

## β-Thalassemia

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β-Thalassemia is caused by reduced (β<sup>+</sup>) or absent (β<sup>0</sup>) synthesis of the β-globin chains of hemoglobin. Three clinical and hematological conditions of increasing severity are recognized: the β-thalassemia carrier state, thalassemia intermedia, and thalassemia major, a severe transfusion-dependent anemia. The severity of disease expression is related mainly to the degree of α-globin chain excess, which precipitates in the red blood cell precursors, causing both mechanic and oxidative damage (ineffective erythropoiesis). Any mechanism that reduces the number of unbound α-globin chains in the red cells may ameliorate the detrimental effects of excess α-globin chains. Factors include the inheritance of mild/silent β-thalassemia mutations, the coinheritance of α-thalassemia alleles, and increased γ-globin chain production. The clinical severity of β-thalassemia syndromes is also

influenced by genetic factors unlinked to globin genes as well as environmental conditions and management. Transfusions and oral iron chelation therapy have dramatically improved the quality of life for patients with thalassemia major. Previously a rapidly fatal disease in early childhood, β-thalassemia is now a chronic disease with a greater life expectancy. At present, the only definitive cure is bone marrow transplantation. Therapies undergoing investigation are modulators of erythropoiesis and stem cell gene therapy.

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**Key Words:** β-thalassemia; ineffective erythropoiesis; iron chelators; iron overload; modulators of erythropoiesis

β-thalassemias are heterogeneous autosomal recessive hereditary anemias characterized by reduced or absent β-globin chain synthesis. Approximately 68,000 children are born with various thalassemia syndromes each year.<sup>1</sup> β-thalassemia is highly prevalent, with 80 to 90 million people reported to be carriers across the world (1.5% of the global population). It includes three main forms: β-thalassemia major (TM), also referred to as “Cooley’s anemia” and “Mediterranean anemia”; β-thalassemia intermedia (TI); and thalassemia minor, called “β-thalassemia carrier,” “β-thalassemia trait,” or “heterozygous β-thalassemia.” Apart from the rare dominant forms, subjects with TM are homozygotes or compound heterozygotes for β<sup>0</sup> or β<sup>+</sup> genes, subjects with TI are mostly homozygotes or compound heterozygotes, and subjects with thalassemia minor are mostly heterozygotes.

The extremely high frequency of hemoglobin disorders compared with other monogenic diseases reflects natural selection mediated by the relative resistance of carriers against *Plasmodium falciparum* malaria with a high frequency of consanguineous marriages in many countries. Gene drift and founder effects are other reasons that thalassemias are most frequent in southeastern and southern Asia, the Middle East, the Mediterranean countries, and North and Central Africa. However, as a result of mass migrations of populations from high-prevalence areas, thalassemias are now encountered in most countries, including the United States, Canada, Australia, South America, and North Europe.<sup>2</sup> Thalassemia care in developed countries has achieved the survival of affected patients well into adult life, mainly by adopting good blood transfusion

and chelation practices but also by adopting follow-up protocols to detect early complications and prevent complications in vital organs. In many endemic countries, however, considerable work, financial backing, and political commitment are still needed to effectively address the control of these disorders and to guarantee affected patients all the opportunities available for those with thalassemia who were born in high-income countries.

### CLINICAL FEATURES

#### β-thalassemia major

Individuals with TM are usually brought to medical attention between ages 6 and 24 months; they subsequently require regular red blood cell (RBC) transfusions to survive. Affected infants fail to thrive and become progressively pale. Feeding problems, diarrhea, irritability, recurrent bouts of fever, and progressive enlargement of the abdomen caused by splenomegaly may occur. In developed countries, if prenatal diagnosis has not been performed, the diagnosis of TM is established at this stage and a regular transfusion program is initiated. The classic clinical picture of TM is currently seen only in some developing countries in which the resources for performing long-term transfusion programs are not available. The most relevant features of untreated or poorly transfused individuals are growth retardation, pallor, jaundice, brown pigmentation of the skin, poor musculature, genu valgum, hepatosplenomegaly, leg ulcers, development of masses from extramedullary hematopoiesis, and skeletal changes that result from expansion of the bone marrow. These skeletal changes include deformities of the

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long bones of the legs, typical craniofacial changes, and osteoporosis. Individuals who have not undergone regular transfusions usually die from high-output heart failure. If a regular transfusion program that maintains a minimum hemoglobin (Hb) concentration of 9.0 to 10.5 g/dl is initiated, then ineffective erythropoiesis is inhibited and growth and development tend to be normal up to 10 to 12 years. However, patients who have undergone transfusions may develop complications related to iron overload, depending on their compliance with chelation therapy. Complications of iron overload in children include growth retardation and failure of sexual maturation; in adults, liver fibrosis and cirrhosis, involvement of the endocrine glands (diabetes mellitus and insufficiency of the parathyroid, thyroid, pituitary, and, less commonly, adrenal glands), and cardiac diseases with dilated cardiomyopathy and arrhythmias are the main complications.<sup>3</sup>

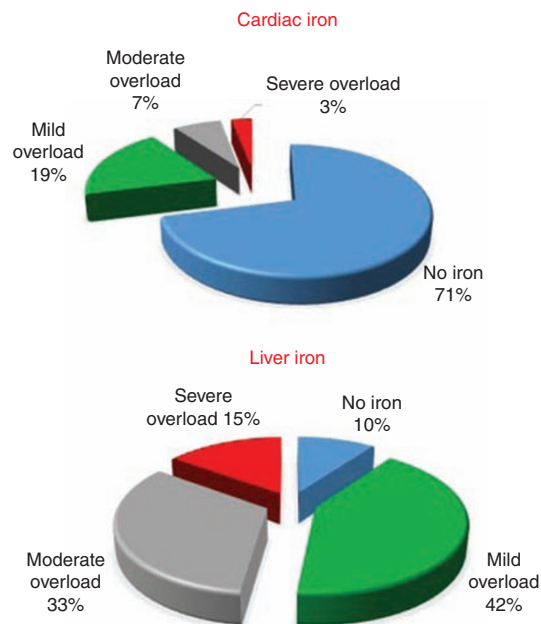
Other complications are hypersplenism, chronic hepatitis (resulting from infection with the viruses that cause hepatitis B and/or hepatitis C), cirrhosis (from iron overload and chronic hepatitis), HIV infection, venous thrombosis, and osteoporosis. Before 2000, approximately 50% of TM patients in the United Kingdom died before age 35 years.<sup>4</sup> The prognosis has dramatically improved over the past decades with the advent of noninvasive methods to measure organ iron before the appearance of clinical symptoms, new chelators, and increased blood safety measures.<sup>5,6</sup>

After 2000, all of these developments led to a significant trend in decreasing cardiac mortality, which was previously reported to cause 71% of deaths for individuals with TM.<sup>5,7,8</sup> Recent studies have shown that despite geographic differences, most individuals with transfusion-dependent thalassemia have normal cardiac iron; however, a significant proportion have simultaneous liver iron overload (**Figure 1**) and the number of patients who die from liver disorders now exceeds that of individuals who die from cardiac diseases in some European countries.<sup>7,11</sup> In particular, the risk for hepatocellular carcinoma has progressively increased secondary to liver viral infection, iron overload, and longer survival.<sup>12,13</sup>

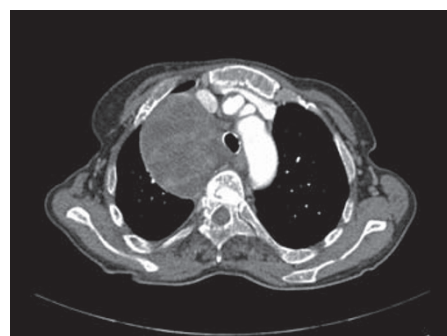
Infections remain a leading cause of death, especially in splenectomized patients.

### $\beta$ -thalassemia intermedia

Individuals with TI present later than TM, have milder anemia, and by definition do not require or only occasionally require transfusions. Sometimes, they are completely asymptomatic until adult life. Clinical features are pallor, mild to moderate jaundice, cholelithiasis, liver and spleen enlargement, moderate to severe bone modifications, leg ulcers, extramedullary masses of hyperplastic erythroid marrow (**Figure 2**), a tendency to develop osteopenia, osteoporosis, and thrombotic complications. Cardiac involvement in TI is mainly characterized by a high-output state and pulmonary hypertension, with systolic left ventricle function usually preserved.<sup>14</sup> Myocardial siderosis is rare.<sup>15</sup> Without appropriate treatment, the incidence of the comorbidities increases with advancing age.<sup>16</sup> Pseudoxanthoma



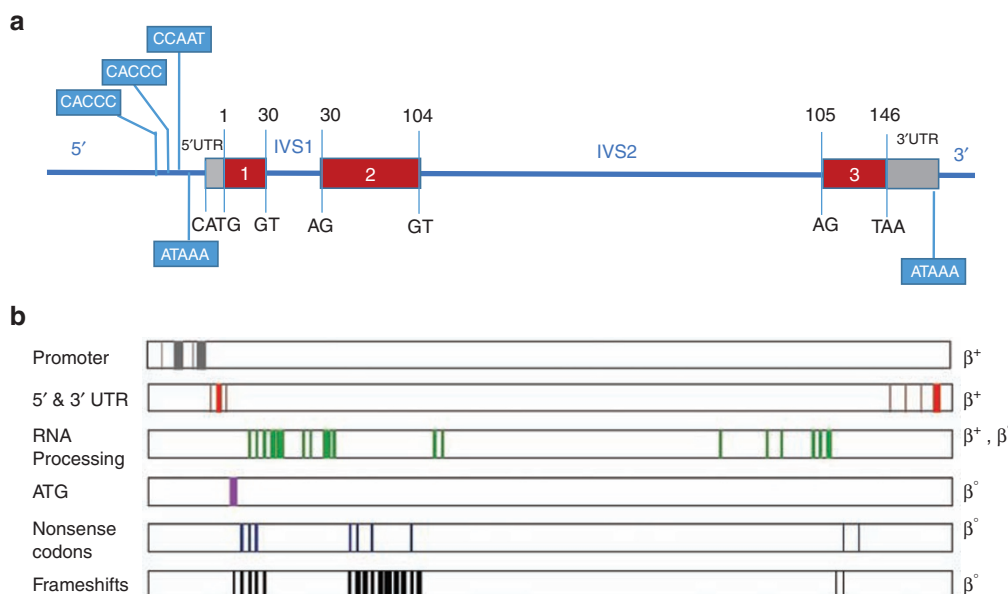
**Figure 1** Heart T2\* (ms) and liver iron concentration (LIC; mg/g dry weight) during magnetic resonance for 246 patients older than 16 years with transfusion-dependent  $\beta$ -thalassemia. Heart: T2\*  $\geq$  20 ms no iron, T2\*  $\geq$  14 < 20 ms mild iron overload, T2\*  $\geq$  8 < 14 ms moderate iron overload, and T2\* < 8 ms severe iron overload. Liver: LIC < 1.8 mg/g dry weight no iron, LIC  $\geq$  1.8 < 7 mg/g dry weight mild iron overload, LIC  $\geq$  7 < 15 mg/g dry weight moderate iron overload, and LIC  $\geq$  15 mg/g dry weight severe iron overload. To convert T2\* to LIC, the formula  $0.202 + 25.4/T2^*$  from ref. 76 was used. All the patients were undergoing regular observation and treatment at the Ospedale Microcitmico-Cagliari (Italy). Adapted from ref. 10.



**Figure 2** Chest computed tomography (CT) showing a pulmonary mass of extramedullary erythropoiesis (10×9×10 cm) in the right hemithorax of a woman with thalassemia intermedia. The patient, aged 64 years, also presented other masses in the same hemithorax and bilaterally at paravertebral level. She was admitted to the hospital with symptoms of acute respiratory insufficiency and right heart failure and subsequently treated with radiotherapy. Courtesy of Susanna Barella.

elasticum, a disorder affecting skin, eyes, blood vessels, and, less frequently, other areas, is characterized by the accumulation of deposits of calcium and other minerals in elastic fibers and has been described in such patients.<sup>17</sup>

Iron overload in nontransfused patients mainly occurs because of increased intestinal iron absorption due to greatly



**Figure 3 Molecular basis of  $\beta$ -thalassemia.** (a) Human  $\beta$ -globin gene in chromosome 11. Grey areas represent transcribed but not translated regions. Red areas represent the exons. Numbers represent the  $\beta$ -globin amino acids residues encoded by the three exons. (b) Schematic representation of the type and distribution of  $\beta$ -thalassemia mutations.

expanded but ineffective erythropoiesis.<sup>18,19</sup> Although the rate of iron loading is slower in TI than in TM, patients with TI can eventually develop complications similar to those of patients with TM, including hepatic, endocrine, and cardiac dysfunction.<sup>20</sup> Even transfusion-independent patients with TI can develop hepatocarcinoma, which is mainly attributed to a state of iron overload in the liver.<sup>13–21</sup>

### $\beta$ -thalassemia trait

Carriers of thalassemia are usually clinically asymptomatic but sometimes have mild anemia.

## MOLECULAR GENETICS AND GENETIC MODIFIERS

The  $\beta$ -globin gene (*HBB*) maps in the short arm of chromosome 11 in a region also containing the  $\delta$ -globin gene, the embryonic  $\epsilon$ -gene, the fetal  $A$ - $\gamma$  and  $G$ - $\gamma$  genes, and a pseudogene ( $\psi B1$ ). The five functional globin genes are arranged in the order of their developmental expression. *HBB*, which spans 1.6 Kb, contains three exons and both 5' and 3' untranslated regions (UTRs) (Figure 3). It is regulated by an adjacent 5' promoter in which TATA, CAAT, and duplicated CACCC boxes are located. A major regulatory region also containing a strong enhancer maps 50 Kb from *HBB*. This region, dubbed the locus control region, contains four (HS-1 to HS-4) erythroid-specific DNase hypersensitive sites (HSs), which are a hallmark of DNA-protein interaction. Each HS site constitutes a combination of several DNA motifs interacting with transcription factors, among which the most important are GATA-1 (GATA indicates the relative recognition motif), nuclear factor erythroid 2, erythroid Krüppel-like factor 1 (KLF1), and friend of GATA 1. The importance of the locus control region

for the control of  $\beta$ -like globin gene expression was discovered by studying a series of naturally occurring deletions that totally or partly remove the HS sites and result in the inactivation of the intact downstream *HBB*. Several transcription factors bind and regulate the function of *HBB*, the most important of which is KLF1, which binds the proximal CACCC box and whose knockout in the mouse leads to a  $\beta$ -thalassemia-like clinical picture.

In addition to the phenotypic level,  $\beta$ -thalassemias are also very heterogeneous at the molecular level, with more than 200 mutations reported to date; a complete, current list is available at the Globin Gene Server (<http://globin.cse.psu.edu/>). The large majority of mutations are single-nucleotide substitutions, insertions of single nucleotides, or small oligonucleotides leading to frameshift in functionally important regions of *HBB*.<sup>22</sup> Gross gene deletions are uncommon.

$\beta^0$ -thalassemias, characterized by the complete absence of  $\beta$ -chain production, result from deletion, initiation codon, nonsense, frameshift, and splicing mutations, especially at the splice-site junction. By contrast,  $\beta^+$ -thalassemias, characterized by reduced production of the  $\beta$ -chains, are produced by mutations in the promoter area (either the CACCC or TATA box), the polyadenylation signal, and the 5' or 3' UTR or by splicing abnormalities. According to the extent of the reduction of the  $\beta$ -chain output, the  $\beta$ -thalassemia mutations may be categorized as severe, mild, or silent (Supplementary Table S1 online).

In rare instances, the  $\beta$ -thalassemia defect does not lie in *HBB* or in *HBB* cluster. In instances in which the  $\beta$ -thalassemia trait is associated with other features, the molecular lesion has been found either in the gene encoding the transcription factor *TFIIF* ( $\beta$ -thalassemia trait associated with xeroderma

**Table 1** Molecular mechanisms leading to thalassemia intermedia

Factors that may have an ameliorating effect on the clinical picture of $\beta$ -thalassemia	Comments
<b>Homozygous or compound heterozygous state for <math>\beta</math>-thalassemia mutations</b>	
Inheritance of mild or silent $\beta$ -thalassemia alleles	Mutations in the promoter of the $\beta$ -globin gene, the poly-A cleavage site, cryptic splice sites in exons, or consensus sequences in introns
Coinheritance of $\alpha$ thalassemia	Reduction of the $\alpha$ -globin chain product decreases the $\alpha$ /non- $\alpha$ -chain imbalance
Increased synthesis of HbF (within the $\beta$ -globin cluster)	
Deletion HPFH	$\delta\beta^0$ -thalassemia due to deletions within the $\beta$ -globin cluster Deletions involving the 5' region of the $\beta$ -globin promoter
Nondeletion HPFH	
Increased synthesis of Hb F (outside the $\beta$ -globin cluster)	Point mutations at G $\gamma$ or A $\gamma$ promoters (-196 C→T A $\gamma$ , -158 C→T G $\gamma$ (XmnI))
BCL11A (chromosome 2)	Involved in the regulation of the globin switching process SNP rs11886868 influences persistence of HbF in Sardinians and in nonanemic Caucasians and ameliorates the phenotype in thalassemia and sickle cell diseases
HBS1L-MYB (chromosome 6)	Regulatory sequences controlling MYB expression Further studies necessary to elucidate the biological role of this gene in the modulation of HbF
KLF1 (chromosome 19)	Zinc-finger erythroid transcriptional regulator that binds to the critical promoter elements of the adult $\beta$ -globin gene Direct activation of $\beta$ -globin and indirect repression of $\gamma$ -globin gene expression in adult erythroid precursors via regulation of BCL11A In China, KLF1 mutations selectively represented in $\beta$ -thalassemia intermedia patients
AHSP	In mice, knockout of AHSP led to reduced life span of circulating RBCs, caused increased apoptosis of erythroid precursors, and exacerbated the severity of heterozygous $\beta$ -thalassemia In humans, contrasting results
<b>Compound heterozygosity for <math>\beta</math>-thalassemia and HbE (thalassemic structural variant)</b>	
<b>Compound heterozygosity for <math>\beta</math>-thalassemia and other <math>\beta</math>-chain structural variant (HbD-Los Angeles <math>\beta</math> 121 Glu→Gln; HbC <math>\beta</math> 6 Glu→Lys; HbO-Arab <math>\beta</math> 121 Glu→Lys unstable variants)</b>	
<b>Heterozygous state for <math>\beta</math>-thalassemia</b>	
Coinheritance of excess $\alpha$ globin genes ( $\alpha\alpha\alpha/\alpha\alpha$ , $\alpha\alpha\alpha/\alpha\alpha\alpha$ , $\alpha\alpha\alpha\alpha/\alpha\alpha\alpha$ )	Worsening of the imbalance of $\alpha$ /non- $\alpha$ -globin chain synthesis, which may cause an excess of unassembled $\alpha$ -chains, thereby resulting in premature destruction of RBC precursors
Dominantly inherited $\beta$ thalassemia	Abnormal hyper-unstable protein product that precipitates in the RBC membrane together with unassembled $\alpha$ -globin chains, resulting in markedly ineffective erythropoiesis
Mosaic somatic deletion of the in trans $\beta$ -globin gene in a subpopulation of hematopoietic cells	Very rare

AHSP,  $\alpha$ -hemoglobin-stabilizing protein; HPFH, hereditary persistence of hemoglobin F; RBC, red blood cell; SNP, single-nucleotide polymorphism.

pigmentosum and trichothiodystrophy) or in the X-linked transcription factor *GATA-1* (X-linked thrombocytopenia with thalassemia).<sup>23,24</sup>

Despite marked molecular heterogeneity, the prevalent molecular defects are limited in each at-risk population, in which 4 to 10 variants usually account for most of *HBB* disease-causing alleles.

Because the main pathophysiological determinant of the severity of the  $\beta$ -thalassemia syndromes is the extent of  $\alpha$ /non- $\alpha$ -globin chain imbalance, any factor capable of reducing this imbalance results in a lesser degree of  $\alpha$ -globin chain precipitation and may ameliorate the clinical picture as shown in **Table 1**.

The clinical phenotype of homozygous  $\beta$ -thalassemia may also be altered by the coinheritance of secondary genetic modifiers mapping outside *HBB* cluster and mainly influencing

the complications of the thalassemia phenotype. The best known of these modifying genes is *UGT1A*, the gene encoding uridin-diphosphoglucuronyltransferase. When combined with TM or TI, the *UGT1A* pathogenic variant causing Gilbert disease—i.e., (TA)<sub>7</sub> configuration instead of the (TA)<sub>6</sub> in the TATA box—leads to increased jaundice and increased risk of gallstones.<sup>25</sup> Genetic variations of the glutathione S-transferase M1 (GSTM1) enzyme seem to be associated with cardiac iron deposition; in individuals with TM who have low body iron, the GSTM1-null genotype is a predisposing factor for myocardial iron overload, possibly due to enhanced entry of iron into the heart when GSTM1 is absent.<sup>26</sup>

Less defined modifying factors are genes coding for HFE-associated hereditary hemochromatosis, probably because their effect on iron overload is hidden as a result of treatment and genes involved in bone metabolism.<sup>27,28</sup>



## **PATHOPHYSIOLOGY**

Imbalance in  $\alpha$ -non- $\alpha$ -globin chains is the basis of  $\beta$ -thalassemia.  $\alpha$ -Globin tetramers accumulate and precipitate in the erythroid precursors forming inclusion bodies that, bound to the membrane skeleton, cause oxidative membrane damage and extensive premature destruction by apoptosis of the RBC precursors in the bone marrow (ineffective erythropoiesis). Hemolysis plays a secondary role. Hypertrophy of erythroid marrow in medullary and extramedullary sites results in characteristic deformities of the skull and face, may cause cortical thinning and pathological fractures of long bones, and may lead to the formation of extramedullary erythropoietic tissue masses. The lipid membrane composition of abnormal RBCs may result in thrombotic complications, especially in splenectomized patients.<sup>29,30</sup> In nontransfused patients, erythropoiesis, anemia, and hypoxia downregulate hepcidin, the master regulator of iron homeostasis.<sup>18,31,32</sup>

Hepcidin deficiency allows excessive duodenal iron absorption and development of systemic iron overload. In regularly transfused patients, iron overload is due mostly to red cell breakdown. When the iron-binding capacity of transferrin is saturated, iron can appear in the serum as non-transferrin-bound iron, which is a powerful catalyst for the formation of free radicals capable of causing oxidative stress and damage to mitochondria, lysosomes, lipid membranes, protein, and DNA, consequently leading to organ compromise typical of thalassemia.

## **DIAGNOSIS**

### **Clinical diagnosis**

TM is suspected in infants or children less than 2 years old with severe microcytic anemia, mild jaundice, and hepatosplenomegaly. TI is suspected in individuals who present at a later age with similar but milder clinical findings.

### **Hematologic diagnosis**

RBC indexes show microcytic anemia. TM is characterized by reduced Hb level ( $<7$  g/dl), mean corpuscular volume (MCV)  $> 50 < 70$  fl, and mean corpuscular Hb (MCH)  $> 12 < 20$  pg. TI is characterized by Hb levels between 7 and 10 g/dl, MCV  $> 50 < 80$  fl, and MCH  $> 16 < 24$  pg. Thalassemia minor is characterized by reduced MCV and MCH, with increased HbA2 level.<sup>33</sup>

The peripheral blood smear in affected individuals demonstrates RBC morphologic changes with microcytosis, hypochromia, anisocytosis, poikilocytosis, and nucleated RBCs. The number of erythroblasts is related to the degree of anemia and is markedly increased following splenectomy.

Carriers demonstrate reduced MCV, MCH, and RBC morphologic changes that are less severe than in affected individuals. Erythroblasts are normally not seen.

Qualitative and quantitative Hb analyses (by cellulose acetate electrophoresis and DE-52 microchromatography or high-performance liquid chromatography (HPLC)) identify the amount and type of Hb present.

The Hb pattern varies by  $\beta$ -thalassemia type. HbA2 is enhanced in  $\beta$ -thalassemia minor and variable in  $\beta$ -thalassemia homozygotes and compound heterozygotes.  $\beta^0$ -Thalassemia omozygotes show complete absence of globin  $\beta$ -chain production and HbF constitutes 92–95% of the total Hb. In  $\beta^+$ -homozygotes and  $\beta^+/\beta^0$  genetic compounds, HbF is 70–90% and HbA 10–30% according to the variable degree of reduction of  $\beta$ -globin chain synthesis.

Hb electrophoresis and HPLC also detect other hemoglobinopathies that may interact with  $\beta$ -thalassemia.

### **Molecular diagnosis**

Molecular testing approaches can include targeted analysis for pathogenic variants or single-gene testing. Because the prevalent pathogenic variants are limited in each at-risk population, targeted analysis for pathogenic variants based on ancestry may be considered first.

Commonly occurring mutations of HBB are detected by polymerase chain reaction–based procedures. The most commonly used methods are reverse dot blot analysis and primer-specific amplification, with a set of probes or primers complementary to the most common mutations in the population from which the affected individual originated.

If targeted mutation analysis fails to detect the mutation, *HBB* sequence analysis can be used to detect mutations in *HBB*. Sequence analysis may be considered first if the affected individual is not of an ancestry at high risk or if targeted analysis reveals only one or no pathogenic variant.

Gene-targeted deletion/duplication analysis of *HBB* may be considered if only one or no pathogenic variant is found after sequence analysis has been performed.

### **Establishing the diagnosis**

The diagnosis of  $\beta$ -thalassemia is established in a proband older than age 12 months based on the hematologic findings of microcytic hypochromic anemia, nucleated RBCs on peripheral blood smear, and Hb analysis that reveals decreased amounts of HbA and increased amounts of HbF.

The diagnosis of  $\beta$ -thalassemia is established in a proband younger than age 12 months by detecting a complete absence of HbA. Definitive diagnosis of  $\beta^+$ -thalassemia by Hb electrophoresis and HPLC is not possible in the newborn period because the diminished amount of HbA overlaps the range for normal babies. Thalassemia may be suspected based on the microcytic hypochromic anemia with nucleated RBCs on peripheral blood smear and confirmed with the molecular analysis of *HBB*.

## **DIFFERENTIAL DIAGNOSIS**

Few conditions share similarities with homozygous  $\beta$ -thalassemia.

- Some molecular lesions of the  $\beta$ -gene, most commonly in exon 3, produce an abnormal hyper-unstable protein product that precipitates in the RBC membrane together with unassembled  $\alpha$ -globin chains, resulting in markedly

ineffective erythropoiesis (dominant  $\beta$ -thalassemias or thalassemic hemoglobinopathies). HBB sequencing establishes the diagnosis.

- The genetically determined sideroblastic anemias (e.g.,  $\delta$ -aminolevulinic acid synthase deficiency) are easily differentiated because of ring sideroblasts in the bone marrow and variably elevated serum concentration of erythrocyte protoporphyrin.
- Congenital dyserythropoietic anemias do not have high HbF and do have other distinctive features, such as multinuclearity of the RBC precursors.
- A few acquired conditions associated with high HbF (juvenile chronic myeloid leukemia, aplastic anemia) may be mistaken for  $\beta$ -thalassemia, even though they have very characteristic hematologic features.

### POPULATION SCREENING

Because of the high carrier rate for HBB mutations in certain populations and the availability of genetic counseling and prenatal diagnosis, population screening is ongoing in several at-risk populations in the Mediterranean. Carrier testing relies on hematological analysis. When the hematological analysis indicates a  $\beta$ -thalassemia carrier state, molecular genetic testing of HBB can be performed to identify a disease-causing mutation. If both partners of a couple have HBB disease-causing mutation, each of their offspring has a 25% risk of being affected. Through genetic counseling and the option of prenatal testing, such a couple can opt to bring to term only the pregnancies in which the fetus is unaffected.

The optimal time for the assessment of genetic risk, definition of carrier status, and genetic counseling is before pregnancy. It is appropriate to offer genetic counseling (including discussion of the availability of prenatal diagnosis, potential risks to offspring, and reproductive options) to young adults who are carriers.

The classic phenotype of heterozygous  $\beta$ -thalassemia may be modified by several genetic determinants, with resulting potential problems in carrier identification (**Supplementary Table S2** online).<sup>34</sup>

A flowchart for thalassemia carrier identification is shown in **Supplementary Figure S1** online.

Population screening associated with genetic counseling is extremely useful because it allows couples at risk to make informed decisions regarding their reproductive choices. Furthermore, in the population at risk targeted by screening, a consistent reduction of the birth rate of affected children has been registered, as has been shown in the Sardinian population.<sup>35</sup>

### Prenatal diagnosis

Prenatal diagnosis for pregnancies at increased risk is possible by analysis of DNA extracted from fetal cells obtained by amniocentesis, usually performed at approximately 15–18 weeks of gestation, or chorionic villi sampling at 11 weeks of gestation. Both disease-causing alleles must be identified

before prenatal testing can be performed. Analyses of fetal cells in maternal blood and of fetal DNA in maternal plasma for the presence of the father's mutation are currently being conducted.<sup>36</sup> Preimplantation genetic diagnosis may be available for families in which disease-causing mutations have been identified.<sup>37</sup>

### MANAGEMENT OF THALASSEMIA INTERMEDIA

Treatment of individuals with TI is symptomatic.<sup>38</sup> Supplementary folic acid can be prescribed to patients with TI to prevent deficiency from hyperactive bone marrow.

Because hypersplenism may cause worsening anemia, retarded growth, and mechanical disturbance from the large spleen, splenectomy is a relevant aspect of TI management. Because of the increased prevalence of cholelithiasis and the risks of cholecystitis in splenectomized patients, the gallbladder should be inspected during splenectomy and removed if appropriate. Treatment of extramedullary erythropoietic masses detected by magnetic resonance imaging (MRI) is based on radiotherapy, transfusions, or hydroxycarbamide. Once a leg ulcer has developed, it is very difficult to manage. Regular blood transfusions, zinc supplementation, pentoxifylline, and the use of an oxygen chamber have been proposed for ulcer treatment. Hydroxycarbamide also has some benefits, either alone or with erythropoietin. Recently promising results have been obtained with platelet-derived growth factor. Because patients with TI are at high risk for thrombosis, exacerbated by splenectomy, it is important to be aware of thrombotic complications. Recommended treatment options include proper anticoagulation prior to surgical or other high-risk procedures, platelet antiaggregating agents in the case of thrombocytosis, and low-molecular-weight heparin in patients with documented thrombosis.

Although clinical trials evaluating the role of transfusion therapy in TI patients are lacking, observational studies also suggest a role for blood transfusions in the prevention and management of the following clinical morbidities frequently encountered in adult patients: leg ulcers, thrombotic events, pulmonary hypertension, silent brain infarcts, and extramedullary hematopoietic pseudotumors.<sup>39</sup>

Some patients may require frequent transfusions, but they are usually temporary and can be tailored or withdrawn when the desired outcomes are achieved. Presence of alloantibodies and autoantibodies may severely compromise transfusion therapy for patients with TI who receive their first transfusions during adolescence or later.<sup>40</sup> Because individuals with TI may develop iron overload from increased gastrointestinal absorption of iron or occasional transfusions, chelation therapy should be started in case of serum ferritin concentration exceeding 800 ng/ml or liver iron concentration >5 mg Fe/g dry weight, which represent thresholds after which the risk of serious iron-related morbidity is increased.<sup>41</sup> Deferasirox is the only iron chelator specifically approved for non-transfusion-dependent patients when deferoxamine therapy is contraindicated or inadequate in patients aged 10 years and older.<sup>42</sup>

## MANAGEMENT OF THALASSEMIA MAJOR

### Transfusions

The goals of transfusion therapy are correction of anemia, suppression of erythropoiesis, and inhibition of gastrointestinal iron absorption, which occurs in transfused patients as a consequence of increased, although ineffective, erythropoiesis.

Before starting transfusions, diagnosis of thalassemia should be confirmed; the molecular defect, the severity of anemia on repeated measurements, the level of ineffective erythropoiesis, and clinical criteria such as failure to thrive or bone changes with facial deformities should be taken into account.<sup>43</sup>

Before the first transfusion, it is absolutely necessary to administer a hepatitis B vaccination and perform extensive RBC antigen typing, including Rh, Kell, Kidd, and Duffy, and serum immunoglobulin determination—the last of which detects individuals with IgA deficiency who need special (repeatedly washed) blood unit preparation before each transfusion.

The recommended treatment for TM involves blood transfusions administered every 2 to 5 weeks to maintain the pre-transfusion Hb level above 9.0–10.5 g/dl. This regimen allows normal growth and physical activities, minimizes transfusional iron accumulation, and suppresses bone marrow expansion in most patients.<sup>44</sup>

The amount of transfused RBCs should not exceed 15 to 20 ml/kg per day, infused at a maximum rate of 5 ml/kg/h, to avoid a rapid increase in blood volume. To monitor the effectiveness of transfusion therapy, some indexes such as pre- and posttransfusion Hb, amount and hematocrit of the blood unit, daily Hb decline, and transfusional interval—parameters important to calculate RBC requirement and iron intake—should be recorded at each transfusion.

Over the past decade, there has been increased development of pathogen-reduction technologies to protect the blood supply from emerging pathogens.

### Splenectomy

Splenectomy should be taken into consideration in only a certain few circumstances because of the observation of an increased risk of venous thrombosis and pulmonary hypertension along with overwhelming infections after splenectomy.<sup>45,46</sup> Whenever possible, splenectomy should be avoided in small children because of a considerably greater risk of fulminant postsplenectomy sepsis, mainly from encapsulated bacteria (*Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Neisseria meningitidis*). Prevention of postsplenectomy sepsis includes immunization against the afore-mentioned bacteria and antibiotic prophylaxis as well as early antibiotic treatment for fever and malaise.

The main indications for splenectomy in TM are an increased blood requirement (annual blood requirement >200–220 ml/kg/year) that prevents adequate control with iron chelation therapy, hypersplenism with cytopenias, and symptomatic splenomegaly with risk of splenic rupture.<sup>45</sup>

### Prevention and treatment of iron overload

Iron overload is an unavoidable consequence of regular transfusions because the human body lacks a mechanism to excrete excess iron. However, the most common complications related to transfusional hemosiderosis, such as heart failure, cirrhosis, growth retardation, and multiple endocrine abnormalities, can be prevented and, to some extent, reverted by adequate iron chelation.

Three iron chelators are currently available. Desferrioxamine B, available in clinical practice for nearly 50 years, was first to unequivocally demonstrate the value of iron chelation therapy for removing excess iron from the body.<sup>7</sup> Because desferrioxamine is a large molecule with low oral availability and a short half-life (approximately 20 min), treatment requires slow subcutaneous infusion via a portable pump for a period of 8–12 hours 5–7 days per week. Ascorbate repletion (daily dose not to exceed 100–150 mg) increases the amount of iron removed after desferrioxamine administration. Side effects of desferrioxamine chelation therapy are more common in the presence of relatively low iron burden and include ocular and auditory toxicity, growth retardation, and, rarely, renal impairment and interstitial pneumonitis. Desferrioxamine administration also increases susceptibility to *Yersinia* infections. However, the major drawback of desferrioxamine chelation therapy is low compliance resulting from the cumbersome method of administration. These limitations have led to the search of oral chelators that are at least as effective as desferrioxamine and have a reasonable tolerability profile.

The first oral chelator to be licensed was three-times-daily deferiprone, a bidentated oral drug approved for TM patients for whom desferrioxamine therapy is contraindicated or inadequate. The main side effects of deferiprone therapy are arthropathy, gastrointestinal symptoms, and, most importantly, neutropenia and agranulocytosis, which demand close monitoring.<sup>47</sup> The effect of deferiprone on liver iron concentration may vary among the individuals treated. Results from independent studies demonstrate that deferiprone is more cardioprotective than desferrioxamine; compared with those treated with desferrioxamine, individuals treated with deferiprone have better myocardial MRI patterns and less probability of developing (or worsening preexisting) cardiac disease.<sup>48,49</sup> These retrospective observations were confirmed in a prospective study.<sup>50</sup>

Deferasirox was developed as a once-daily oral monotherapy for the treatment of transfusional iron overload. It is effective in adults and children and has a defined safety profile that is clinically manageable with appropriate monitoring. The most common treatment-related adverse events are gastrointestinal disorders, skin rash, and a mild, nonprogressive increase in serum creatinine concentration.<sup>51</sup> Cases of renal failure, hepatic failure, cytopenias, and gastrointestinal hemorrhage have been reported in the postmarketing phase. If adequate doses are administered, there is a good response to deferasirox across the full range of baseline iron concentration values in the

liver. Prospective data demonstrate the efficacy of deferasirox in improving myocardial T2\* and maintaining a normal left ventricle ejection fraction.<sup>52</sup> Deferasirox has not been evaluated in formal trials for affected individuals with symptomatic heart failure or low left ventricular ejection fraction.

Strategies of chelation using a combination of desferrioxamine and deferiprone have been effective in individuals with severe iron overload. Retrospective, prospective, and randomized clinical studies have shown that combined iron chelation with deferiprone and desferrioxamine rapidly reduces myocardial siderosis, improves cardiac and endocrine function, reduces liver iron and serum ferritin concentrations, reduces cardiac mortality, and improves survival; furthermore, toxicity is manageable.<sup>52–54</sup>

When deferiprone cannot be used, combination desferrioxamine and deferasirox may be considered for patients with severe transfusional myocardial siderosis. In a prospective trial, almost one-third of severely overloaded patients treated with this combination exhibited a reduction in their cardiac overload, along with a rapid decrease in liver iron concentration.<sup>55</sup>

Recent studies show that the deferiprone and deferasirox combination is as effective as the deferiprone and desferrioxamine combination in decreasing liver iron and serum ferritin, and it is superior in increasing myocardial iron, patient satisfaction, and quality of life.<sup>56</sup>

The possibility that cardiac siderosis and amlodipine combined with chelation therapy reduces cardiac iron more effectively than chelation therapy alone is currently under investigation.<sup>57</sup>

Chelation dosing and regimens require adjustments for changing circumstances. These can be identified via careful monitoring of iron and its distribution while avoiding the risk of underchelation with increased iron toxicity or of overchelation and increased chelator toxicity. The iron status of multitransfused patients can be assessed by several methods, including serum ferritin and liver iron concentration measurements by liver biopsy. In recent years, MRI techniques for assessing iron loading in the liver and heart have been introduced (**Figure 4**).<sup>58</sup> R2 and T2\* parameters have been validated for liver iron concentration. Cardiac T2\* is reproducible, transferable between different scanners, correlates with cardiac function, and relates to tissue iron concentrations. The clinical utility of

monitoring T2\*, as well as its prognostic value in patients with siderotic cardiomyopathy has been demonstrated.<sup>59</sup> MRI R<sub>2</sub>\* (1/T2\*) has recently been calibrated against myocardial iron in humans.<sup>60</sup> Magnetic biosusceptometry (SQUID) is another option for obtaining a reliable measurement of hepatic iron concentration, but it is currently available in only a limited number of centers worldwide.

Methods of managing iron-related complications and follow-up are listed in **Supplementary Table S3** online.

### Pregnancy management

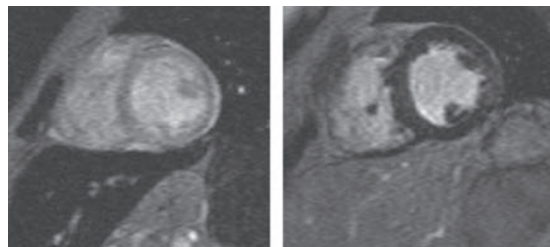
An increasing number of women with TM have expressed a desire to have children, and recent reports indicate that most may have a successful pregnancy with no major complications. Although hypogonadotropic hypogonadism remains a common condition, gonadal function is usually intact and fertility is usually retrievable. A multidisciplinary team, including a cardiologist, an endocrinologist, and a gynecologist, with the supervision of an expert in  $\beta$ -thalassemia, should follow these women throughout the duration of pregnancy.<sup>61</sup>

Pregnancy also appears to be safe for most women with TI, although larger and more detailed studies are needed. Indeed, an increased risk for certain complications cannot yet be excluded. For example, women with TI who had never previously received a blood transfusion or who had received a minimal quantity of blood are reported to be at risk for severe alloimmune anemia if blood transfusions are required during pregnancy.<sup>61</sup>

### Bone marrow transplantation

Bone marrow transplantation (BMT) from an HLA-identical sibling, a widely applied alternative to traditional transfusion and chelation therapy, is the only available curative option for TM. Traditionally, the outcome of BMT is related to the pre-transplantation clinical conditions, specifically the presence of hepatomegaly, extent of liver fibrosis, and magnitude of iron accumulation.<sup>62</sup> In children who lack these risk factors, disease-free survival is more than 90%. Treosulfan-based conditioning has improved overall and thalassemia-free survival, even in high-risk patients. Adults with  $\beta$ -thalassemia have a greater probability of transplant-related toxicity due to an advanced phase of the disease, and the cure rate is approximately 65% with current treatment protocols.<sup>63</sup> BMT from unrelated donors has been carried out in several individuals with  $\beta$ -thalassemia. Provided that donor selection is based on stringent criteria of HLA compatibility, and that individuals have limited iron overload, the results are comparable to those obtained when the donor is a compatible sibling.<sup>64</sup> Affected individuals without matched donors could also benefit from haploidentical mother-to-child transplantation, the results of which appear encouraging.<sup>65</sup>

Cord blood transplantation from a related donor is associated with a low risk for graft-versus-host disease and offers good probability of a successful cure if an adequate number of nucleated cells are harvested and infused; however, data on



**Figure 4** Lack of correlation between liver and cardiac iron at magnetic resonance in patients with  $\beta$ -thalassemia. (Left) A patient with no iron in the interventricular septum and severe liver overload. (Right) A patient with severe iron overload in the interventricular septum and no liver overload.



unrelated cord blood transplantation in individuals with thalassemia are conflicting.<sup>66</sup>

When considering the very significant costs of lifelong blood transfusions, chelation, and management of the complications for optimal thalassemia care, transplantation is a cost-effective option, even in developing countries. At present, more than 30% of procedures are regularly performed in nonindustrialized countries, with no significant differences from those performed in industrialized countries in terms of patient outcomes.<sup>67</sup>

### Therapies under investigation

Induction of fetal Hb synthesis can reduce the severity of  $\beta$ -thalassemia by improving the imbalance between  $\alpha$ -globin and non- $\alpha$ -globin chains. Several pharmacologic compounds including 5-azacytidine, decytidine, and butyrate derivatives have had encouraging results in clinical trials. These agents induce Hb F by different mechanisms that are not yet well defined. Their potential in the management of  $\beta$ -thalassemia syndromes is under investigation.<sup>68</sup>

Hydroxyurea is used in persons with TI to reduce extramedullary masses, increase Hb levels, and, in some cases, improve leg ulcers. A retrospective study found no pulmonary hypertension in 50 individuals with TI treated with hydroxyurea for 7 years. A good response, correlated with particular polymorphisms in the  $\beta$ -globin cluster, has been reported in individuals with transfusion dependence. However, controlled and randomized studies are warranted to establish the role of hydroxyurea in the management of thalassemia syndromes.<sup>68</sup>

Recent studies have shown that interrupting the vicious cycle between ineffective erythropoiesis and iron overload may be of therapeutic benefit in thalassemias. Induction of iron restriction by means of transferrin infusions, minihepcidins, or manipulation of the hepcidin pathway prevents iron overload, redistributes iron from parenchymal cells to macrophage stores, and partially controls anemia in  $\beta$ -thalassemic mice.<sup>32–69</sup>

Modulators of erythropoiesis, such as TGF- $\beta$ -like molecules or inhibitors of JAK2, could soon revolutionize the treatment of  $\beta$ -thalassemia and related disorders.

Activins, members of TGF- $\beta$  family signaling, are key regulators of human hematopoiesis and modulate various cellular responses such as proliferation, differentiation, migration, and apoptosis. A modified activin type IIB receptor inhibiting signaling induced by some members of the TGF- $\beta$  superfamily promotes maturation of terminally differentiating erythroblasts. In thalassemic mice, it ameliorates hematologic parameters as well as comorbidities that develop as a consequence of the erythroid hyperplasia.<sup>70</sup>

The discovery that JAK2 plays an important role in the progression and exacerbation of ineffective erythropoiesis suggests that drugs inhibiting JAK2 activity could mitigate ineffective erythropoiesis and reverse splenomegaly. In fact, in preclinical studies it has been shown that a JAK2 inhibitor dramatically decreased spleen size and modulated ineffective erythropoiesis.<sup>71</sup>

The possibility of correction of the molecular defect in hematopoietic stem cells by transfer of a normal gene via a suitable vector or by homologous recombination is being actively investigated. The most promising results in the mouse model have been obtained with lentiviral vectors.

Several clinical trials of gene therapy for  $\beta$ -TM are ongoing in France, Italy, and the United States. One individual with transfusion-dependent HbE/ $\beta$ -thalassemia treated in France exhibited a therapeutic effect after transplantation with autologous CD34<sup>+</sup> cells genetically modified with a  $\beta$ -globin lentiviral vector and had not required blood transfusions as of 4 years after the transplantation.<sup>72</sup> Very encouraging preliminary data on individuals with HbE/ $\beta$ -thalassemia and homozygous  $\beta$ -thalassemia transplanted with autologous CD34<sup>+</sup> cells transduced with a replication-defective, self-inactivating lentiviral vector containing an engineered HBB ( $\beta$ A-T87Q) were recently reported by the same group.<sup>73</sup>

Other approaches being investigated for gene therapy of the  $\beta$ -hemoglobinopathies include pharmacological or genetic induction of  $\gamma$ -globin production through interference with the BCL11A pathway or disruption of the BCL11A erythroid enhancer by CRISPR/CAS9 technology as well as zinc finger or transcription activator–like effector nuclease, and even using genome editing in attempts at repairing the defective HBB in hematopoietic stem cells.<sup>74</sup>

### SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/gim>

### DISCLOSURE

The author declares no conflict of interest.

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