

89. Chang J-G, Lee L-S, Lin C-P, Chen P-H, Chen C-P: Rapid diagnosis of  $\alpha$ -thalassaemia-1 of southeast Asia type and hydrops fetalis by polymerase chain reaction. *Blood* 78:853, 1991

90. Bowden DK, Vickers MA, Higgs DR: A PCR-based strategy to detect the common severe determinants of  $\alpha$  thalassaemia. *Br J Haematol* 81:104, 1992

91. Ko T-M, Tseng L-H, Hsieh F-J, Hsu P-M, Lee T-Y: Carrier detection and prenatal diagnosis of alpha-thalassaemia of Southeast Asian deletion by polymerase chain reaction. *Hum Genet* 88:245, 1992

92. Winichagoon P, Fucharoen S, Kanokpongsakdi S, Fukumaki Y: Detection of  $\alpha$ -thalassaemia-1 (Southeast Asian type) and its application for prenatal diagnosis. *Clin Genet* 47:318, 1995

93. Kattamis AC, Camaschella C, Sivera P, Surrey S, Fortina P: Human  $\alpha$ -thalassaemia syndromes: Detection of molecular defects. *Am J Hematol* 53:81, 1996

94. Old J: Haemoglobinopathies. *Prenat Diagn* 16:1181, 1996

95. Ko T-M, Tseng L-H, Hwa H-L, Hsu P-M, Li S-F, Chu J-Y, Lu P-J, Lee T-Y, Chuang S-M: Misdiagnosis of homozygous alpha-thalassaemia 1 may occur if polymerase chain reaction alone is used in prenatal diagnosis. *Prenat Diagn* 17:505, 1997

96. Cheung M-C, Goldberg JD, Kan YW: Prenatal diagnosis of sickle cell anaemia and thalassaemia by analysis of fetal cells in maternal blood. *Nature Genet* 14:264, 1996

97. Williamson B: Towards non-invasive prenatal diagnosis. *Nature Genet* 14:239, 1996

98. Ghosh A, Tang MHY, Liang ST, Ma HK, Chan V, Chan TK: Ultrasound evaluation of pregnancies at risk for homozygous  $\alpha$ -thalassaemia-1. *Prenat Diagn* 7:307, 1987

99. Saltzman DH, Frigoletto FD, Harlow BL, Barss VA, Benacerraf BR: Sonographic evaluation of hydrops fetalis. *Obstet Gynecol* 74:106, 1989

100. Kanokpongsakdi S, Fucharoen S, Vatanasiri C, Thonglairoam V, Winichagoon P, Manassakorn J: Ultrasonographic method for

detection of haemoglobin Bart's hydrops fetalis in the second trimester of pregnancy. *Prenat Diagn* 10:809, 1990

101. Tongsong T, Wanapirak C, Srisomboon J, Piyamongkol W, Sirichotiyakul S: Antenatal sonographic features of 100 alpha-thalassaemia hydrops fetalis fetuses. *J Clin Ultrasound* 24:73, 1996

102. Lam YH, Ghosh A, Tang MHY, Lee CP, Sin SY: Second-trimester hydrops fetalis in pregnancies affected by homozygous  $\alpha$ -thalassaemia-1. *Prenat Diagn* 17:267, 1997

103. Ko T-M, Tseng L-H, Hsu P-M, Hwa H-L, Lee T-Y, Chuang S-M: Ultrasonographic scanning of placental thickness and the prenatal diagnosis of homozygous alpha-thalassaemia 1 in the second trimester. *Prenat Diagn* 15:7, 1995

104. Lam YH, Ghosh A, Tang MHY, Lee CP, Sin SY: Early ultrasound prediction of pregnancies affected by homozygous  $\alpha$ -thalassaemia-1. *Prenat Diagn* 17:327, 1997

105. Lam YH, Tang MHY: Prenatal diagnosis of haemoglobin Bart's disease by cordocentesis at 12-14 weeks' gestation. *Prenat Diagn* 17:501, 1997

106. Kan YW, Allen A, Lowenstein L: Hydrops fetalis with alpha thalassaemia. *New Engl J Med* 276:18, 1967

107. Beris P, Darbellay R, Dornier C, Hochmann A, Miescher P: Prenatal diagnosis of thalassaemia and hemoglobinopathies in Switzerland. *Eur J Haematol* 46:163, 1991

108. Legras B, Lucas-Clerc C, Doualin G, Audiau C, Ruelland A, Vialard J, Cloarec L: Découverte fortuite d'une  $\alpha\alpha$  thalassémie homozygote chez un foetus de vingt-et-une semaines. *Pathol Biol* 39:50, 1991

109. Hofstaetter C, Gonser M, Goelz R: Perinatal case report of unexpected thalassaemia Hb Bart. *Fetal Diagn Ther* 8:418, 1993

109a. Hartevelde KL, Losekoot M, Heister AJGAM, Van der Wielen M, Giordano PC, Bernini LF:  $\alpha$ -Thalassaemia in the Netherlands: A heterogeneous spectrum of both deletions and point mutations. *Hum Genet* 100:465, 1997

110. Pearson HA, Shanklin DR, Brodine C: Alpha-thalassaemia as cause of nonimmunological hydrops. *Am J Dis Child* 109:168, 1965

111. Dozy AM, Forman EN, Abuelo DN, Barsel-Bowers G, Mahoney MJ, Forget BG, Kan YW: Prenatal diagnosis of homozygous  $\alpha$ -thalassaemia. *JAMA* 241:1610, 1979

112. Boer HR, Anido G: Hydrops fetalis caused by Bart's hemoglobin. *South Med J* 72:1623, 1979

113. Miller PD, Smith BC, Marinoff DN: Theca-lutein ovarian cysts associated with homozygous  $\alpha$ -thalassaemia. *Am J Obstet Gynecol* 157:912, 1987

114. Maberry MC, Klein VR, Boehm C, Warren TC, Gilstrap LC III: Alpha-thalassaemia: Prenatal diagnosis and neonatal implications. *Am J Perinatol* 7:356, 1990

115. Manoukian AA, Bhagavan NV, Oshiro TH, Scottolini AG: Concentrations of alpha-fetoprotein increased in maternal serum but normal in amniotic fluid in pregnancy involving hemoglobin Bart's disease. *Clin Chem* 36:822, 1990

116. Feng C-S, Tsoi W-C: A survey of pregnancies that ended in haemoglobin Bart's hydrops foetalis and Cooley's anaemia. *HKMJ* 1:22, 1995

117. Liu T-C, Lin S-F, Yang T-Y, Lee J-P, Chen T-P, Chang J-G: Prenatal diagnosis of thalassaemia in the Chinese. *Am J Hematol* 55:65, 1997



**blood**<sup>®</sup>

1998 91: 2213-2222

## **Hydrops Fetalis Caused by $\alpha$ -Thalassemia: An Emerging Health Care Problem**

David H.K. Chui and John S. Wayne

---

Updated information and services can be found at:  
<http://www.bloodjournal.org/content/91/7/2213.full.html>

Articles on similar topics can be found in the following Blood collections  
[Review Articles](#) (738 articles)

---

Information about reproducing this article in parts or in its entirety may be found online at:  
[http://www.bloodjournal.org/site/misc/rights.xhtml#repub\\_requests](http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests)

Information about ordering reprints may be found online at:  
<http://www.bloodjournal.org/site/misc/rights.xhtml#reprints>

Information about subscriptions and ASH membership may be found online at:  
<http://www.bloodjournal.org/site/subscriptions/index.xhtml>