Alpha-thalassemia

Renzo Galanello, MD[1], and Antonio Cao, MD[2]

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Abstract: Alpha-thalassemia is one of the most common hemoglobin genetic abnormalities and is caused by the reduced or absent production of the alpha globin chains. Alpha-thalassemia is prevalent in tropical and subtropical world regions where malaria was and still is epidemic, but as a consequence of the recent massive population migrations, alpha-thalassemia has become a relatively common clinical problem in North America, North Europe, and Australia. Alpha-thalassemia is very heterogeneous at a clinical and molecular level. Four clinical conditions of increased severity are recognized: the silent carrier state, the alpha-thalassemia trait, the intermediate form of hemoglobin H disease, and the hemoglobin Bart hydrops fetalis syndrome that is lethal in utero or soon after birth.

Alpha-thalassemia is caused most frequently by deletions involving one or both alpha globin genes and less commonly by nondeletional defects. A large number of alpha-thalassemia alleles have been described and their interaction results in the wide spectrum of hematological and clinical phenotypes. Genotype-phenotype correlation has been only partly clarified. Carriers of alpha-thalassemia do not need any treatment. Usually, patients with hemoglobin H disease are clinically well and survive without any treatment, but occasional red blood cell transfusions may be needed if the hemoglobin level suddenly drops because of hemolytic or aplastic crisis likely due to viral infections. Hemoglobin Bart hydrops fetalis syndrome currently has no effective treatment although attempts at intrauterine transfusion and hematopoietic stem cell transplantation have been made. ***Genet Med* 2011:13(2):83–88.**

Key Words: *alpha-thalassemia, HbH disease, Hb Bart, hydrops fetalis*

Alpha-thalassemia is one of the most common hemoglobin genetic abnormalities. The primary defect is the reduced or absent production of the alpha globin chains, which constitute the moieties of several hemoglobin (Hb) types, including the adult HbA (alpha2 beta2), fetal HbF (alpha2 gamma2), and the minor component HbA_2 (alpha2 delta2). Similar to other common globin gene disorders (i.e., beta-thalassemia and sickle cell anemia), alpha-thalassemia is prevalent in tropical and subtropical world regions, where malaria was and still is epidemic, and it is thought that carriers of hemoglobinopathies are relatively protected in a malarial environment.[1,2] Despite extensive studies, the mechanism underlying this protection is still unknown. It should be pointed out that the different molecular defects causing alpha-thalassemia occur at variable frequencies in the populations and this results in a different occurrence of the clinically significant forms, namely, HbH disease and Hb Bart hydrops fetalis syndrome. As a consequence of the recent massive population migrations, alpha-thalassemia has become a relatively common clinical problem in North America, North Europe, and Australia.[3]

CLINICAL FORMS

Four clinical conditions of increased severity are recognized: two carrier states (i.e., alpha$^+$-thalassemia usually caused by the deletion or dysfunction of one of the four normal alpha globin genes and alpha°-thalassemia resulting from deletion or dysfunction of two alpha genes in *cis* [see "Molecular genetics"]) and two clinically relevant forms (i.e., HbH disease [only one functioning alpha gene] and Hb Bart hydrops fetalis syndrome [no functioning alpha genes]; Table 1).

At phenotypic level, the carrier states are divided into silent carrier and - alpha/-alpha thalassemia trait. The silent carrier state most frequently results from the presence of a single alpha globin gene deletion (-alpha/alpha alpha) and is characterized in the newborn by a very mild increase (1–2%) of Hb Bart, a tetramer of globin chains (gamma4), which is present when there is an excess of gamma chains relative to alpha chains. However, sometimes failure to demonstrate Hb Bart in cord blood does not exclude the silent carrier state.[4] In adults, the one gene deletion genotype may be completely silent or associated with a moderate microcytosis and hypochromia with normal HbA_2 and F (Table 2). Subjects with two residual functional alpha genes, either in *cis* (- -/alpha alpha) or in *trans* (- alpha/- alpha), clearly show the alpha-thalassemia trait (alpha trait), characterized by a moderate increase (5–6%) of Hb Bart in the newborn and by alpha-thalassemia-like red blood cell indices with normal HbA_2 and F in the adult, and reduced alpha/beta globin chain synthesis ratio in the range of 0.7–0.8 (Table 2). Carriers of nondeletion defects (see later) have quite variable hematologic phenotypes ranging from the alpha trait to the silent carrier state. Double heterozygotes for deletion and nondeletion alpha-thalassemia have the alpha-thalassemia trait phenotype, whereas homozygotes for nondeletion defects may have the alpha trait phenotype and sometime a mild HbH disease (see

From the [1]Dipartimento di Scienze Biomediche e Biotecnologie, Università di Cagliari, Ospedale Regionale Microcitemie ASL8; and [2]Istituto di Neurogenetica e Neurofarmacologia, Consiglio Nazionale delle Ricerche, Cagliari, Italy.

Renzo Galanello, Ospedale Regionale Microcitemie, Via Jenner s/n, 09121 Cagliari, Italy. E-mail: renzo.galanello@mcweb.unica.it.

Disclosure: The authors declare no conflict of interest.

Submitted for publication August 3, 2010.

Accepted for publication September 9, 2010.

Published online ahead of print January 5, 2011.

DOI: 10.1097/GIM.0b013e3181fcb468

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Table 1 Clinical classification of α-thalassemia

Condition	Clinical characteristics
Silent carrier	Clinically and hematologically normal
Thalassemia trait	Microcytosis, hypochromia, and mild anemia
HbH disease	Moderate to severe microcytic, hypochromic, hemolytic anemia, mild jaundice, moderate hepatosplenomegaly
Hb Bart hydrops fetalis syndrome	Severe anemia, generalized edema, ascites, marked hepatosplenomegaly, skeletal and cardiovascular malformations, usually death in utero

below).[5] Homozygotes for the Hb Constant Spring mutation, the most common nondeletion defect in the Oriental population, have a clinical syndrome that is similar to HbH disease.[6] The -alpha-thalassemia carrier state should be differentiated from iron deficiency and from delta- and beta-thalassemia interaction (see "Carrier detection"). This differentiation has important practical consequences.

HbH disease is a clinical condition resulting from the presence of only one residual functioning alpha globin gene (- -/- alpha) or (- -/alphaND alpha). As a consequence, there is a relative excess of beta globin chains, which form beta4 tetramers (HbH). HbH is unstable and mainly precipitates inside the older red cells, which are prematurely destroyed in the spleen, resulting in moderate to severe hemolysis. HbH disease shows a considerable variability in clinical and hematological severity.[7] The most significant features are microcytic and hypochromic hemolytic anemia, hepatosplenomegaly, jaundice, and sometime moderate alpha-thalassemia-like bone modifications. The hemoglobin concentration is usually in the range of 7–10 g/dL, and mean corpuscular volume (MCV) varies with age (being around 58 fl in childhood and around 64 fl in adulthood), whereas mean corpuscular hemoglobin (MCH) is around 18 pg irrespective of age[7] (Table 2). Reticulocytes range between 5% and 10% and the alpha to beta-globin chain synthesis ratio is markedly reduced, in the order of 0.20–0.60. Anemia may worsen during pregnancy and suddenly as a consequence of increased hemolysis with infections and after administration of oxidant drugs, which should therefore be avoided. A variable spleen enlargement is almost always present, whereas liver enlargement is less common. Iron overload is uncommon but has been reported in older individuals, usually as a result of repeated blood transfusions or increased iron absorption. The severity of HbH disease correlates with the degree of alpha chain deficiency (see "Genotype-phenotype correlation"). A very few cases of unusual severe HbH disease associated with hydrops fetalis have been described.[8,9] Because of different prevalence and interactions between the various molecular defects underlying alpha-thalassemia (particularly the alpha°-thalassemia), HbH disease is predominantly seen in Southeast Asia, although it is not rare in Mediterranean.

Two peculiar types of HbH disease have been reported. One is acquired, associated with myelodysplasia, and characterized by the presence of HbH inclusion bodies in the red blood cells and severe microcytic and hypochromic anemia. The alpha globin genes and their flanking regions are normal. Recent studies have shown that some patients have point mutations and/or splicing abnormalities in the *ATRX* gene: located at Xq13.1-q21.1 (see below),[10] whereas one patient showed a large deletion of the chromosome 16 telomeric region including both alpha globin genes.[11] The other is the -alpha-thalassemia associated with mental retardation syndromes, which includes two different forms.[12,13] The first is characterized by a relatively mild mental retardation and a variety of facial and skeletal abnormalities and developmental delay. This form, known as ATR 16 syndrome, is due to extended deletions (1–2 Mb) of the short arm of chromosome 16 removing both alpha genes and other flanking known and unknown genes.[12] A second group of patients has a complex phenotype with quite uniform clinical features (hypertelorism, flat nasal bridge, triangular upturned nose, wide mouth, and genital abnormalities) and severe mental retardation.[13] No structural changes of the alpha cluster or 16p chromosome have been reported in these subjects, and the transmission is X-linked (ATRX syndrome). Mutations in the *ATRX* gene that encodes a chromatin-associated protein belonging to the SNF2 family of helicase/adenosine triphosphatases, members of which are involved in a wide variety of cellular processes, such as transcriptional regulation, control of cell cycle, DNA repair, and mitotic chromosome segregation.[13]

Table 2 Hematological data and HbA_2 in the most common alpha-globin genotypes

Genotype	Sex	Hb (g/dl)	MCV (fl)	MCH (pg)	A_2 (%)	α/β ratio[a]
- $\alpha^{3.7}/\alpha$	M	14.4 ± 0.9	75.4 ± 4.8	25.4 ± 2.1	2.5 ± 0.3	0.74 ± 0.08
	F	12.0 ± 1.0				
- $\alpha^{3.7}$/- α	M	13.6 ± 0.8	71.3 ± 3.0	23.8 ± 2.0	2.4 ± 0.3	0.60 ± 0.09
	F	11.8 ± 0.9				
$\alpha^{ND}\alpha/\alpha\alpha$	M	14.4 ± 1.1	75.7 ± 3.0	25.6 ± 1.4	2.5 ± 0.3	0.79 ± 0.1
	F	12.2 ± 0.8				
- -/$\alpha\alpha$	F	13.2 ± 1.6	65.0 ± 3.3	21.0 ± 1.3	2.4 ± 0.1	0.70 ± 0.03
- -/-α^{b}	M + F	10.3 ± 0.8	61.0 ± 4.0	19.0 ± 1.0	<2.0	
- -/$\alpha^{ND}\alpha$	M + F	9.0 ± 0.7	64.0 ± 6.0	19.0 ± 1.0	<2.0	

[a] α/β ratio by in vitro globin chain synthesis analysis.
[b] Subjects with these genotypes show at hemoglobin analysis variable amounts of HbH (up to 30%).
ND indicates Non Deletion defect; Hb, hemoglobin; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin.

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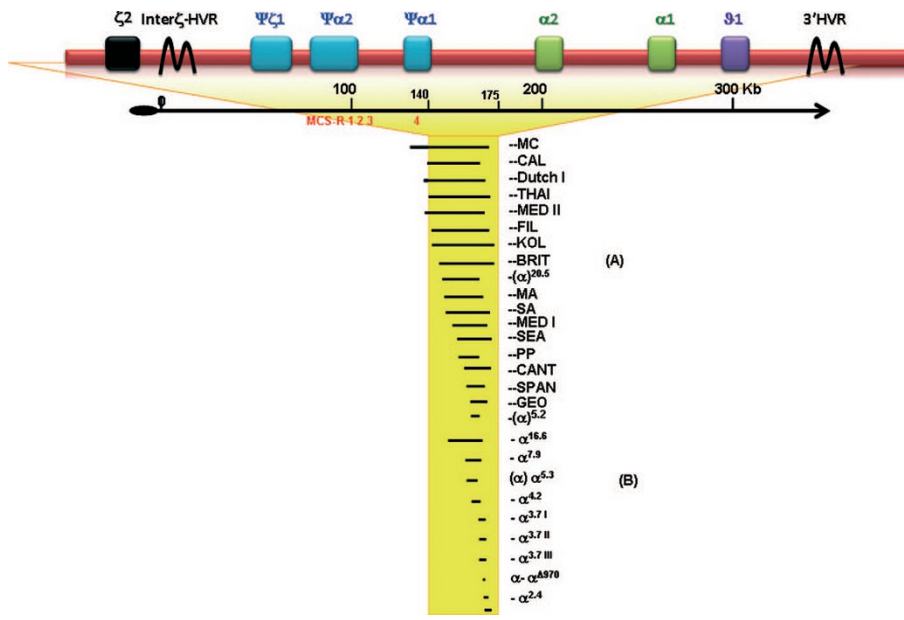

Fig. 1. α-globin gene cluster and deletions associated with α° (A) and α^{+} (B) thalassemia.

Hb Bart hydrops fetalis syndrome is the most severe -alpha-thalassemia clinical condition. Usually, it is associated with the absent function of all four alpha globin genes (- -/- -). The affected fetus is unable to produce any alpha globin chains to make HbF or HbA. Fetal blood contains mainly Hb Bart (gamma4) and small amounts of hemoglobins Portland 1 and 2 (zeta2 gamma2 and zeta2 beta2). The clinical picture is characterized by very severe anemia (Hb level, 3–8 g/dL), marked hepatosplenomegaly, hydrops fetalis, and cardiac failure.[14] Other congenital abnormalities, particularly of the cardiac and skeletal and urogenital system, have been reported. This condition is usually not compatible with postnatal life and affected fetuses are either stillborn or die soon after birth.[14] Maternal complications during pregnancy have been reported, including preeclampsia (hypertension and fluid retention with or without proteinuria), poly-oligohydramnios (increased or reduced accumulation of amniotic fluid, respectively) hemorrhage, anemia, and sepsis. Given the severity of this syndrome and of the maternal complications during the pregnancy, early termination of at-risk pregnancies is recommended, and in some population, universal prenatal screening to address homozygous alpha-thalassemia has been initiated.[15,16] Hb Bart hydrops fetalis is relatively common in Southeast Asia, whereas in Mediterranean population, it is relatively rare because of the low frequency of alpha°-thalassemia.[17,18] However, as a result of change in demographics, the problem of alpha-thalassemia-associated hydrops fetalis is increasing worldwide. At present, there is no effective treatment for Hb Bart hydrops fetalis syndrome (see "Management").

MOLECULAR GENETICS

In normal individuals, alpha globin genes encoding the alpha globin chains are duplicated and localized in the telomeric region of chromosome 16 (16p 13.3), in a cluster containing also an embryonic zeta2 gene, encoding the embryonic zeta globin chains, three pseudogenes (pseudo zeta1, pseudo alpha1, and pseudo alpha2) and one gene (theta1) of unknown function (Fig. 1).[19] The functional genes are arranged in the order—telomer-zeta-alpha2-alpha1–centromer—and their expression is regulated by four remote highly conserved noncoding regions (named multispecies conserved sequences [MCS]-R1–R4) located about 40 kb upstream in the introns of a flanking, widely expressed gene (Fig. 1).[20] The level of transcription of the two alpha genes differs, as the alpha2 gene encodes two to three times more alpha globin than alpha1 gene.[21] The different expression of the two alpha genes has implications for the amount of hemoglobin variant present in carriers of alpha1 or alpha2 globin mutations and for the pathophysiology of the deletional and nondeletional forms of alpha-thalassemia.

Deletion α-alpha-thalassemia

Alpha-thalassemia is caused most frequently by deletions involving one or both alpha globin genes. The most common deletions remove a single alpha globin gene, resulting in the mild alpha^{+}-thalassemia phenotype (- alpha/alpha alpha). Reciprocal recombination between highly homologous regions called (Z boxes) results in a chromosome with a 3.7-kb deletion containing only one alpha gene (-alpha$^{3.7}$), whereas recombination between mispaired homologous X boxes produces a 4.2-kb deletion (-alpha$^{4.2}$).[22] These recombinational events also result in the production of chromosomes containing three alpha globin genes.[23] The -alpha$^{3.7}$ and -alpha$^{4.2}$ deletions are the most common alpha^{+} alpha-thalassemia defects. Other rare deletions totally or partially remove one of the two alpha globin genes (Fig. 1). Extended deletions, varying from 100 to >250 kb, removing all or part of the cluster including both alpha globin genes and sometimes the embryonic zeta2 gene, result in the complete absence of alpha chain synthesis (alpha°-thalassemia; Fig. 1). Such deletions are the result of several molecular mechanisms including illegitimate recombination, reciprocal translocation, and truncation of chromosome 16. More than 40 different alpha°-thalassemia deletions have been described, the most common being the Southeast Asian, Filipino, and Mediterranean types (Fig. 1). Two deletions [-(alpha)$^{5.2}$ and -(alpha)$^{20.5}$] removing the alpha-2 and partially the alpha1 globin

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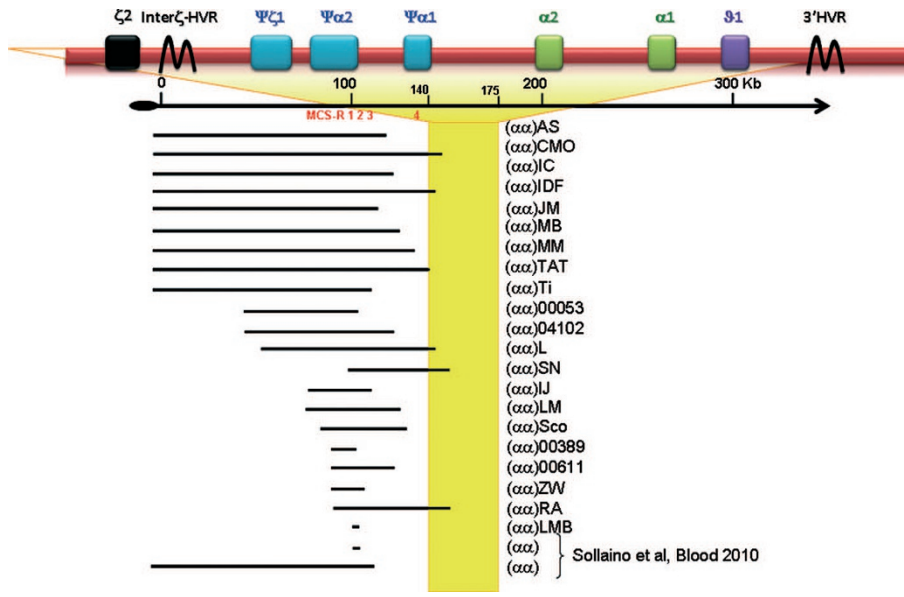

Fig. 2. Deletions of the MCS-R region.

gene also result in alpha°-thalassemia. Rare large deletions extending from 100 to >200 kb and removing the entire alpha globin cluster, and other genes that flank the cluster, including a DNA repair enzyme (methyladenine DNA glycosylase), and inhibitor of GDP dissociation from Rho (Rho GDI γ), a protein disulfide isomerase (PDI-R) and other anonymous housekeeping genes, have been reported in single families.[23] Despite the removal of several genes, such patients seem to have a normal phenotype apart from having alpha-thalassemia. A deletion removing the alpha1 gene, the theta gene, and extending downstream centromeric from the alpha cluster results in alpha°-thalassemia. The silencing of intact alpha2 gene is related to an antisense RNA transcribed from the widely expressed *LUC7L* gene, becoming juxtaposed to the normal alpha2 gene by the deletion and running through the alpha2 gene sequences.[24]

Several different deletions involving the MCS-R regulatory regions, but leaving both alpha genes intact, have also been reported, and all result in alpha°-thalassemia[23,25–27] (Fig. 2).

Nondeletion alpha-thalassemia

Nondeletion defects less frequently cause alpha-thalassemia. These defects include single nucleotide substitutions or oligonucleotide deletions/insertions in regions critical for alpha globin gene expression.[23] Several molecular mechanisms (abnormalities of RNA splicing and of initiation of mRNA translation, frameshift and nonsense mutations, in-frame deletions, and chain termination mutations) have been described, the majority occurring in the predominant alpha2 gene and producing alpha$^+$-thalassemia. The most common nondeletional variants are the T→C initiation codon mutation and the -5nt alpha -IVS1 deletion in Mediterranean, polyadenylation site mutations in Mediterranean and Middle East populations, stop codon mutations resulting in elongated alpha globin variants, including the T>C (stop→glu) of the alpha2 gene that results in Hb Constant Spring, and other elongated variants (Hb Icaria, Hb Seal Rock, and Hb Koya Dora) found in Mediterranean, middle East Asia, and Southeast Asia.[23] Hb Constant Spring, the most common (up to 4%) nondeletion defect present in Southeast Asian population, is an alpha chain variant elongated by 31 amino acids, which is produced in a very low amount (~1%). The instability of the mRNA, due to disruption of the untranslated region may be the reason for the reduced production of Hb Constant Spring.[28] As for beta globin gene, mutations of alpha genes, which result in the production of hyperunstable globin variants, such as Hb Quong Sze, (alpha 109 Leu→Pro), Hb Heraklion (alpha 137 pro→0), and Hb Agrinio (alpha 29 Leu→Pro), unable to assemble in stable tetramers and thus rapidly degraded, might produce the phenotype of alpha-thalassemia. At present, about 30 alpha globin chain hyperunstable variants have been described. The complete list of nondeletional mutations that cause alpha-thalassemia has been recently reported by Harteveld and Higgs.[20]

DIAGNOSIS

Hematologic testing

Initial laboratory testing for alpha-thalassemia carrier identification should include MCV and MCH determination and quantitative Hb analysis (usually done by high-performance liquid chromatography [HPLC]; Table 2). However, identification of alpha-thalassemia carriers is difficult because they have microcytosis and hypochromia but do not have typical changes in HbA_2 or HbF, characteristics of beta and delta-beta alpha-thalassemia carriers, respectively. Carriers with -alpha/-alpha and - -/alpha alpha genotypes have always reduced MCV and MCH, whereas -alpha/alpha alpha carriers may have normal red cell indices or only slightly reduced MCV and MCH. HbA_2 is normal or slightly reduced, and HbF is normal. After incubation of erythrocytes with 1% brilliant cresyl blue supravital stain, some RBC with inclusion bodies (precipitated beta4 tetramers) can be detected by microscope in alpha alpha-thalassemia carriers. In vitro globin chain synthesis analysis shows reduced α/β ratio (0.9–0.6). Sometimes, especially in regions where thalassemias are uncommon, as the hematological parameters are quite similar, alpha-thalassemia trait may be confused with iron-deficiency anemia. Iron status assessment (i.e., serum iron and transferrin saturation or red blood cell zinc protoporphyrin

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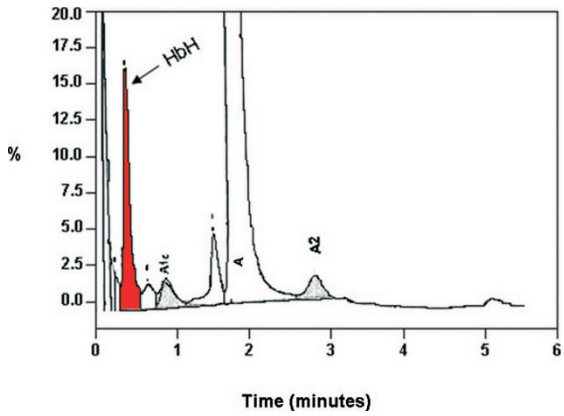

Fig. 3. HPLC pattern of a patient with HbH disease. The peaks of the different hemoglobins are indicated.

determination) is usually sufficient to make a correct diagnosis. Newborn carriers with alpha-thalassemia usually, but not always, have at hemoglobin electrophoresis or HPLC a slight to moderate (1–5%) increase in Hb Bart.

Patients with HbH disease have microcytic hypochromic anemia and reduced (<2%) HbA_2, but the typical finding is the presence of variable amounts (up to 30%) of HbH. HbH is easily detected as a fast-moving band by cellulose acetate electrophoresis or slow eluting peak at HPLC (Fig. 3). Another simple and very sensitive test consists in the detection of inclusion bodies in a variable proportion of red blood cells after incubation with supravital stains. In the neonatal period, subjects with HbH disease genotype can be detected by hemoglobin electrophoresis because they have elevated levels (about 25%) of Hb Bart. Hematologic diagnosis of Hb Bart syndrome is characterized by the presence of severe macrocytic anemia and Hb Bart (85–90%), and absence of HbF and HbA at hemoglobin analysis with electrophoresis or HPLC.

Molecular analysis

Polymerase chain reaction-based methods have been developed for the most common alpha-thalassemia mutations. GAP-polymerase chain reaction, using specific primers flanking the deletion break-points, detects deletions associated with alpha$^+$- or alpha°-thalassemia.[29] Primer panels targeted to the population specific mutations can be used.[30–32]

Alpha globin gene sequence analysis can be performed to identify nondeletional point mutations. For suspected rearrangements (deletions or duplications) of the alpha gene cluster or of the MCS-R regions, the recently available multiplex ligation-dependent probe amplification method can be used.[33] Definition of alpha globin genotype in carriers is useful for genetic counseling, whereas, in patients with HbH disease, is useful for prognosis, as the nondeletional forms are more severe than the deletional forms.[34–36]

PHENOTYPE-GENOTYPE CORRELATION

The different alpha-thalassemia mutations vary widely in severity, and the resulting phenotype depends on the degree of alpha globin chain deficiency relative to beta-globin production. Overall, there is a rank in severity (from least to most severe): one alpha gene deletion (silent carrier or alpha-thalassemia trait), nondeletion defects (alpha-thalassemia trait), two alpha gene deletions either in *cis* or in *trans* (alpha-thalassemia trait), three missing or dysfunctional alpha genes (HbH disease), and all four alpha genes deleted (Hb Bart hydrops fetalis syndrome).

However, it should be pointed out that -alpha/alpha alpha carriers have a variable phenotype ranging from completely normal red blood cell indices to a moderate thalassemia-like hematological picture (reduced MCV and MCH and very mild anemia) with normal HbA_2 and F.

In general, nondeletional defects involving the alpha2 gene are more severe because the alpha2 gene encodes two or three times more alpha2 globin than alpha1 gene.[21] Moreover, when in the deletional defects the alpha2 gene is removed, the output from the remaining alpha1 gene seems to be increased. For these reasons, interactions involving nondeletional defects result in a more severe phenotype than those with deletional defects. Therefore, patients with HbH disease with nondeletional defects (- -/alphaND alpha) have a more severe clinical expression with earlier presentation, more marked anemia, jaundice, hepatosplenomegaly, bone changes, and more frequent needs of red cell transfusions when compared with patients with deletion HbH disease (- -/-alpha).[34–36]

The phenotype of patients with HbH disease with deletions of the MCS-R region is usually like that of the deletion type of HbH, but sometimes it can be more severe.[25–27] Hb Bart syndrome usually results from deletion of all four alpha globin genes but rarely involve nondeletion defects.[9,10]

GENETIC COUNSELLING

Genetic counseling in alpha-thalassemia is particularly relevant for couples where both partners are alpha° carriers, as they are at risk (25%) of their offspring having Hb Bart hydrops fetalis syndrome. For this condition, prenatal diagnosis is always indicated not only for its severity and absence of an effective treatment but also to avoid the severe maternal toxemic complications during pregnancy. For these reasons, several countries have initiated universal prenatal screening programs to address homozygous alpha°-thalassemia.[15,16] For couples at risk of having offspring with HbH disease (- -/alpha alpha in one parent and - alpha/alpha alpha or alphaND alpha/alpha alpha genotypes in the other), prenatal diagnosis is not indicated because this condition is usually mild and compatible with an almost normal postnatal life.

Only very rarely, the interaction of alpha°-thalassemia with a nondeletional allele or homozygosity for nondeletional alleles has led to individuals with hydrops fetalis syndrome. Therefore, in these cases prenatal diagnosis can be considered.[9,10,37]

MANAGEMENT

Carriers of alpha-thalassemia, both alpha° or alpha$^+$ generally do not need treatment. Management of HbH disease is influenced by the marked clinical variability of this condition. Most individuals with HbH disease are clinically well and survive without any treatment. As for other hemolytic anemias, folic acid supplementation is recommended by some clinician. Patients should be advised to avoid oxidant drugs (the same drugs to be avoided by subjects with glucose-6-phosphate dehydrogenase deficiency) because of the risk of hemolytic crisis. Occasional red blood cell transfusions may be needed if the hemoglobin level suddenly drops because of hemolytic or aplastic crisis likely due to viral infections (i.e., parvovirus B19). Repeated red blood cell transfusions are considered in selected individuals (usually with the nondeletional forms) with severe anemia, sometimes affecting cardiac function and massive erythroid expansion, causing severe bone changes and extramed-

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ullary erythropoiesis. Splenectomy may be indicated in the presence of hypersplenism, but the potential life-threatening complication of venous thrombosis, reported in some patients with HbH disease following splenectomy, should be considered.[37,38] Iron overload is uncommon in HbH disease but has been reported in older patients and in those on chronic transfusions regimen.

Hb Bart hydrops fetalis syndrome currently has no effective treatment. Attempts at intrauterine transfusions, after early prenatal detection with Doppler ultrasonography of this condition, have been conducted, but most survivors experienced a high prevalence of congenital malformations.[40,41] In a few cases, unrelated and cord blood transplants have been performed.[42,43] These have resulted in ethical dilemmas for the family and the provider. Therefore, similar attempts should be discouraged until more effective therapies (e.g., somatic gene therapy) are available.

ACKNOWLEDGMENTS

This study was supported by Grants from L.R.7, 2007 Regione Autonoma Sardegna.

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