Alpha-thalassemia syndromes: from clinical and molecular diagnosis to bedside management

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A B S T R A C T

Heterozygotes for α^0-and α^+-thalassemias are usually asymptomatic or have microcytic-hypochromic red blood cells. Interactions of α^0-and α^+-thalassemias result in a non-fatal form of alpha-thalassemia syndrome; hemoglobin H (Hb H) disease. Patients with this condition present with a diverse clinical severity, from mild to moderate severity, included in the broader syndrome of non-transfusion dependent thalassemia (NTDT). In general, patients with non-deletional (--/$\alpha^T\alpha$) Hb H are usually more severe than deletional Hb H (--/-α) types. Moreover, certain non-deletional Hb H patients have the most severe phenotype, referred to as Hb H hydrops fetalis. In these rare cases, intrauterine and neonatal complications develop with hydropic features. These patients require regular blood transfusion for survival similar to patients with beta(β)-thalassemia major. Other mechanisms beside imbalanced globin synthesis might influence the Hb H disease pathophysiology resulting in heterogeneous clinical phenotypes. Hb Bart's hydrops fetalis characterized by a complete loss of all α globin loci (--/--) usually leads to death *in utero* or soon after birth. Due to advanced perinatal and neonatal care, the number of surviving Hb Bart's hydrops is increasing, raising concerns regarding the long-term outcome, in particular cognitive and neurological development. Although stem cell transplantation offers a curative measure for these severe α-thalassemia syndromes, its application has been limited by donor availability. Management guidelines for α-thalassemia syndromes are proposed here.

Learning goals

At the conclusion of this activity, participants should be able to:

- provide an understanding of the molecular basis underlying α-thalassemia and how interactions of α-thalassemia genes give rise to syndromes with different clinical phenotypes;
- acquire the ability to clinically diagnose and interpret relevant hematology laboratory and molecular studies in order to correctly identify the different types of α-thalassemia syndromes;
- provide an appropriate management plan, from supportive care, blood transfusion, iron chelation, up to stem cell transplantation, for patients with α-thalassemia syndromes, with special emphasis on Hb H disease.

Introduction

Hemoglobin (Hb) is a tetramer of two α-like and two β-like globin chains which are covalently linked to heme, the oxygen-binding molecule.[1,2] In normal adult erythropoiesis, approximately 95% of the Hb produced is Hb A ($\alpha 2\beta 2$), followed by approximately 2%-3% Hb A2 ($\alpha 2\delta 2$) and less than 1% fetal Hb (Hb F, $\alpha 2\gamma 2$).[1] The α globin gene cluster is located on the subtelomeric region of the short arm of chromosome 16 (16p 13.3) and consists of four functional genes, from 5' to 3': *HBZ* (OMIM 142310), *HBA2* (OMIM 141850), *HBA1* (OMIM 141800), and *HBQ1* (OMIM 142240).[3] These genes produce ζ, α and θ globin chains, respectively, and are under the control of the upstream regulatory sequences, a multispecies conserved, non-coding regulatory sequence (MCS-R 1 and 2) (see Figure 1 and review in Higgs[4]). The β globin gene cluster is located on chromosome 11 p15.4 and composed of five functional genes, from 5' to 3': *HBE1* (OMIM 142100), *HBG2* (OMIM 142250), *HBG1* (OMIM 142200), *HBD* (OMIM 142000), and *HBB* (OMIM 141900).[1] These genes encode ε, Gγ, Aγ, δ, and β globin chains, respectively. Similar to the α-globin gene cluster, a stage-specific temporal expression of these β-like globin chains is under the control of an upstream regulatory region, known as the β globin locus control region (LCR).[5] During erythroid development, from embryonic to fetal and adult hematopoiesis, a precise co-ordinated expression of both globin clusters is required to generate a balanced and adequate amount of stage-specific hemoglobins required for the red blood cell function. This process is highly critical since 200 billion red blood cells are produced daily to support continuous oxygen flow and supply.[1]

A great deal has been learnt about the normal regulation of globin gene expression from the analysis of naturally occurring mutations of the globin clusters, which cause α and β thalassemia.[6] Over the last 40 years, more than 120 mutations that cause α thalassemia and over 270 mutations that cause β thalassemia

have been characterized.[1] Thalassemia most frequently results from deletions or point mutations which affect the normal structures of the α and β globin genes.[2] These mutations fall into three main groups. First, there are deletions of the structural genes that are a particularly common cause of α thalassemia and, in a few cases, of β thalassemia. Second, point mutations of the structural genes and their critical elements, which in contrast are extremely common in β thalassemia (>220 different mutations) and less common in α thalassemia. Third, rare deletions involving the regulatory elements (MCS-R 1 and 2 and β-LCR, see below).[2] Studies of such natural mutations that can inactivate or severely down-regulate gene expression provide important insights into all aspects of gene structure regulation, including transcription and mRNA processing. The importance of promoter and enhancer elements, the role of upstream and downstream untranslated region (UTR) in mRNA transcription, stabilizing nascent mRNA and the translation process were also derived from studies of thalassemia. These mutations thus generate 'natural models' which help our understanding of globin gene expression.[2]

Molecular basis of α-thalassemia

There are two copies of the α globin gene per haploid genome, annotated αα/αα. The α2 gene lies upstream of the α1 gene and is expressed 2-3 fold more than the α1 gene. Alpha-thalassemia syndromes are remarkable for their variable molecular basis and phenotypic diversity depending on the degree of α globin deficit according to the number of the affected α globin genes.[2-4, 7-9]

In α^0 thalassemia (a condition in which α globin expression from one chromosome is completely abolished), both of the linked α globin genes are lost (--/αα) due to deletions that involve part or the entire α globin gene cluster (Figure 1). Heterozygotes for α^0 thalassemia are clinically normal but have a mild hypochromic, microcytic anemia (mean cell volume, MCV, <78 fL; mean corpuscular hemoglobin, MCH, <27 pg).[10,11] Other molecular mechanisms that can result in a similar degree of the α-globin deficit akin to that of α^0-thalassemia include: 1) upstream deletions that remove the major regulatory elements of the α globin cluster;[12-14] 2) an interstitial deletion (>18 kb, ZF deletion) that removes only the α1 gene but causes *de novo* methylation and downregulation of the remaining α2 gene;[15] and 3) large deletions that extend beyond the α globin gene cluster, identified in patients with dysmorphism and alpha-thalassemia mental retardation syndrome (ATR-16)[16] (shown in Figure 1 and comprehensively reviewed by Higgs[4]).

In the less severe condition (α^+-thalassemia), the α globin expression from one chromosome is reduced but not abolished. There are two types of α^+-thalassemia; deletional α^+ and non-deletional α^+-thalassemia.[4] The high

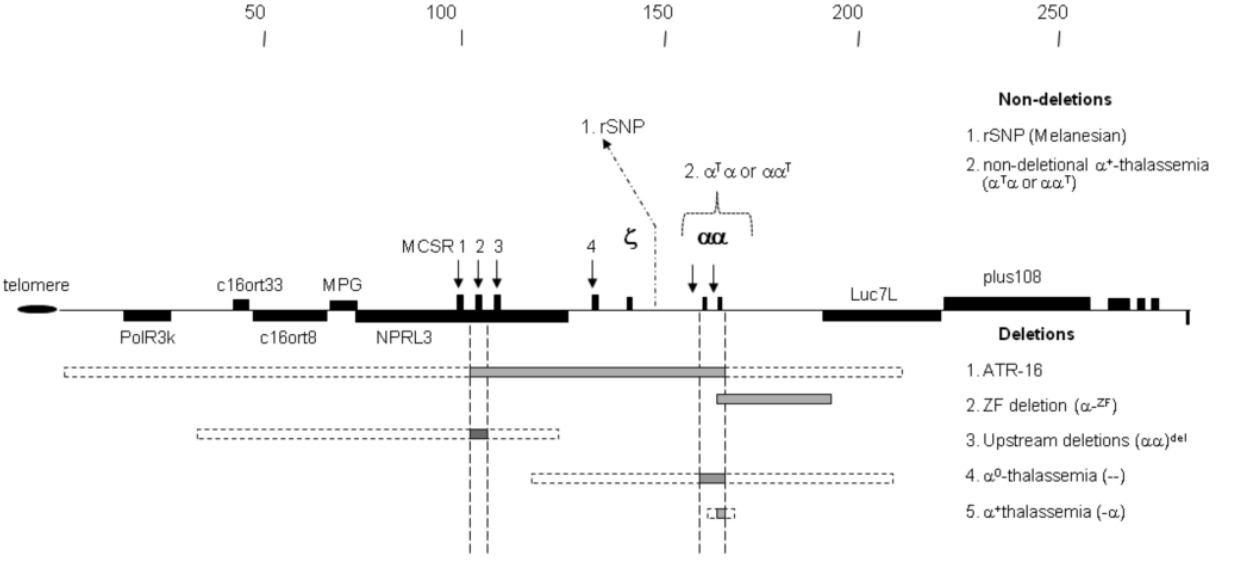


Figure 1. Summary of all reported deletional and non-deletional mutations causing a significant reduction of the α-globin gene expression and α^0- and α^+-thalassemia phenotypes. A schematic diagram of the subtelomeric region (black oval) of chromosome 16 (16p13.3) showing the human α-globin cluster (5'- ζ-α2-α1-3') flanked by ubiquitously expressed genes (as denoted with gene annotations above and below the line by Higgs[4]). Two major molecular mechanisms of α-thalassemia; deletional and non-deletional mutations are demonstrated (see text). Below the line, several gray boxes showed the critical regions removed by different deletions that involved (1). A multispecies conserved non-coding regulatory sequence (MCS-R2) essential for α-globin expression, (2). Single or both linked α-globin genes and (3). A region 3' to the α1 gene that caused epigenetic dysregulation in ZF deletion. ATR-16 is the large deletion that extended beyond the α-globin cluster and removed all critical regions. Above the line, two types of non-deletional mutations are shown; the rSNP; regulatory single nucleotide polymorphism or Melanesian mutation and the conventional nucleotide mutations involving coding sequences of either α2 or α1 genes. Black arrows show erythroid specific hypersensitive sites along the α-globin cluster.

homology of two α globin loci and local chromosomal constraints make the α globin cluster vulnerable to homologous recombination.[17] The majority of α^+-thalassemias results from deletions removing either the α2 gene, leaving the α1 gene intact ($-\alpha^{4.2}$ or 4.2 kb-deletion) or part of both α2 and α1 genes, generating a new hybrid α globin gene ($-\alpha^{3.7}$ or 3.7 kb-deletion) (Figure 1).[4,18] Both types of deletions have been found worldwide with a few others, such as 3.5 kb-deletion, found at a lower incidence.[9]

Less commonly, α^+ thalassemia results from mutations in one or a few nucleotides in critical regions of the α genes usually, but not always, affecting the highly expressed α2 gene ($\alpha^T\alpha$) rather than the α1 gene ($\alpha\alpha^T$). This is called 'non-deletional α thalassemia' and more than 70 different non-deletional mutations have been reported so far (as regularly up-dated at the globin gene server. Available from: http://globin.cse.psu.edu/).[9]

Recently, De Gobbi and Viprakasit *et al.* have described a regulatory single nucleotide polymorphism (rSNP) located in the region in between the ζ and the α2 gene that creates a new GATA binding site as an underlying cause of α-thalassemia in Melanesian population.[14] This rSNP demonstrated a novel mechanism for downregulation of the downstream α globin genes, creation of a GATA site competes with the α-globin promoters in the interaction with the MSC-Rs and has a 'stealing effect' on the associated erythroid specific and basal transcription machinery.[4,14] Heterozygotes for this rSNP have the phenotypes similar to non-deletional α^+-thalassemia. In addition, homozygotes and compound heterozygotes of this rSNP and α^0-or α^+-thalassemia can cause clinical Hb H disease (V Viprakasit, unpublished data, 2008).

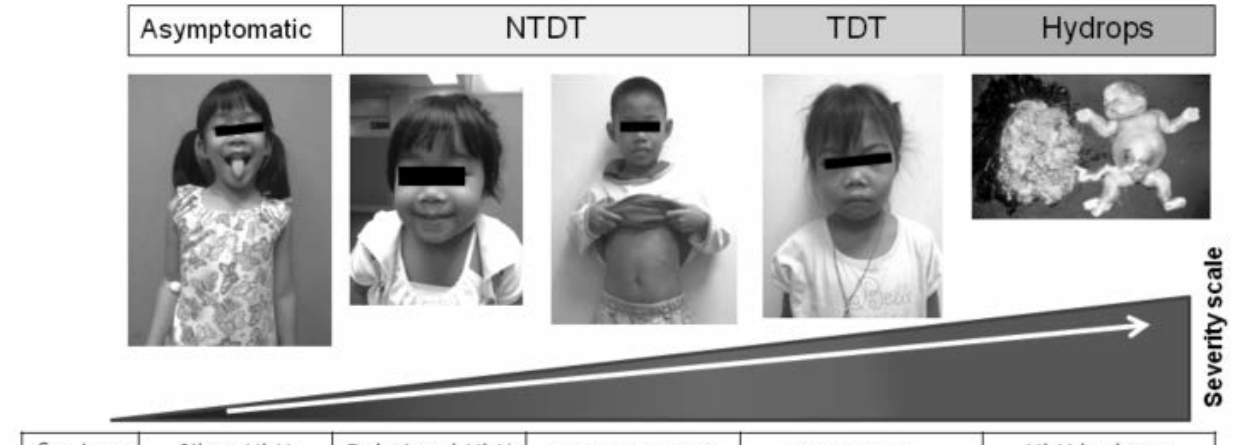
Molecular genotype-phenotype correlation in α-thalassemia syndromes

Hemoglobin (Hb) Bart's hydrops fetalis, characterized by a complete loss of four α-globin genes (--/--), is the most severe form of α-thalassemia syndromes.[8,19,20] The complete deficit of the α-globin chains is caused by deletional loss (homozygote or compound heterozygotes for the different molecular genotypes of α^0 thalassemia. A complete absence of α globin that is critically required for fetal erythropoiesis to produce Hb F ($\alpha_2\gamma_2$) causes intrauterine death of the affected fetus or death soon after birth. The free γ-globin chains in the fetus combine to form tetrameric hemoglobin known as Hb Bart's (γ_4) comprising 100% of total hemoglobin in affected patients. In general, such fetuses survive until the third trimester of pregnancy because they continue to produce small amounts of the embryonic Hbs Portland I ($\zeta_2\gamma_2$) and Portland II ($\zeta_2\beta_2$). However, at this stage they often have multiple congenital abnormalities and die of heart failure as a result of anemia.[20,21] Moreover, hydropic changes of the fetus can also result in several maternal complications including preeclampsia and hemorrhage.[9] In the past, the majority of Hb Bart's hydrops perished *in utero*. However, there are increasing reports of cases of Hb Bart's hydrops, which, with or without intrauterine intervention, had survived until delivery.[22] These patients require immediate care and effective neonatal resuscitation including blood transfusion during the neonatal period.

A loss of three out of four copies of the α-globin genes (--/-α) due to compound heterozygosity for α^0- and deletional α^+-thalassemia is the most common molecular mechanisms underlying Hb H disease.[2,18,20,23] The excess β-globin chains form tetrameric hemoglobin (β4) called Hb H. This classical form of deletional Hb H disease affects millions of people worldwide due to a high frequency of α-thalassemia alleles.[2,24] However, this condition is quite benign and may require the occasional blood transfusion during hemolytic episodes.[18,25,26] This α-thalassemia syndrome is the most common cause of non-transfusion dependent thalassemia (NTDT) around the world.[27] Interaction of rare mechanisms of α^0-thalassemia described above with α^+-thalassemia can also result in clinical Hb H disease.[13,28] Less commonly, non-deletional Hb H disease (--/$\alpha^T\alpha$ or --/$\alpha\alpha^T$) results from interactions of α^0- and non-deletional α^+-thalassemia. The common non-deletional mutations include; Hb Constant Spring ($\alpha^{CS}\alpha$, termination codon, TAA-CAA),[29] the most prevalent non-deletional α thalassemia identified to date in several countries, Hb Paksé ($\alpha^{PS}\alpha$, another termination codon mutation, TAA-TAT),[30] an initiation codon mutation (ATG to A-G),[31,32] Hb Quong Sze ($\alpha^{QS}\alpha$, codon 125, CTG-CCG)[33] and different types of polyadenylation site of the α 2 gene mutation including the $\alpha^{TSaudi}\alpha$ (AATAAA to AATAAG).[34] Patients with non-deletional Hb H disease have a more severe phenotype than deletional Hb H as demonstrated by the greater degree of anemia and jaundice, earlier presentation, greater degree of hepatosplenomegaly, greater need for blood transfusion, and splenectomy[25,26,35-43] In addition, non-deletional Hb H patients have higher levels of Hb H inclusion bodies and many of the patients with Hb H disease who have transfusion dependent thalassemia (TDT) or are thalassemia major (TM)-like fall into the non-deletional group.[18] The deficit in α globin expression in these patients appears to be greater than in deletional Hb H disease (--/-α). Sometimes, non-deletional mutations have additional deleterious effects on terminal erythroid differentiation and red cell metabolism.[44,45] These effects might include globin instability as in Hb Constant Spring (CS), Hb Paksé (PS), Hb Quong Sze and Hb Adana, $\alpha^{Adana}\alpha$, codon59,GGC-GAC, which results in a more severe phenotype. Other non-deletional mutations, such as those involving the initiation codon or splice site mutation, only reduce α-globin mRNA expression without generating unstable variants, and might not be as severe as the former ones.[46] These findings suggest that a precise molecular characterization will be required to provide appropriate counseling and a management plan for future patients. Nevertheless, there is considerable clinical diversity in both deletional (--/-α) and non-deletional (--/$\alpha^T\alpha$) Hb H disease which remains unexplained.[18,47] Until recently, mutations in erythroid specific transcription factor erythroid krüpple-like factor, EKLF or KLF-1, have been identified in several pedigrees of patients with Hb H disease with unexpectedly severe phenotype.[48] It is plausible that these *trans* acting mutations might play a role as major genetic modifiers in patients with α-thalassemia syndromes. Homozygotes for many types of non-deletional α^+ thalassemia ($\alpha^T\alpha/\alpha^T\alpha$) usually have a mild hypochromic, microcytic anemia with no detectable Hb H on electrophoresis whilst others may have small amounts of Hb H.[49-52] However, homozygotes for a mutation affecting the polyA addition site of the α2 gene, first described

in patients from Saudi Arabia, $\alpha^{TSaudi}\alpha$ consistently have Hb H disease of variable severity and 8.0%-26.6% Hb H detectable on electrophoresis.[53] In one pedigree from Turkey, homozygosity for the $\alpha^{Tsaudi}\alpha$ chromosome led to fetal loss raising questions for the reason for phenotypic discrepancies.[54] Similar unexplained findings were also observed in patients with homozygous Hb CS.[55]

The most severe viable form of α-thalassemia syndromes is Hb H hydrops, a transfusion-dependent Hb H disease that is caused by specific non-deletional α-thalassemia mutations.[34,56-59] The α globin expression is severely reduced but not absent in these rare infants with non-deletional Hb H disease ($--/\alpha^T\alpha$), a result being the profound anemia *in utero* (3.4-9.7 g/dL) and hydropic features, with 31-65% Hb Bart's. This clinical syndrome has been seen in patients with rare non-deletional mutations such as; $\alpha^{Cd\ 59Gly\text{-}Asp}\alpha$, $\alpha^{\Delta Cd\ 30}\alpha$,[12] $\alpha^{Cd\ 66\ Leu\text{-}Pro}\ \alpha$ and $\alpha^{Cd\ 35Ser\text{-}Pro}\alpha$.[57] In multiple affected pedigrees, this interaction resulted in fetal lethality in late gestation or in death in the early neonatal period,[59] whereas the few survivors had a severe transfusion-dependent type of Hb H disease. This suggests that additional environmental and genetic factors may modify the outcome of this clinical syndrome. For example the interaction of Hb Pak Num Po (PNP), a rare α1 gene mutation, with α^0-thalassemia (--/) results in transfusion-dependent phenotype[60] while interactions with either deletional ($-\alpha^{4.2}/$) or non-deletional α^+-thalassemias ($\alpha^{PS}\alpha/$) causes a non transfusion-dependent phenotype with variable severity.[61] Molecular genotyping of non-deletional α-thalassemia is of clinical importance and should be performed in all severe Hb H patients. Clinical heterogeneity of α-thalassemia syndromes from silent Hb H disease to Hb H hydrops and the associated genetic determinants are summarized in Figure 2.



Genotypes	Silent HbH	Deletional HbH	Non-deletional HbH	Transfusion – dependent HbH		HbH hydrops
1^0	$--/-\alpha$ $--/\alpha^T\alpha$	$--/-\alpha$	$--/\alpha^T\alpha$ $--/\alpha\alpha^T$	$--/-\alpha$	$--/\alpha^T\alpha$ $--/\alpha\alpha^T$ $\alpha^T\alpha/\alpha^T\alpha$	$--/\alpha^T\alpha$ $\alpha^T\alpha/\alpha^T\alpha$
2^0	β thal	+ β globin variants such as Hb E, Hb C, Hb S, homozygous Hb E, β thal/Hb E etc.		-		-
3^0	-			KLF-1		-

Figure 2. Heterogeneous clinical presentation and severity of patients with α-thalassemia syndromes from Hb H hydrops to silent Hb H disease. The table shows three levels of partially known genetic basis underlying the clinical heterogeneity of Hb H disease. Primary defects are based on the types of α-globin mutations (deletional and non-deletional α-thalassemias) and their interaction. The secondary level of genetic control is the co-inheritance of β-thalassemia[62-64] or β-hemoglobinopathy such as Hb E.[65,66] The presence of β-thalassemia generally causes more balanced globin synthesis resulting in a milder phenotype with possible absence of Hb H (silent Hb H), while inheritance of unstable β-globin variants in particular Hb E or homozygous Hb E causing AE Bart's and EF Bart's disease results in a more severe phenotype than simple deletional Hb H disease.[67] The tertiary level involves other genetic modifiers outside the globin gene clusters. At present, only KLF-1 was found to deteriorate the clinical course of patients with deletional and non-deletional Hb H disease.[48] Other genetic modifiers that might affect other complications such as bone disease (vitamin D receptor gene), iron overload (Hfe and others), jaundice and gall stone formation (UGT1A1 and others) are not shown and were reviewed previously.[18] NTDT: non-transfusion dependent thalassemia; TDT: transfusion-dependent thalassemia.

Diagnosis of α-thalassemia syndromes

Heterozygotes for single α gene deletions (-α/αα) are clinically and hematologically normal and cannot be diagnosed correctly without molecular and DNA studies while α^0-thalassemia traits can be diagnosed using aforementioned MCV and MCH cut offs, but not RBC and RDW values (Figure 3A). Patients with Hb H disease have hypochromic microcytic anemia with reticulocytosis similar to patients with β-thalassemia syndromes. Peripheral blood smear shows numerous target cells, aniso-poikilocytosis with polychromasia mimicking alterations found in β-thalassemia disease (Figure 3B).[18] Of note, patients with Hb H-Hb CS usually have numerous basophilic stippling positive red blood cells.[35] The key diagnostic marker is the presence of Hb H (from <2% to >25% of total Hb) in the peripheral blood that is visualized by using a special staining (brilliant cresyl blue) or by hemoglobin electrophoresis or chromatography.[8] It should be noted that due to the unstable nature of Hb H tetramer, the identification of this Hb species can be jeopardized by the quality and age of the blood samples; old blood or inappropriately stored samples could provide false negative results. Quantitation of Hb H might be problematic on some hemoglobin analysis platforms, such as high performance liquid chromatography (HPLC), since the instrument is not pre-set to detect and quantify Hb H species.[18] A new generation of capillary electrophoresis (CE) is better suited for measuring the amount and detection of Hb H in hemolysate.[68] Ultimately, a molecular diagnosis using DNA testing such as GAP-polymerase chain reaction (GAP-PCR) for common deletional α-thalassemias is

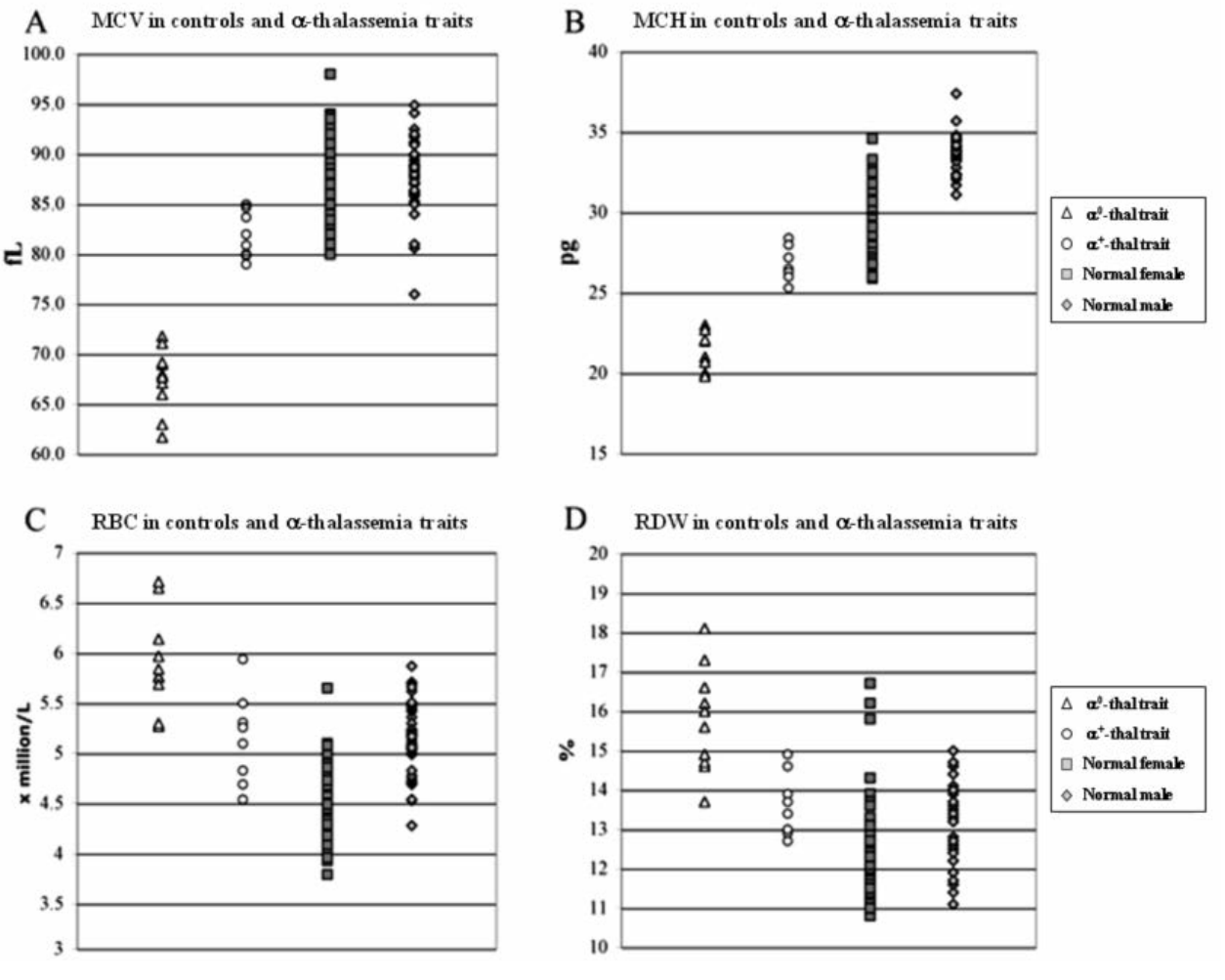


Figure 3A. Red blood cell indexes comparing between α^0-thalassemia traits (--/αα), deletional α^+-thalassemia (-α/αα) traits and normal age-matched controls. Red blood cell indexes composed of mean corpuscular volume (MCV), mean cell hemoglobin (MCH), red blood cell count (RBC), and red cell distribution width (RDW), n=350. Adapted from Viprakasit.[10]

highly recommended[69] in cases that are not easily diagnosed by complete blood count (CBC) and hemoglobin analysis. Detailed molecular techniques to identify α-globin gene defects have recently been reviewed.[9] Moreover, precise molecular characterization of either deletional or non-deletion Hb H disease including the type of the non-deletional mutations can be useful to roughly predict the clinical severity and provide some guidance for clinical management (Figure 4). Patients with non-deletional mutations should be closely followed up every 2-3 months in view of worsening clinical severity with age, while the clinical course in deletional Hb H patients is more stable and a regular follow up on a 4-6 monthly basis may be adequate.

Clinical management of α-thalassemia syndromes

Hb Bart's hydrops fetalis[8,19]

Only a few surviving Hb Bart's hydrops cases have been documented in the literature. It has been suggested that due to marked anemia in early gestation, Hb Bart's hydrops patients could suffer from other physical complications including limb anomalies, abnormal urogenital and most seriously, neurological development.[21,70] Moreover, all rescued Hb Bart's hydrops would be dependent on life-long transfusion. At Siriraj Hospital, Bangkok, Thailand, 5 surviving patients with Hb Bart's

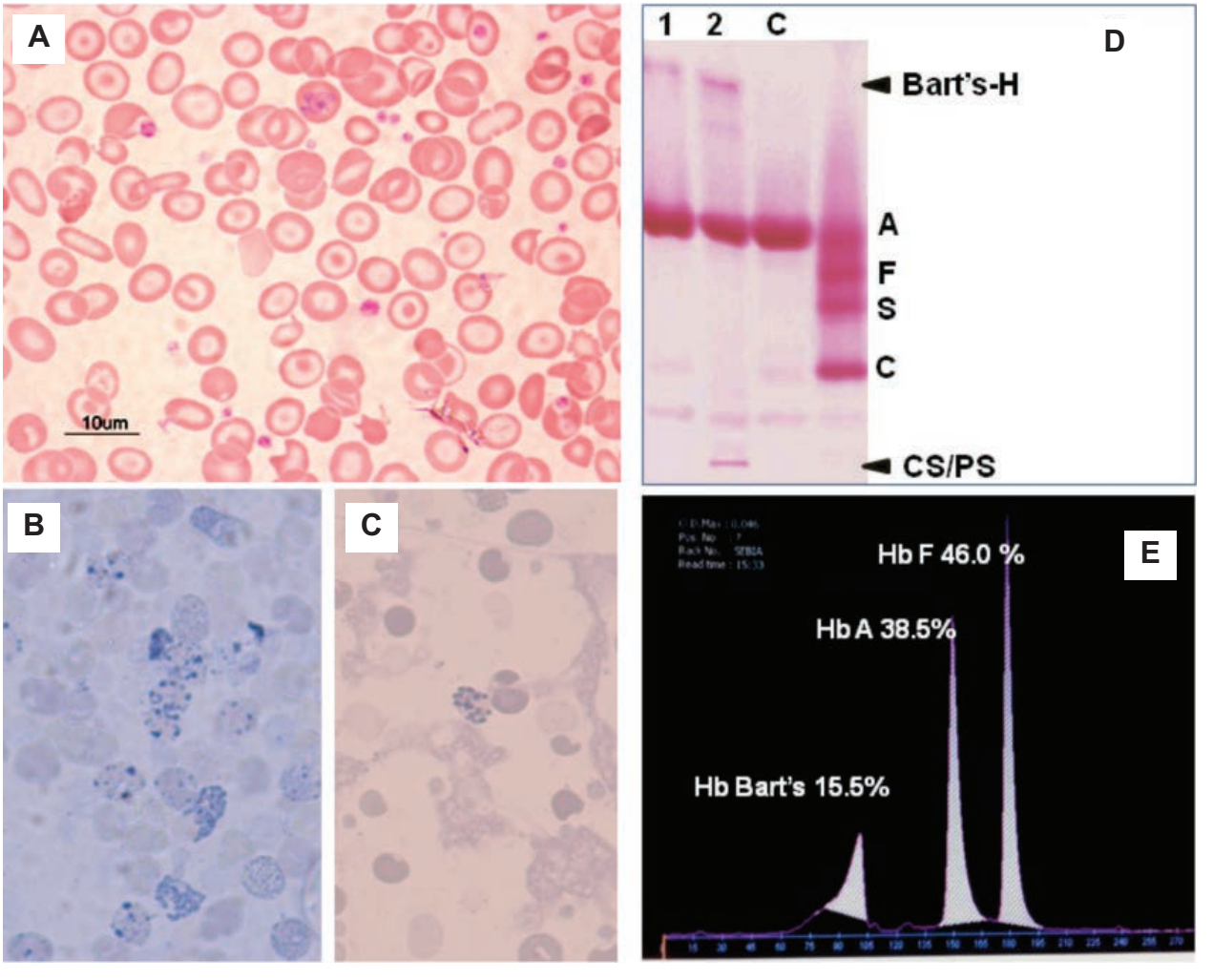


Figure 3B. (A) Peripheral blood smear shows marked hypochromic microcytosis with anisopoikilocytosis and numerous targets and fragmented red blood cells. (B) A supravital staining shows numerous HbH inclusion bodies with a golf-ball appearance in a patient with deletional Hb H ($--^{SEA}/-\alpha^{3.7}$). The presence of Hb H inclusion bodies can be rare in a patient who also co-inherited Hb E (AE Bart's disease; $--^{SEA}/-\alpha^{3.7}/\beta^{E}/\beta^{A}$), as shown in (C). (D) Hemoglobin analysis by cellulose acetate electrophoresis from a patient with deletional Hb H (1; $--^{SEA}/-\alpha^{4.2}$) and non-deletional Hb H (2; $--^{SEA}/\alpha^{CS}\alpha$) with the presence of Hb Bart's and Hb H. Using this analysis, the presence of a slow moving hemoglobin at the end of the strip suggests coinheritance of non-deletional mutation such as Hb Constant Spring or Hb Pakse'.[30] (E) Capillary electrophoresis (CE) of a cord blood sample from a patient with Hb H disease ($--^{SEA}/-\alpha^{3.7}$) at birth. The patient developed severe anemia and neonatal jaundice requiring blood transfusion. Hb Bart's is approximately 15%

hydrops are under regular transfusion. They had a wide range of associated anomalies, in particular of the limbs. However, none had delayed mental and/or neurological development (Vπ Viprakasit, unpublished data, 2013). These possible risks and associated complications must be well known and carefully weighted by physicians and patients' parents when deciding about rescuing affected fetus by intrauterine blood transfusion. Although successful stem cell transplantation in Hb Bart's hydrops has been reported from several centers using different sources of stem cells from matched and mismatched related bone marrow and cord blood to unrelated donors,[71-74] it is important to follow these 'cured' Hb Bart's hydrops patients on a long-term follow up, particularly with regard to their neurological development and cognitive function.

Hb H hydrops/transfusion dependent Hb H disease

Similar to surviving Hb Bart's hydrops patients, patients with Hb H hydrops or severe Hb H disease such as Hb PNP[60,61] or Hb Adana[59] become transfusion-dependent later in life. In contrast to other types of Hb H disease (see below), patients with this severe form of α-thalassemia seldom respond to splenectomy, and surgery should not be provided unless patients show clinical signs of hypersplenism. Regular transfusion with iron chelation therapy similar to protocols used in patients with β-thalassemia major seems a more appropriate treatment for Hb H hydrops. Recently, stem cell transplantation was performed to provide cure in a patient within this category owing to the fact that the transplantation-related morbidity and mortality is rather low, especially when an HLA-matched sibling donor is available.[61]

Hb H disease

In general, patients with Hb H disease have a rather mild anemia. The majority of these patients should receive supplementary folic acid (up to 5 mg/day), multivitamins including vitamin D, antioxidant (vitamin E 10 U/kg/day) and nutritional supplement (calcium and zinc) to support their increased bone marrow activity and increased oxidative stress.[18,75,76] However, clinical presentation in patients with Hb H disease can be heterogeneous and some might suffer more from clinical anemia, especially patients with non-deletional Hb H disease.

Patients under six years of age with clinical anemia (Figure 4) should receive regular blood transfusion with appropriate iron chelation therapy similar to those for patients with transfusion-dependent thalassemia (TDT).[77] Splenectomy after six years of age has been proven to be effective in patients with Hb H disease who have moderately severe phenotype,[78] but it is associated with increased risk of thrombosis and vasculopathy in later life. Splenectomy could restore transfusion independence in Hb H disease patients in the long term apart from

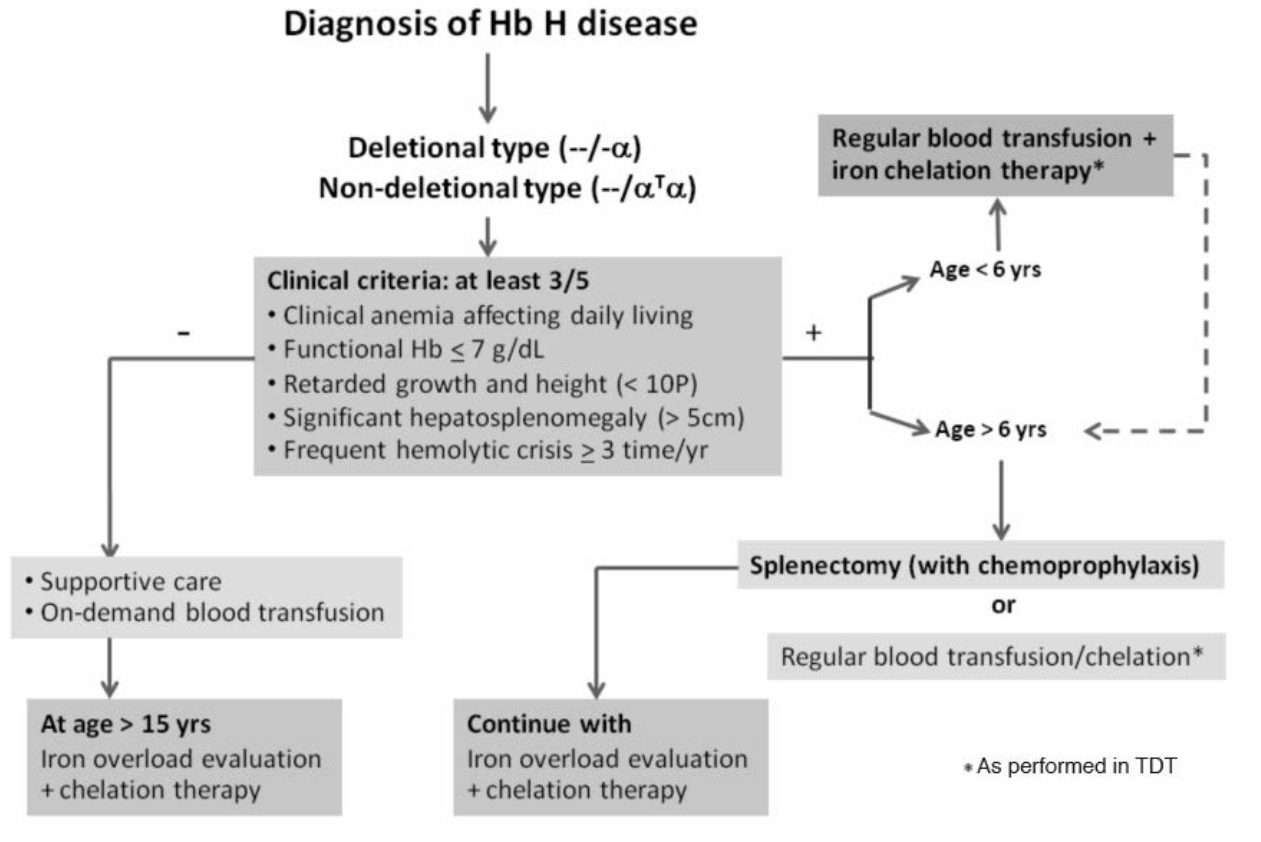


Figure 4. Management guideline in a new patient with Hb H disease. Diagnosis of Hb H disease requires a comprehensive hematology, hemoglobin and DNA analyses. [+] = with ≥3 and [-] = with <3 out of 5 criteria.

instances related to acute hemoglobin reduction during a hemolytic episode. Therefore, in a resource limited setting for a long-term safe blood transfusion support, splenectomy remains a standard of care for selected patients with Hb H disease.[77] This procedure should be performed after appropriate vaccination (pneumococcal and meningococcal vaccines) followed by antibiotic prophylaxis (penicillin V chemoprophylaxis; 250 mg/day twice a day for body weight over 20 kg for at least 3-5 years), anti-platelet drug using aspirin (80 mg/day) and regular vaccination booster every five years.[77]

During hemolytic crisis, the hemoglobin level in Hb H patients may drop significantly. Several factors, including infections and pyrexia (during or after), oxidative challenge, hypersplenism, or pregnancy may contribute to the hemolytic crisis.[79,80] Increased body temperature is the major mechanism generating Hb H inclusion bodies that can induce oxidative damage to the red blood cells and cause further extravascular hemolysis.[81,82] Moreover, if the acute hemolytic crisis is more profound with evidence of severe jaundice, hemoglobinemia and hemoglobinuria, it may result in renal damage and acute renal insufficiency.[83] This serious complication requires immediate intervention. In addition, patients with homozygous non-deletional mutations (especially Hb CS) also develop hemolytic crisis after infection as well.[52] An empirical antibiotic should be started immediately until the causative pathogens are identified. In the tropics, dengue hemorrhagic fever is probably one of the most lethal inter-current infections that cause hemolytic crisis in patients with Hb H disease.[83] Contrary to clinical dengue hemorrhagic fever or dengue shock syndrome in a normal child, Hb H patients have no evidence of hemoconcentration. They would, instead, develop hemolytic crisis that is usually misdiagnosed as gram-negative septicemia.[83] In addition, fragmented red blood cell vesicles from hemolysis can cause an erroneous count of the platelets when an automated cell counter is used, resulting in a delayed detection of thrombocytopenia.[83] More significantly, patients with evidence of poor tissue oxygenation or hypoxia must receive supportive pre-storage filtered blood transfusion at the amount of 5-12 ml/kg/dose that should be repeated if the hemolysis continues. Adequate intravenous hydration with urine alkalinization is recommended to prevent possible kidney damage from the precipitation of hemoglobin passing through the renal glomerular and tubule structures. Details of management of acute hemolysis in Hb H disease have been described previously.[18]

Iron overload may develop in Hb H disease.[25,43] However, due to milder anemia, less transfusion than in other NTDT genotypes and a lower level of ineffective erythropoiesis, iron overload in Hb H disease develops at a much slower rate.[43] Therefore, it is rare to find patients with significant iron overload before 15 years of age, except patients who have received regular or frequent blood transfusion supports (Figure 4). As in other non-transfusion-dependent thalassemias, single measurements of serum ferritin can underestimate the total body iron store in Hb H disease. Therefore, direct monitoring using magnetic resonance imaging (MRI)-evaluation is the approach of choice.[84] The use of liver biopsy to assess iron overload in Hb H patients is not recommended due to procedure-related complications and a possible bias of sampling error unless an open biopsy can be acquired during splenectomy. Once iron overload is detected, it should be treated and monitored using the same recommendation as for other types of NTDT patients.[85]

Prevention and control for severe α-thalassemia syndromes

Due to fetal lethality at mid-gestation and predisposition of the mothers to several obstetric complications including hypertension and antenatal hemorrhage,[86] a prevention and control program for Hb Bart's hydrops fetalis is now operative in Asian countries such as China and Thailand.[87-90] Through the program, carriers for α^0-thalassemia are detected at antenatal care level using a screening by osmotic fragility (OF) or MCV and MCH values.[64] A correct genotype of α^0-thalassemia will be further confirmed by DNA analysis. However, identification of α^0-thalassemia can be complicated by co-inheritance of β-thalassemia traits. Therefore, it is highly recommended to perform a combination of hemoglobin analysis and a common set of α-thalassemia genotype by DNA study as confirmation tests in individuals who come from a region with high prevalence of both α and β thalassemia. This approach can prevent a possible error by missing correct α and β globin genotypes in these individuals and successfully identify couples at risk for producing infants affected with Hb Bart's hydrops.[64] Prenatal diagnosis of Hb Bart's hydrops can be achieved by DNA analysis of chorionic villous samples or cord blood hemoglobin analysis by cordocenthesis.[88,91] For couples who would like to avoid prenatal diagnosis and a termination of pregnancy with affected fetus, an assisted *in vitro* reproduction with embryo selection after pre-implantation genetic diagnosis (PGD) for Hb Bart's hydrops is now available with modest success rates (< 30%) due to allelic drop-out and low pregnancy rate.[92] However, this technology still has to be improved and confirmation by prenatal diagnosis of this assisted pregnancy is still recommended.

As Hb H disease is generally mild and does not require life-long blood transfusion, a prenatal diagnosis for both common deletional and non-deletional types might not be ethical and is not recommended. However, concerning the rare non-deletional α-thalassemias mentioned above, a prenatal diagnosis for couples at risk of Hb H hydrops or transfusion dependent Hb H should be offered, in particular to those families with previously affected offspring. Nevertheless, it remains a challenge to provide such a service to a new couple since the molecular characterization of these rare non-deletional mutations is not routinely performed nor is it widely available. In addition, heterozygotes for these non-deletional mutations have normal or borderline MCV and MCH and simply might not be diagnosed without DNA studies.[18]

Summary

A definitive diagnosis of the disease-causing mutations in α-thalassemia syndromes is important for disease management and genetic counseling. Patients with severe α-thalassemia syndromes such as Hb H hydrops should be treated with regular blood transfusion with appropriate iron chelation therapy. Stem cell transplantation as cura-

tive therapy should be offered if a matched donor is available. The majority of patients with Hb H disease can do well using only supportive care with 'on demand' blood transfusion. Splenectomy should be reserved to more severely affected patients; in particular, to those with non-deletional Hb H disease. Couples at risk of having an affected child with severe form of α-thalassemia syndromes, such as Hb Bart's hydrops fetalis and Hb H hydrops/transfusion dependent Hb H, should be offered genetic counseling and an informed choice on reproductive options, including prenatal diagnosis, which involves fetal sampling to determine the fetal genotype. In addition, assisted reproductive technology that combines preimplantation genetic diagnosis (PGD) with *in vitro* fertilization (IVF) may help parents who have thalassemia or who are carriers of a severe defective α globin gene to give birth to healthy babies by embryo selection.

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