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Update in the genetics of thalassemia: What clinicians need to know

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Thalassemia is a significant health problem worldwide. Prenatal diagnosis is the only effective way to prevent the birth of a fetus with severe thalassemias, which include hemoglobin Bart's hydrops fetalis and thalassemia major. However, accurate prenatal diagnosis depends on the comprehensive consideration of the molecular basis of thalassemias. To make a correct decision, the obstetrician should have a certain understanding of the genetics of thalassemias. Here we present a brief introduction of some fundamental genetic knowledge of thalassemias, including the production of hemoglobin, structure and location of globin genes, hemoglobin switch, epidemiology, clinical classification, molecular and cellular pathology, genotype–phenotype correlation, and genetic modifiers. Furthermore, some unusual clinical cases that cannot be explained by Mendel's laws are described. On the basis of a thorough understanding of the above information, clinicians should have the ability to precisely diagnose thalassemia patients and provide applicable genetic counselling to the affected families.

Introduction

Inherited hemoglobin (Hb) disorders are the most common inherited blood disorders globally and account for approximately 3.4% of deaths in children under 5 years of age [1]. This group of diseases is

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caused by mutations in human globin genes, which are classified into two categories: those that produce structurally abnormal globin (Hb variants) and those with impaired globin synthesis (thalassemia). Thalassemia is characterized by the absence or decreased accumulation of one of the globin subunits. The most common forms are α-thalassemia (OMIM: #604131) and β-thalassemia (OMIM: #613985), which affect the synthesis of α- and β-globin subunits, respectively.

Thalassemias are prevalent in tropical and subtropical areas where malaria was and still is epidemic. The high frequency may be due to carriers of hemoglobinopathies who have a survival advantage in malarial endemic areas [2]. People carrying thalassemia variants are concentrated in Southeast Asia, the Mediterranean area, the Indian subcontinent, the Middle East, and Africa [3,4]. Moreover, it is noteworthy that as a consequence of recent massive population migrations, thalassemia is not restricted to traditional high-incidence regions and is now a relatively common clinical problem in North America, North Europe, and Australia [2]. The clinical management of thalassemia, such as its diagnosis and treatment, has challenged the local health system. For example, screening for Hb H disease (one form of α-thalassemia) has been added for newborns in California [5]. An analysis of 86 Hb H disease patients performed by Lal's group supported the usefulness of universal newborn screening and suggested that the screening should be extended to other populations [6].

The inheritance mode of thalassemias is autosomal recessive (AR). Carriers of thalassemia mutations are clinically normal. However, when both parents are carriers, for every pregnancy, there is a 25% chance that the child will be a thalassemia patient, a 50% chance that the child will be a thalassemia carrier, and a 25% chance that the child will be normal. To date, prenatal diagnosis is the only way to prevent the birth of an affected child. Therefore, in highly prevalent regions, an ideal and effective strategy to decrease the birth rate of thalassemia patients is to identify high-risk couples, who are both carriers, before pregnancy by screening (or carrier testing) and then perform a prenatal diagnosis during pregnancy.

Basic genetic structures of Hemoglobin gene clusters

All normal human Hbs are tetramers of two pairs of globin chains: one pair of α-like globins and one pair of β-like globins. At the molecular level, Hb synthesis is controlled by two multigene clusters (Fig. 1A). The α-cluster contains an embryonic gene (ζ2), two fetal/adult α genes (α2 and α1), two pseudo genes (Ψζ1 and Ψα1), and two minor globin-like genes (Ψα2 and θ), which are all arranged in the following order: 5′-ζ2–Ψζ1–Ψα2–Ψα1–α2–α1–θ-3′. HS-40 is the major regulatory element of the α-globin locus. The β-cluster contains an embryonic gene (ε), two fetal genes ($^{G}\gamma$ and $^{A}\gamma$), one pseudo gene (Ψβ), and two adult genes (δ and β), which are arranged in the following order: 5′-ε–$^{G}\gamma$–$^{A}\gamma$–Ψβ–δ–β–3′. Locus control region (LCR) is the important upstream regulatory region.

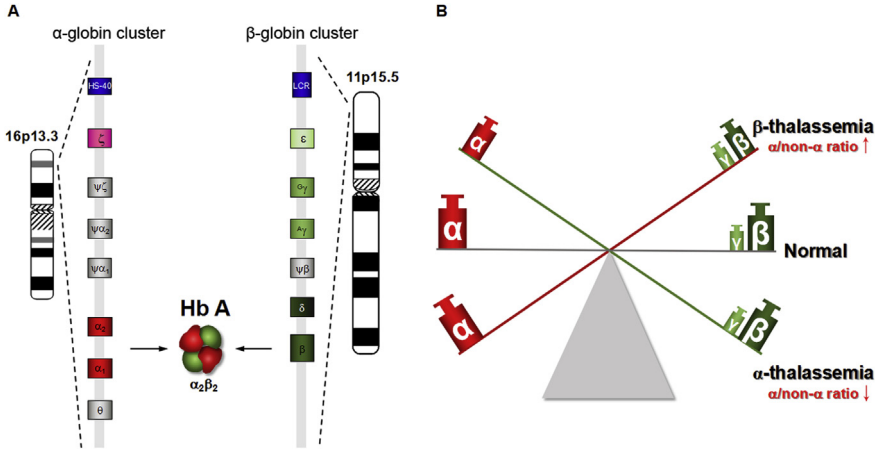

Fig. 1. Structure of the α- and β-globin gene cluster (A) and the pathophysiology of thalassemia (B).

Clinically, thalassemias display a wide spectrum of phenotypes ranging from asymptomatic to lethal. According to the clinical severity, thalassemias are generally divided into three groups: (1) Thalassemia trait: they are carriers who are often asymptomatic and do not need any treatment. (2) Thalassemia intermedia (TI): they have moderate anemia (Hb 60–100 g/L) and occasionally require red blood cell transfusion; in α-thalassemia, it is known as Hb H disease. (3) Thalassemia major (TM): they have severe anemia and require transfusions for survival; in α-thalassemia, this clinical form was named Hb Bart's hydrops fetalis. The fetus usually dies in utero or shortly after birth. In general, the latter two groups are defined as thalassemia patients. The common symptoms of these patients include pallor, jaundice, splenomegaly, and skeletal deformities.

The imbalance of subunits is central to the pathophysiology of thalassemia. For normal Hb synthesis, the ratio of α:non-α subunits should be 1:1 (Fig. 1B). In α-thalassemia, the quantity of β-like chains is greater than that of α chains; on the contrary, in β-thalassemia, the quantity of β-like chains is less than that of α chains. The degree of imbalance is proportional to the severity of the disease. In Hb Bart's hydrops fetalis, because of the absence of the α-globin, fetal blood contains mainly Hb Bart (γ4). Hb Bart's cannot release oxygen even in a hypoxic state. Thus, the fetus suffers from severe anemia and hypoxia and often develops fetal anomalies. Such a fetus always dies either in utero (23–38 weeks) or shortly after birth. This disease causes up to 90% of all fetal hydrops in Southeast Asia [7]. In Hb H disease, which is the intermedia α-thalassemia clinical form, the affected individual usually produces less than 30% of the normal amount of α-globin, whereas a relative excess of β-globin chains form Hb H (β4). Hb H is unstable and precipitates inside the red cells, which are prematurely destroyed, resulting in moderate hemolysis. The main pathophysiological mechanisms of β-thalassemia are hemolysis and ineffective erythropoiesis. Insufficiency of β-globin results in an excess of free α-globin. The unstable free α-globin forms α-hemichromes, generates reactive oxygen species (ROS), and triggers cascades of events that lead to hemolysis and ineffective erythropoiesis. Further complications include iron overload, splenomegaly, skeletal deformities, and erythroid marrow expansion.

Hemoglobin switch process

Because all normal Hbs are tetramers of two pairs of globin chains, the production of α-like and β-like chains is balanced in each stage of development. The structure of human Hb changes during development, as shown in Fig. 2. In the embryonic stage, there are Hb Gower1 (ζ2ε2), Hb Gower2 (α2ε2), and Hb Portland (ζ2γ2). These embryonic Hbs are confined to the yolk-sac stage and thereafter are replaced by the fetal hemoglobin (Hb F) (α2γ2). Hb F is the dominant Hb in utero. After birth, Hb F is replaced by adult hemoglobin (Hb A) (α2β2, major Hb, approximately 97%) and Hb A_2 (α2δ2, minor Hb approximately 2%–3%) over the first year of life. Hb F is present during the first 6 months such that the babies do not develop β-thalassemia at birth. In normal adults, Hb F continues to be present, constituting approximately 1% of the total Hb [3,8]. The entire process is called Hb switch. It is very interesting that the globin genes in the two clusters are arranged along the chromosome in the same order (Fig. 1) according to which they are expressed during development. The sequential activation and

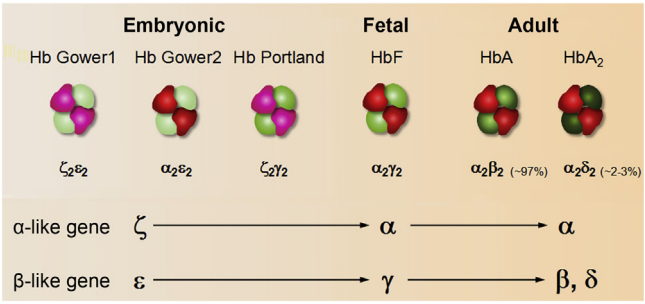

Fig. 2. The globin switch process.

silencing of globin genes are precisely controlled. Studies of the expression of globin genes had shown that HS-40 in α-cluster and LCR in β-cluster play important roles as cis-regulating elements [3]. Each element is bound by the complex formed by many proteins that serve as trans-acting factors.

The gene switch in the α-cluster is relatively simple. The two α genes are continuously expressed throughout life, except for the first 6 weeks of embryogenesis in which ζ protein is produced. In contrast, the gene switch in the β-cluster is more complex. It consists of a change from ε→γ→β. In particular, the γ to β transition is more important in clinical practice because a high level of Hb F could be curative for β-thalassemia. How to reactivate the γ-genes to bind the excess α-globin is one of the main directions of thalassemia treatment. Studies have established two major mechanisms for γ silencing in adults, including the competitive interaction of the γ- and β-genes with the LCR during the fetal to adult switch and gene-autonomous γ-globin silencing [9]. The latter mechanism provides the basis for a gene-based strategy to increase the Hb F level after birth to cure patients with TM. Many transcription factors are involved in this mechanism, including BCL11A, KLF1, MYB, LRF, and others [10,11] (Fig. 3).

BCL11A acts as a major repressor of γ-globin expression. Loss of function of BCL11A, regardless of whether it occurs in human erythroid precursor or in transgenic mice, is sufficient to prevent γ-globin repression [12]. It appears to exert its repressive function at a distance. BCL11A binds to the LCR, but it does not bind to the γ-globin or β-globin gene themselves [12]. BCL11A is necessary for configuring the β-locus. It promotes long-range interactions between the LCR and β-globin gene. Thus, when BCL11A is knocked out, the LCR interacts with γ-globin genes instead of the β-globin gene, and the γ-globin expression is reactivated.

KLF1 is a master regulator of adult β-globin transcription. Inactivation of the *Klf1* gene in mice showed that it is essential for the activation of β-globin expression [13]. It also mediates the γ to β switch by binding the *BCL11A* gene promoter and activating its transcription. Knocking down the expression of *KLF1* inhibited the expression of *BCL11A* gene and increased the γ:β ratio in erythroblasts [14]. It is suggested that the KLF1/BCL11A regulatory axis plays a crucial role in the Hb switch [15]. In the normal development process, KLF1 activates BCL11A, which next represses γ gene expression, thereby promoting the switch from Hb F (α2γ2) to Hb A (α2β2). At the same time, KLF1 itself activates β-globin expression. In some cases of hereditary persistence of fetal hemoglobin (HPFH), a haploinsufficiency of KLF1 results in reduced BCL11A expression, which in turn increases the Hb F level and decreases the Hb A level.

The mechanism of MYB in affecting γ-globin expression is still not clear. However, the disruption of MYB in mice produced an increase in ε- and γ-globin expression, indicating that MYB accounts for γ-globin silencing during development [16]. Recently, LRF has been identified as a new transcription factor that represses γ-globin expression [11]. It interacts with the γ-globin genes and maintains the

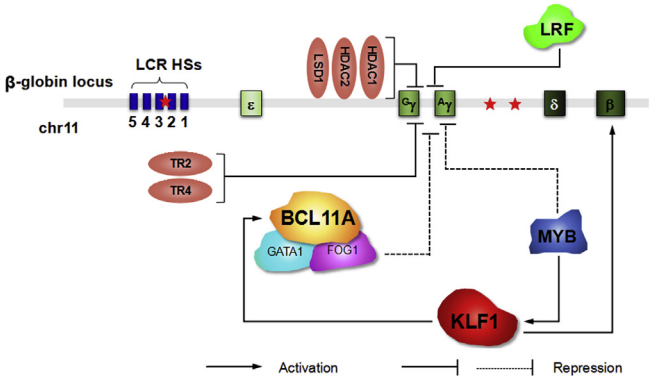

Fig. 3. Major transcription factors involved in the γ to β switch. LCR comprises the hypersensitive sites 1–5 (blue boxes). BCL11A binding sites are indicated with red stars. Some factors, including the BCL11A complex, repress γ-globin through indirect mechanisms of action and are therefore shown with dotted lines.

nucleosome density necessary for γ-globin gene silencing in adults. The function of LRF repressing γ-globin is not dependent on BCL11A protein; this suggests that there may be more elements or factors contributing to the Hb switch. Epigenetics alteration and microRNAs should be considered in the future.

Molecular basis of pathogenesis of thalassemia

Thalassemias are caused by two types of defects in globin genes: deletion defects and nondeletion defects. The range of deletion defects usually involves more than 1 kb. Nondeletion defects consist of single nucleotide substitutions or oligonucleotide deletions/insertions. It is interesting that a different spectrum of α- and β-thalassemia mutations is often found in different populations, although thalassemia is a common worldwide disorder. Therefore, reference data of the mutations found in specific populations are characteristics of these populations. When performing molecular diagnosis, the ethnic origin of the patients should be a concern.

α-thalassemia

The vast majority of α-thalassemia is caused by deletion. Because there are two α-globin genes in one chromosome, the haplotype can be written as αα/. Considering one haplotype, α-thalassemia mutations are classified into three groups [4,17]: (1) α^{+}-thalassemia deletion (-α/), which removes only one α-globin gene; (2) α^{0}-thalassemia deletion (--/), which removes both α-globin genes; and (3) nondeletion mutation ($\alpha^{T}\alpha$/ or $\alpha\alpha^{T}$/, depending on whether the α2 or α1 gene is affected). The common mutations are listed in Table 1. The output from the α2 gene accounts for two-thirds of the production of the whole α-globin, whereas the α1 gene accounts for the remaining one-third. Thus, α2 gene mutations would have more severe effects than α1 gene mutations. In addition, the nondeletion may give rise to a more severe reduction in α-chain synthesis than the α^{+} deletion. According to these rules, a haplotype order was established on the basis of its relative effect on α-globin production: $\alpha\alpha^{T}$/<-α/ <$\alpha^{T}\alpha$/<--/.

More than 40 different α^{0} deletions have been reported [2], the most common being the $-^{SEA}$ (Southeast Asia) and $-^{MED}$ (Mediterranean) mutations. These deletions can be grouped into those that lie entirely within the α-globin cluster (e.g., $--^{SEA}$) and deletions that extend to the telomere of chromosome 16 (e.g., $--^{235}$) [17,18]. Although $--^{235}$ deletions delete other genes, the affected heterozygotes appear phenotypically normal apart from their α-thalassemia [18]. Another type of rare deletions causing α^{0}-thalassemia removes the regulatory region HS-40 and leaves the α-globin genes intact [19]. More than 10 α^{+} deletions have been reported; $-\alpha^{3.7}$ and $-\alpha^{4.2}$ are the most common worldwide [2,4]. These deletions are the products of reciprocal recombination. These recombinational events also generate α-triplication $\alpha\alpha\alpha^{anti3.7}$ and $\alpha\alpha\alpha^{anti4.2}$. Further recombination events may even result in

Table 1
Thalassemia: deletions and common mutations.

Locus	Mutation/deletion types	Common mutations
α-globin	α^{0}-deletion	$--^{SEA}$ (Southeast Asia), $--^{MED}$ (Mediterranean)
	α^{+}-deletion	$-\alpha^{3.7}$, $-\alpha^{4.2}$ (worldwide)
	$\alpha^{T}\alpha$ (α2 gene)	Hb CS (Southeast Asia), $\alpha^{IVS1(-5nt)}\alpha$ (Mediterranean), $\alpha^{PA(AATAAG)}\alpha$ (Middle East Asia)
	$\alpha\alpha^{T}$ (α1 gene)	Hb Q-Thailand (Southeast Asia)
β-globin	β^{++}-mutation	$\beta^{-101(C>T)}$ (Mediterranean)
	β^{+}-mutation	$\beta^{IVS1-110(G>A)}$ (Mediterranean), Hb E (Southeast Asia)
	β^{0}-mutation	$\beta^{CD39(C>T)}$ (Mediterranean), $\beta^{CD41-42(-CTTT)}$ (Southeast Asia)
	deletion (β gene)	619 bp deletion (Asian Indian)
	Deletion (HPFH/ζβ)	SEA-HPFH, Chinese $^{G}\gamma^{+}$ ($^{A}\gamma\delta\beta)^{0}$ deletion (Chinese)

Deletion (β gene): deletions affecting only the β-globin gene.
Deletion (HPFH/ζβ): large deletions involving part or all of the β-globin gene clusters.

quadruplicated α genes (αααα) or quintuplicated (ααααα) [17]. Such deletions can also be generated by other molecular mechanisms such as nonhomologous recombination [20] and replication errors [21]. Furthermore, more complex crossover events occur in this cluster, such as "patchwork" α2 and α1 genes (α212 and α121) [22], the HKαα allele (a rearrangement that contains both the -$\alpha^{3.7}$ and ααα$^{anti4.2}$ unequal crossover junctions), and the anti-HKαα allele (the reciprocal product that contains both the -$\alpha^{4.2}$ and ααα$^{anti3.7}$ unequal crossover junctions) [23].

At least 90 nondeletion mutations have been found, including mutations that affect mRNA processing, mRNA translation, and α-globin stability. Some Hb variants causing similar thalassemia phenotype were also classified into this group, such as Hb Constant Spring (Hb CS, $\alpha^{CS}\alpha$) and Hb Quong Sze (Hb QS, $\alpha^{QS}\alpha$) [19]. Among the currently known nondeletion mutations, the majority of them occur in the α2 gene, and mutations in the α1 gene are rare. The most common nondeletion mutations are $\alpha^{IVS1(-5nt)}\alpha$ (Mediterranean), $\alpha^{PA(AATAAG)}\alpha$ (Middle East Asia), and $\alpha^{Constant\ Spring}\alpha$ (Southeast Asia) [2,4,19]. The Hb Q-Thailand, a G>C mutation in codon 74, is a relatively common α1 mutation in Southeast Asia [24].

β-thalassemia

In contrast to α-thalassemia, nondeletion defects account for the vast majority of β-thalassemia. More than 300 nondeletion variants have been described in different populations (http://globin.bx.psu.edu/hbvar/). Most of them are point mutations, and only a minority of them are small deletions in the exons of the β-globin gene, such as $\beta^{CD54-58(-13\ bp)}$ and $\beta^{CD89-93(-14\ bp)}$ [25].

Depending on the degree of quantitative reduction in the synthesis of normal β-globin, β-thalassemia mutations are classified into three groups: (1) β^0-thalassemia mutation (β^0/), which results in the absence of β-globin; (2) β^+-thalassemia mutation (β^+/), which severely reduces the output of β-globin; and (3) β^{++}-thalassemia mutation (β^{++}/, also known as silent β-mutation), which mildly reduces the output of β-globin. The common β-mutations are also listed in Table 1. Some Hb variants are synthesized at a reduced rate or are highly unstable and lead to thalassemia phenotypes, such as Hb E ($\beta^{CD26(G>A)}$). This mutation occurs in β-codon 26 (GAG>AAG) and results in amino acid substitution from Glu to Lys; it also activates a new splice site that causes abnormal mRNA processing. It is usually considered a β^+-thalassemia mutation [3,26]. According to the mechanism by which they affect the β-globin gene's function, the mutations are divided into different groups: (1) mutants that affect transcription, such as $\beta^{-101(C>T)}$ in the promoter [26] or $\beta^{CAP+39(C>T)}$ in the 5′UTR [27]; (2) mutants that affect RNA processing, such as $\beta^{IVS1-110(G>A)}$ that generate cryptic splice sites, $\beta^{PA(GATAAG)}$ that decreases the efficiency of the cleavage-polyadenylation process, and $\beta^{Term\ CD+32(A>C)}$ in the 3′UTR [26,27]; and (3) mutants that affect RNA translation, such as initiation codon mutation $\beta^{(ATG>GTG)}$, nonsense mutation $\beta^{CD39(C>T)}$, and frameshift mutation $\beta^{CD41-42(-CTTT)}$.

Rare deletion mutations of β-thalassemia have also been identified. One group of deletions is restricted to the β-globin gene itself. For example, the 619 bp deletion removes the 3′-end of the β-globin gene. It is a common mutation in Asian Indians and accounts for approximately 30% of the β-thalassemia cases in this population [26]. This group of deletions is also often termed β^0-mutations. The other group of deletions consists of large deletions involving a part of or the entire β-globin gene clusters. These large deletions are responsible for δβ-thalassemias or HPFH. These large deletions are often associated with an absence of β-globin chain production but are associated with the production of a high quantity of γ-globin. Thus, δβ-thalassemias and HPFH are clinically milder than the typical cases of β^0-thalassemia [26]. More than 40 large deletions have been reported in different populations. SEA-HPFH and the Chinese $^{G}\gamma^{+}$ $(^{A}\gamma\delta\beta)^0$ deletion are common in southern Chinese populations [27].

Genotype–Phenotype correlation

Clinically, thalassemia cases are usually subclassified into three groups according to the phenotypic severity: thalassemia of mild state (α- or β-thalassemia trait), thalassemia of intermediate state with moderate anemia (Hb H disease and TI), and thalassemia of severe state with severe anemia, which can even be lethal (Hb Bart's hydrops fetalis and TM) (Fig. 4). Thalassemia is caused by the reduced synthesis of α- or β-subunits and subsequent α:non-α chain imbalance. Phenotypic severity is proportional to the degree of imbalance, which is determined by the corresponding globin genotype.

α-thalassemia

In α-thalassemia, the phenotypic severity increases with the number of nonfunctional α-globin genes. Normal individuals have four functional α-globin genes (αα/αα). Four clinical conditions of increasing severity are shown in Fig. 4: silent α-carrier, α-thalassemia trait, Hb H disease, and Hb Bart's hydrops fetalis. The first two types often can be recognized as α-thalassemia trait. The last two types are the clinically relevant forms. Silent α-carriers only lose one α gene (-α/αα) and are phenotypically silent. These cases are clinically and hematologically normal [normal Hb, mean corpuscular volume (MCV), mean cell Hb (MCH), Hb A_2, and Hb F). They usually cannot be detected by routine screening (full blood counts and Hb analysis) on the basis of the hematological phenotype and can only be identified by genotyping assay. The α-thalassemia trait loses two α genes (--/αα or -α/-α) and is asymptomatic, but shows microcytosis (MCV↓) and hypochromia (MCH↓). The cis genotype (--/αα) is highly prevalent in the Southeast Asian population, whereas the trans genotype (-α/-α) is common in the African population [4]. Carriers of nondeletion defects ($\alpha\alpha^{T}/\alpha\alpha$) have variable hematologic phenotypes that range from the silent carrier state ($\alpha^{Westmead}\alpha/\alpha\alpha$) to the α-thalassemia trait ($\alpha^{CS}\alpha/\alpha\alpha$ or $\alpha^{QS}\alpha/\alpha\alpha$) [28]. Hb H disease loses three α genes (--/-α or -- /$\alpha^{T}\alpha$) and shows moderate to severe microcytic, hypochromic, and hemolytic anemia [6]. The band of Hb H (γ4) can be detected in an adult's blood by Hb analysis. However, some Hb H cases are due to the homozygosity or compound heterozygosity of nondeletional α-globin gene, such as $\alpha^{CS}\alpha/\alpha^{CS}\alpha$, $\alpha^{QS}\alpha/\alpha^{QS}\alpha$, or $\alpha^{CS}\alpha/\alpha^{QS}\alpha$ [7]. Patients who are affected by the deletion of the three α genes suffer from "deletional Hb H disease," whereas patients resulting from α^{0}-deletion plus nondeletional mutation suffer from "nondeletional Hb H disease." Traditionally, nondeletional Hb H disease is considered a more severe form than the deletional form, particularly the --/$\alpha^{CS}\alpha$ genotype, which is also known as Hb H Constant Spring (HCS). HCS patients have a more severe clinical phenotype than patients with other Hb H diseases [6]. They experience a significant growth delay and require intermittent transfusions. Hb Bart's hydrops fetalis loses all four α genes (--/--), and these patients usually die in utero [7].

β-thalassemia

Similar to α-thalassemia, β-thalassemia can also be divided into four clinical conditions of increasing severity (Fig. 4): silent β-carrier, β-thalassemia trait, TI, and TM. Silent β-carriers are the

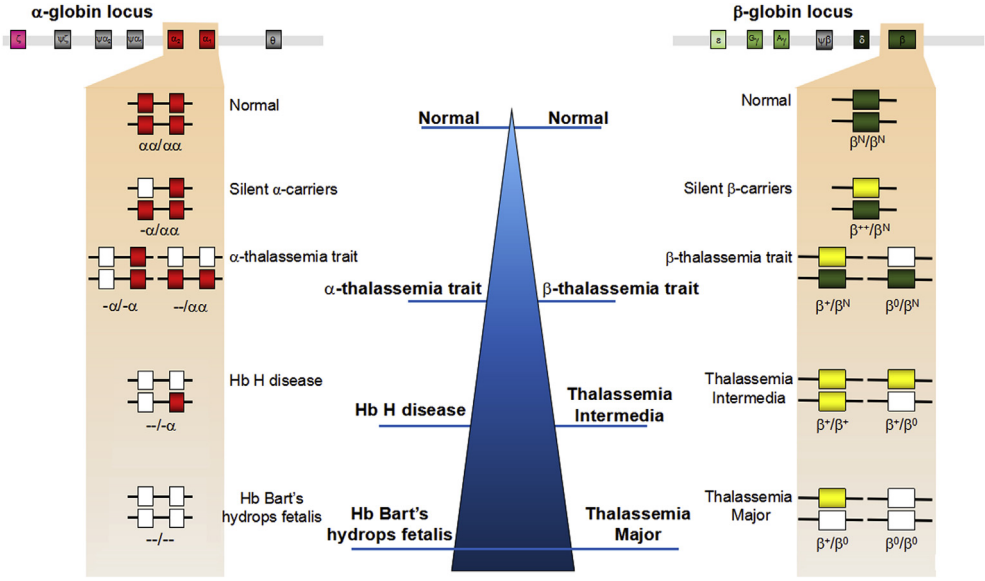

Fig. 4. Clinical classification and genotype–phenotype correlation of α- (left) and β-thalassemia (right). In this figure, the genotype–phenotype correlation of β-thalassemia is focused on the β-globin gene itself. The effect of genetic modifiers has been excluded.

heterozygotes of β^{++} mutation (β^{++}/β^{N}). They have near-normal red cell indices and Hb A_2 levels and can only be identified by molecular diagnosis, similar to silent α-carriers. β^{++} alleles are not common, except for $\beta^{-101(C>T)}$, which is common in the Mediterranean population [26]. β-thalassemia trait, also known as thalassemia minor, affects individuals who inherit a single β^{+} or β^{0} allele (β^{+}/β^{N} or β^{0}/β^{N}). They are asymptomatic but show microcytosis (MCV↓), hypochromia (MCH↓), and increased Hb A_2 levels. TI is a clinical condition of intermediate gravity between thalassemia trait and TM [8,27]. TI cases encompass a wide phenotypic spectrum that spans from mild anemia to more severe anemia and require only occasional blood transfusions. They generally have a later age of onset (over the age of 2 years) than TM patients. The blood profile shows a Hb concentration of 70–100 g/L. Corresponding to the phenotypic diversity, the molecular basis of TI is also variable [27]. The vast majority of them are usually homozygous for β^{+} (β^{+}/β^{+}) or compound heterozygous for β^{+} and β^{0} (β^{+}/β^{0}). Heterozygous for dominant β-thalassemia mutations (β^{D}/β^{N}), homozygous for β^{0} (β^{0}/β^{0}) coinherited with α-thalassemia, or heterozygous for β^{+} or β^{0} (β^{+}/β^{N} or β^{0}/β^{N}) coinherited with α-triplication also showed the TI phenotype. However, the genotype–phenotype correlation of TI is so complex that the pathogenesis of some patients remains uncertain and cannot be explained by known mechanisms [27]. The study of the role of the genetic modifier in modulating β-thalassemia phenotype has brought us considerable novel and interesting information in this area, which is described in detail in the "Genetic modifiers" section. TM is the most severe form of β-thalassemia. Affected individuals usually present with severe anemia (<60 g/L) and require regular transfusions to survive. Children with untreated or partially treated disease die in the first or second decade of life [8]. Most TM cases are homozygous for β^{0} (β^{0}/β^{0}), a small portion of whom are compound heterozygous for β^{+} and β^{0} (β^{+}/β^{0}).

Genetic modifiers

The phenotypic severity of β-thalassemia widely varies from mild to severe forms, and the genotype–phenotype correlations of β-globin genes have been described above. However, individuals with the same β-thalassemia genotype show wide phenotypic variability that ranges from moderate to severe disease due to various genetic modifiers of disease severity, which are linked or unlinked to the β-globin locus. Thein [29] summarized the genetic modifiers into two groups: the primary modifiers at the level of the α:non-α chain imbalance and the secondary modifiers at the level of complications related to disease and therapy. In this review, we focus on the primary modifiers.

The central pathophysiological mechanism of β-thalassemia involves the degree of globin chain imbalance and the excess of α-globins. Factors that can alter the quantity of free α-globin or reduce the degree of imbalance would have a significant effect on the phenotype [8]. Identifying these modifiers plays an important role in the precise diagnosis of β-thalassemia. Two major categories of modifiers have been identified: copy number variations of α-globin genes and variations affecting Hb F production (Table 2).

The coinheritance of α-thalassemia could ameliorate the severity of β-thalassemia because it lowers α-globin production and reduces the damage to red cells caused by free intracellular α-globin. Coinheritance of α- and β-thalassemia is not uncommon in areas in which both types of thalassemia are highly prevalent [3]. The results obtained for various ethnic groups have shown that coinheritance with

Table 2
Genetic modifiers of β-thalassemia.

Category	Factors aggravating severity	Factors ameliorating severity
Copy numbers of α-globin genes	α-triplication/α-quadruplication	α-thalassemia mutations
Variations affecting Hb F production		rs382144 (T allele) rs2071348 (C allele) rs11886868 (C allele) rs766432 (C allele) rs4895441 (G allele) rs9399137 (C allele) KLF1 $^{wt/var}$

α^0- or α^+- thalassemia (--/αα or −α/αα) can ameliorate the severity of β^0/β^0 patients from TM to TI [30]. On the contrary, coinheritance with α-triplication (ααα/αα) or α-quadruplication (αααα/αα) would aggravate the severity because the extra α-globin genes increase α-globin production. When heterozygotes for β-thalassemia (β^0/β^N or β^+/β^N) are coinherited with this variation, their phenotype would deteriorate from thalassemia trait to TI [3,29]. However, individuals who only carry α-triplication are phenotypically normal; therefore, the frequency of this variation is not known in detail in most populations.

The production of Hb F after birth is an important factor in modifying the clinical severity because an increased γ-globin level will bind the additional α-globin and form Hb F. Many factors are involved in setting the Hb F level; some are located in the β-cluster and others on different chromosomes.

A well-known factor affecting the Hb F level in the β-cluster is a polymorphism at position −158 (C>T) in the Gγ-gene (rs382144), which has also been called *XmnI* polymorphism. It is a relatively common polymorphism that is present in many populations. It appears to have little effect in normal individuals, but it significantly up-regulates Hb F production in β^0 thalassemia [31]. Its genetic contribution to the Hb F level is estimated to be approximately 10% in European populations [29]. A (A>C) polymorphism in Ψβ gene (rs2071348) was also reported to increase the Hb F levels, leading to milder symptoms [32].

Factors regulating γ-gene expression also act as genetic modifiers to the phenotype. The results of genome-wide genetic association study confirmed that two loci unlinked to the β-cluster, i.e., BCL11A on 2p16 and HBS1L-MYB on 6q23, are quantitative trait loci (QTL) controlling Hb F. SNPs (rs11886868, rs766432, rs4671393, rs7557939, rs6732518, and rs1427407) in BCL11A have been reported to be associated with F-cell numbers or Hb F levels in different populations [33–36]. The genetic contribution is estimated to be approximately 15% in nonanemic North Europeans [34] and 7%–12% in African Americans having sickle cell anemia [37]. The rs11886868 "C" allele is strongly associated with high Hb F and is significantly more frequent in TI patients than in TM patients in the Sardinian population [38]. The rs766432 "C" allele is associated with augmented Hb F/F-cells in Chinese patients [35]. Similarly, SNPs (rs9399137, rs4895441, and rs1320963) in the HBS1L-MYB intergenic region have also been reported to be associated with Hb F production in different populations [33,34,36,37,39], with a genetic contribution of approximately 19% in Europeans [34] and 3%–7% in African Americans [37]. Recently, the effects of coinherited KLF1 variation on the severity of thalassemia patients were analyzed systematically [40,41]. The effects of KLF1 variants on hematologic parameters of normal individuals and thalassemia cases are summarized in Table 3. The association between KLF1 variations and elevated Hb A_2 and Hb F levels in groups of both α- and β-thalassemia carriers was established. It was also suggested that coinherited KLF1 variation ($KLF1^{wt/var}$) could increase the production of Hb F, which in turn ameliorates the clinical severity of β-thalassemia [41]. However, an association of KLF1 mutations with Hb H disease severity was excluded [40].

Rare clinical cases

Dominantly inherited β-thalassemia

There is a special form of β-thalassemia defects called dominant β-thalassemia mutations. Generally, β-thalassemia is considered an AR disorder because the inheritance of two mutant β-globin genes

Table 3
Impact of KLF1 variants on hematological phenotype of thalassemia.

	Genotype	Effect of coinheritance with $KLF1^{wt/var}$
Normal	αα/αα, β^N/β^N	MCV↓, MCH↓, Hb A2↑, Hb F↑
α-thalassemia	-α/αα, β^N/β^N	MCV↓, MCH↓, Hb A2↑, Hb F↑
	-α/αα, β^N/β^N	
	$\alpha^T\alpha$/αα, β^N/β^N	
	--/-α, --/$\alpha^T\alpha$	No significant effects were observed
β-thalassemia	αα/αα, β^0/β^N	Hb A2↑, Hb F↑
	αα/αα, β^0/β^0	KLF1 is a positive genetic modifier in ameliorating the severity

is necessary to produce significant clinical features. However, heterozygotes for dominant β-thalassemia mutations showed typical anemia symptoms. They are inherited in affected family members in autosomal dominant (AD) mode. Dominant β-thalassemia mutations have been found in many different ethnic groups [26]. Although studies of dominant mutants are ongoing, these forms are still uncommon, and many of them have only been reported once. This group includes four subgroups: (1) Missense mutations involving amino acids that are important for maintaining the tertiary structure of β-globin, e.g., Hb Hawakki [42]. This mutation occurs in codon 31 of exon 2 of the β-globin gene. The codon CTG is replaced by CGG, which leads to the replacement of amino acid Leu by Arg. (2) Minor insertions or deletions of entire codons that allow the reading frame to remain in phase, e.g., Hb Fairfax [43]. A 15-base-pair tandem duplication of the GAGCTGCACTGTGAC sequence is inserted between codons 94 and 95, coding for additional Glu-Leu-His-Cys-Asp amino acids. (3) Nonsense mutations that lead to premature termination, such as the GAA>TAA termination codon at codon 121 [26]. (4) Frameshift mutations resulting in elongated or truncated β-globin chains, e.g., a mutation of codon 53 (−T) that was first detected in a Chinese Miao family. It results in a β-globin chain with a 59-amino acid truncation [44].

Uniparental isodisomy

Typically, the average age of onset of patients with β-thalassemia major is under 2 years. However, late-onset β-thalassemia major patients have been occasionally found. In particular, some patients come from families in which only one parent is a carrier of β-thalassemia. This condition is inconsistent with the Mendelian laws. The mechanism involves uniparental isodisomy (UPD). It was first identified in a girl whose age at onset was over 20 years [45]. She was homozygous for CD17 (A>T) mutation ($\beta^{CD17(A>T)}/\beta^{CD17(A>T)}$), but her mother was normal (β^{N}/β^{N}) and her father was heterozygous for CD17 mutation ($\beta^{CD17(A>T)}/\beta^{N}$). Molecular and cellular analyses demonstrated that paternal UPD and mosaicism of the chromosomal region 11p14.3–11p15.5 should be considered for the occurrence of β-thalassemia major. Later, three patients who were born as asymptomatic carriers and developed late-onset β-thalassemia major were reported in 2013 [46]. The age of onset in a female patient was 26 years, and her father was a carrier of CD76 (−C). A male patient's age at onset was 38 years; his parents were deceased, but he had two sons who carried the CD39 (C>T) mutation. The age of onset of another male patient was 46 years, and his father was heterozygous for CD39 (C>T). It had been identified that these patients had the segmental paternal UPD (47.2 Mb–49.8 Mb) of 11p. These results showed that segmental UPD often plays a role in explaining the sporadic late onset of β-thalassemia major without a clear Mendelian inheritance. Furthermore, the only identification of paternal UPD suggests that the loss of maternally imprinted genes may be responsible for a selective advantage in hematopoietic cells containing paternal isodisomy of 11p and carrying the β-thalassemia mutation. UPD resulting in α-thalassemia was also described in 2016 [47]. A fetus was diagnosed with Hb Bart's hydrops fetalis by ultrasound examination and cordocentesis. The mother was an α^{0}-thalassemia carrier ($--^{SEA}/\alpha\alpha$), the father was an α^{+}-thalassemia carrier ($-\alpha^{3.7}/\alpha\alpha$), and the fetus was homozygous for α^{0}-thalassemia ($--^{SEA}/--^{SEA}$). STR analysis demonstrated maternal UPD from 16pter to 16p13.2.

De novo mutation

There is another group of sporadic thalassemia patients whose inheritance mode contradicted Mendelian laws because of de novo mutation. Although parentally inherited alterations are responsible for a large majority of thalassemia cases, a few de novo abnormalities can result in thalassemia phenotypes. Large de novo deletions were defined as the cause of ATR-syndrome [17], γδβ-thalassemias, and εγδβ-thalassemias [26]. More de novo mutations have been found as point mutations and resulted in α- and β-thalassemias. A novel CD43/44(−C) at the α2-globin gene was first detected in a Chinese boy with Hb H disease ($--^{SEA}/\alpha^{CD43/44(-C)}\alpha$). His father had a normal genotype ($\alpha\alpha/\alpha\alpha$), and his mother was an α^{0} carrier ($--^{SEA}/\alpha\alpha$). Neither parent had this variant, and nonpaternity was excluded. Thus, it was shown to be a spontaneous mutation in the α-globin genes [48]. Similar de novo mutation cases have also been found in β-globin genes, such as CD31 (T>C) mutation in a Guatemalan boy [42] and a 22-bp duplication in a Thai girl [49].

Summary

Thalassemia is one of the most common genetic disorders worldwide, with at least 60,000 severely affected babies born each year and up to 90% of these births occurring in developing countries. Hb A consists of pairs of α- and β-subunits (α2β2), and Hb F has two α- and γ-subunits (α2γ2). For normal Hb synthesis, α- and non-α-globin production should be balanced. Thus, the imbalance of the subunits is central to the pathophysiology of thalassemia.

α-Thalassemia occurs because of the absence or decreased production of α-globin subunits and is most frequently caused by deletions that remove a part of or the entire α-globin gene cluster. The severity is well correlated with the number of functional copies of the α-globin genes, which range from asymptomatic to lethal. Clinically relevant forms of α-thalassemia include Hb H disease and Hb Bart's hydrops fetalis. Hb H disease is a mild form, and most patients have a moderate hemolytic anemia. Hb Bart's hydrops fetalis is a more severe form, and patients usually die in utero or shortly after birth.

On the contrary, β-thalassemia results in an insufficiency of β-globin and is usually caused by point mutations in the β-globin gene. The phenotype severity is mostly determined by the genotypes of the β-globin gene. However, some genetic modifiers, such as the α-globin and KLF1 genes, play a role in modulating the phenotype.

Finally, we discussed some rare clinical cases including dominantly inherited β-thalassemia, uniparental isodisomy, and de novo mutation.

Conflicts of interest

Author confirms that there is no conflict of interest.

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Abbreviations used

Hb: hemoglobin
MCV: mean corpuscular volume
MCH: mean cell Hb

Practice points

- The mode of inheritance of thalassemias is usually AR, but rare dominant or de novo mutations and uniparental isodisomy could be found in this group of diseases.
- α-Thalassemia is usually caused by deletion mutations of α-globin genes, whereas β-thalassemia is usually caused by point mutations of the β-globin gene.
- The clinical severity of β-thalassemia is mainly related to the genotypes of β-globin genes, but the coinheritance of α-thalassemia mutations or genetic determinants that could increase the Hb F levels can reduce the severity.
- Prenatal diagnosis is very useful in preventing the birth of infants with Hb Bart's hydrops fetalis and TM.

Research agenda

- Discovery of rare thalassemia mutations
- Effect of the transcription factor on the expression of Hb F in adults
- Genetic contribution of the modifiers to the phenotype of thalassemia

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