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β -Thalassemia: Genotypes and Phenotypes

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Abstract

β -Thalassemias are extremely heterogeneous at the molecular level. More than 200 disease-causing mutations have been identified. The majority of mutations are single nucleotide substitutions. Rarely, β -thalassemia results from gross gene deletion. The degree of globin chain imbalance is determined by the nature of the mutation of the β -gene. β^0 refers to the complete absence of production of β -globin on the affected allele. β^+ refers to alleles with some residual production of β -globin (around 10%). In β^{++} , the reduction in β -globin production is very mild. The broad spectrum of β -thalassemia alleles can produce a wide spectrum of different β -thalassemia phenotypes. In this chapter, we review the molecular basis of the marked heterogeneity of the thalassemia syndromes or in other words the genotype-phenotype relationship in β -thalassemia.

Keywords: β -Thalassemia, genotype, phenotype, mutation, disease

1. Introduction

β -Thalassemia syndromes are a group of hereditary blood disorders characterized by reduced or absent β -globin chain synthesis, resulting in reduced Hb in red blood cells (RBCs), decreased RBC production, and anemia. β -Thalassemia includes three main forms: Thalassemia Major, variably referred to as “Cooley’s Anemia” and “Mediterranean Anemia,” Thalassemia Intermedia, and Thalassemia Minor also called “ β -thalassemia carrier,” “ β -thalassemia trait,” or “heterozygous β -thalassemia” [1].

The β -thalassemia syndromes are much more diverse than the α -thalassemia syndromes due to the diversity of the mutations that produce the defects in the β -globin gene. The severity of β -thalassemia relates to the degree of imbalance between the α - and non- α -globin chains. The

β -globin gene maps in the short arm of chromosome 11, in a region that contains also the delta globin gene, the embryonic epsilon gene, the fetal gamma genes, and a pseudogene ($\psi B1$) [1].

Unlike the deletions that constitute most of the α -thalassemia syndromes, β -thalassemias are caused by hundreds of mutations that affect all aspects of β -globin production: transcription, translation, and the stability of the β -globin product [2].

2. Classification of β -thalassemias

1. β -Thalassemia

- Thalassemia major
- Thalassemia intermediate
- Thalassemia minor

2. β -Thalassemia with associated Hb anomalies

- HbC/ β -thalassemia
- HbE/ β -thalassemia
- HbS/ β -thalassemia

3. Hereditary persistence of fetal Hb and β -thalassemia

4. Autosomal dominant forms of β -thalassemia

5. β -Thalassemia associated with other manifestations

- β -Thalassemia-trichothiodystrophy
- X-linked thrombocytopenia with thalassemia

2.1. Epidemiology of β -thalassemias

The frequency of β -thalassemia varies widely, depending on the ethnic population. The disease is reported most commonly in Mediterranean, African, and Southeast Asian populations. The highest carrier frequency is reported in Cyprus (14%), Sardinia (10.3%), and Southeast Asia [1].

Population migration and intermarriage between different ethnicities have introduced thalassemia in almost every country of the world, including Northern Europe, where thalassemia was previously absent [2].

About 1.5% of the global population (80–90 million) are β -thalassemia carriers, with about 60,000 symptomatic individuals born annually. Incidence of symptomatic individuals is estimated at 1 in 100,000 worldwide and 1 in 10,000 in Europe [2].

It is the most common chronic hemolytic anemia in Egypt (85.1%), and its carrier rate has been estimated at 9–10.2% from an examination of 1000 normal random subjects from different geographic areas of the country [3].

2.2. Etiology of β-thalassemia

β-Thalassemia is inherited as an autosomal recessive disorder. There are hundreds of mutations within the β-globin gene, but approximately 20 different alleles comprise 80% of the mutations found worldwide. Within each geographic population, there are unique mutations. The large majority of mutations are point mutations. Deletions of β-globin gene are uncommon. Mutations in β-globin gene cause a reduced or absent production of the β-globin chains [4]. **Table 1** displays the list of common mutations according to severity and ethnic distribution.

β-Gene mutation	Ethnicity	Severity
-619 del	Indian	β ⁰
-101 C→T	Mediterranean	β ⁺⁺
-88 C→T	Black	β ⁺⁺
-87 C→G	Mediterranean, African	β ⁺⁺
-31 A→G	Japanese	β ⁺⁺
-29 A→G	African	β ⁺⁺
-28 A→C	Southeast Asian	β ⁺⁺
IVS1-nt1 G→A	Mediterranean, Asian Indian	β ⁰
IVS1-nt5 G→C	East Asian, Asian Indian	β ⁰
IVS1-nt6 T→C	Mediterranean	β ^{+/+}
IVS1-nt110 G→A	Mediterranean	β ⁺
IVS2-nt654 C→T	Chinese	β ⁺
IVS2-nt745 C→G	Mediterranean	β ⁺
Codon 39 C→T	Mediterranean	β ⁰
Codon 5 -CT	Mediterranean	β ⁰
Codon 6 -A	Mediterranean, African-American	β ⁰
Codon 41/42 -TTCT	Southeast Asian	β ⁰
AATAAA to AACAAA	African-American	β ⁺⁺
AATAAA to AATGAA	Mediterranean	β ⁺⁺
Codon 27 G→T Hb (Hb Knossos)	Mediterranean	β ⁺⁺
Codon 79 G>A (Hb E)	Southeast Asian	β ⁺⁺
Codon 19 G>A (Hb Malay)	Malaysian	

Table 1. Common mutations of β-thalassemia according to severity and ethnicity.

3. Genotype phenotype relationship in β -thalassemia

Mutations causing thalassemia can affect any step in the pathway of globin gene expression. The most common forms arise from mutations that derange splicing of the mRNA precursors or prematurely terminate translation of the mRNA. The resulting phenotype reflect the effects of the β^0 thalassemia in which there is no B-globin gene production and B^+ , B^{++} thalassemia in which there is marked or mild reduction in production of β -chain [5].

3.1. Genetic modifiers

Several modifier genes have been identified which are able to influence the severity of β -thalassemia, so at phenotypic level β -thalassemias are considered multigenic diseases. Improved understanding of the influence of modifier genes involved in modulating the complex pathophysiology of β -thalassemia may allow prediction of disease phenotype [6].

3.1.1. Primary modifiers

Primary genetic modifiers in homozygous β -thalassemia include genetic variants able to reduce the globin chain imbalance, therefore resulting in a milder form of thalassemia.

1. The presence of silent or mild β -thalassemia alleles associated with a high residual output of β -globin.
2. The coinheritance of α -thalassemia.
3. Genetic determinants able to sustain a continuous production of gamma globin chains (HbF) in adult life.

3.1.2. Secondary modifiers

The clinical phenotype of homozygous β -thalassemia may also be modified by the coinheritance of other genetic variants mapping outside the globin clusters.

1. TA_7 polymorphism in the promoter region of the uridine diphosphate-glucuronosyl transferase gene is associated with cholelithiasis in thalassemic patients [7].
2. Apolipoprotein E ϵ 4 allele seems to be a genetic risk factor for left ventricular failure in β -thalassemia [8].
3. Genes involved in iron (i.e., C282Y and H63D HFE gene mutations) and bone metabolism [9].
4. Glutathione-S-transferase M1 gene polymorphism has been associated with an increased risk of cardiac iron overload in patients with thalassemia major [10].
5. Excess functional α -globin genes (α gene triplication or quadruplicating) in heterozygous β -thalassemia may lead to thalassemia intermedia phenotype instead of the asymptomatic carrier state [11].

4. Pathophysiology of β -thalassemia

The basic defect in β -thalassemia is a reduced or absent production of β -globin chains with relative excess of α -chains. Because α - and non- α chains pair with each other at a ratio close to 1:1 to form normal Hb, the excess unmatched α chains accumulate in the cell as an unstable product, leading to cell destruction in the bone marrow and in the extramedullary sites. This process is referred to as ineffective erythropoiesis (IE) and is the hallmark of β -thalassemia [12].

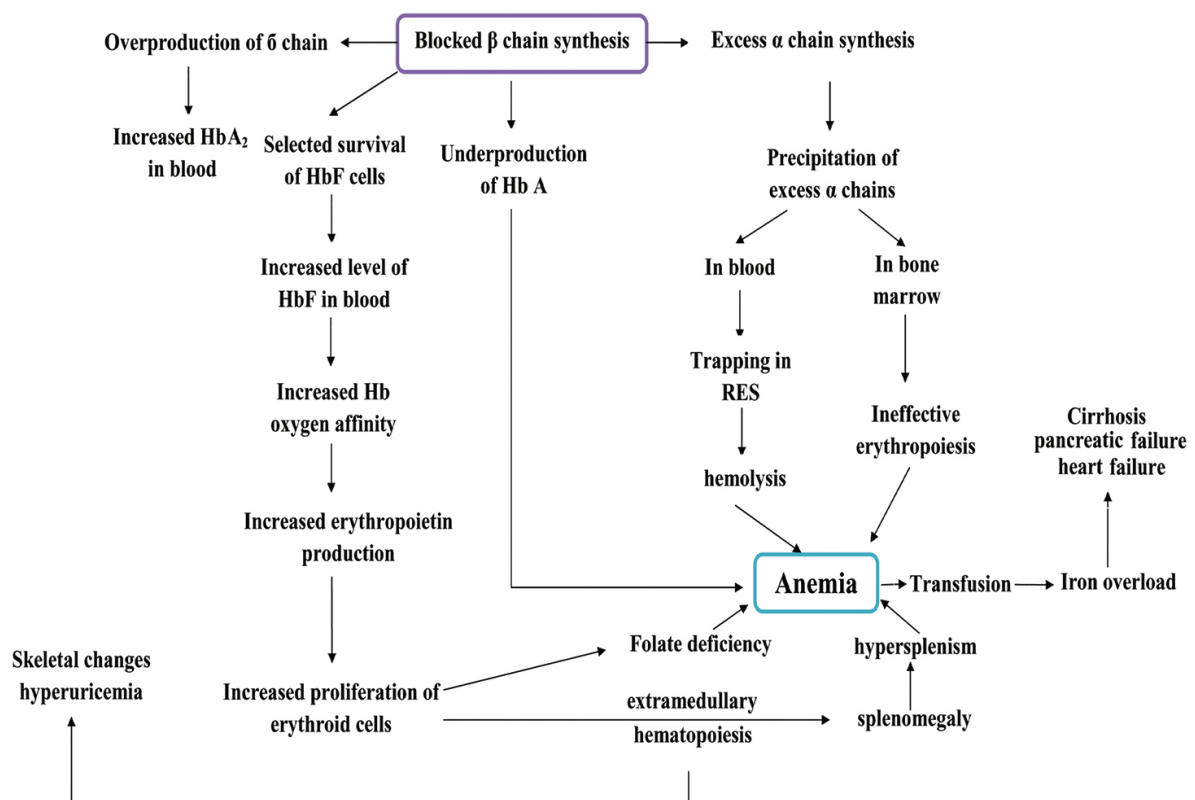


Figure 1. Pathophysiology of β -thalassemia.

The excess α -chains may, in minor amounts, combine with residual β - (in β^+ -thalassemia) and γ -chains (whose synthesis persists usually in small quantity after birth), undergo proteolysis, or in large part become associated with the erythroid precursors with deleterious effects on erythroid maturation and survival. Also excess α -chain precipitation in the red cell membrane causes structural and functional alterations with changes in deformability, stability, and red cell hydration [12].

Alterations of erythroid precursors result in an enhanced rate of apoptosis, which is a programmed cell death. Apoptosis could contribute significantly to ineffective erythropoiesis and occurs primarily at the polychromatophilic erythroblast stage. The ineffective erythropoiesis (IE) and anemia have several consequences producing the clinical picture of the disease. The first response to anemia is an increased production of erythropoietin, causing a marked

erythroid hyperplasia, which may range between 25 and 30 times normal. Anemia may produce cardiac enlargement and sometimes severe cardiac failure [12].

Increased erythropoietin synthesis may stimulate the formation of extramedullary erythropoietic tissue, primarily in the thorax and paraspinal region. Marrow expansion also results in characteristic deformities of the skull and face, as well as osteopenia [13].

High levels of iron, closely associated with denatured hemoglobin, have been found in the membrane of β -thalassemic red cells [14].

Severe IE, chronic anemia, and hypoxia also cause increased gastrointestinal (GI) tract iron absorption. This is combined with increased iron from the breakdown of RBCs and the increased iron introduced into the circulation by the transfusions necessary to treat thalassemia, plus inadequate excretory pathways lead to progressive deposition of iron in tissues and hemosiderosis occurs [13].

Free iron species, such as labile plasma iron as well as labile iron pool in the RBCs accumulate when transferrin saturation exceeds 70%. These free iron species generate reactive oxygen species with eventual tissue damage, organ dysfunction, and death (**Figure 1**) [13].

5. Clinical presentation of β -thalassemia

5.1. History

The history in patients with thalassemia widely varies, depending on the severity of the condition and the age at the time of diagnosis. In most patients with thalassemia traits, no unusual signs or symptoms are encountered, children with thalassemia major usually present between 3 months and 1 year of life, and occasionally presentation is delayed to 4–5 years [15].

Some patients, especially those with somewhat more severe forms of the disease, manifest some pallor and slight icteric discoloration of the sclerae with splenomegaly, leading to slight enlargement of the abdomen. Thalassemia should be considered in any child with hypochromic microcytic anemia that does not respond to iron supplementation [15].

5.2. Physical

Patients with thalassemia minor are often asymptomatic. They have mild anemia and their Hb level is usually not less than 9–10 g/dl therefore pallor and splenomegaly are rarely observed.

The stigmata of severe untreated α -thalassemia major included the following:

- Severe anemia, with an Hb level of 3–7 g/dl
- Massive hepatosplenomegaly
- Severe growth retardation
- Bony deformities

Patients with signs of iron overload may also demonstrate signs of cardiomyopathy and endocrinopathy caused by iron deposits. Diabetes and thyroid or adrenal disorders have been described in these patients [16].

6. β -Thalassemia workup

6.1. Laboratory studies

6.1.1. Complete blood count and peripheral blood film examination

In the severe forms of thalassemia, the Hb level ranges from 2 to 8 g/dl. MCV and MCH are significantly low. Reticulocyte count is elevated to 5–8% and leukocytosis is usually present. Platelet count is usually normal, unless the spleen is markedly enlarged. Peripheral blood film examination reveals nucleated RBCs and occasional immature leukocytes.

6.1.2. Hemoglobin electrophoresis

High performance liquid chromatography (HPLC) is now usually used as first-line method to diagnose hemoglobin disorders. HPLC or hemoglobin electrophoresis reveals absence or almost complete absence of Hb A, with almost all the circulating hemoglobin being Hb F. The Hb A2 percentage is normal, low, or slightly raised.

6.1.3. Biochemical studies

1. **Serum iron and ferritin:** Serum iron level and ferritin levels are elevated. However, an assessment using serum ferritin levels may underestimate the iron concentration in the liver. Liver iron concentration (LIC) could be estimated by liver biopsy or T2* MRI, which provides a noninvasive alternative to liver biopsy. LIC could also be monitored by the use of superconducting quantum interference device (SQUID).
2. **Transferrin saturation:** Transferrin saturation is a surrogate marker for NTBI [17]. Transferrin saturation >50% is suggestive of a high iron load.
3. **NTBI and LPI:** NTBI and LPI are very specific for iron overload and can be used to monitor the response to chelation therapy [18].
4. **Hepcidin measurement:** Hepcidin can be measured in serum and urine using mass spectrometry, and this may be a feasible marker in the near future [19].
5. **Other biochemical changes:** Serum zinc, serum and leukocytic ascorbic acid, vitamin E, and folic acid are low. LDL is elevated as consequence of ineffective erythropoiesis [20].

6.2. Imaging studies

Findings show skeletal changes, including thinning of the cortex, widening of the medulla, and coarsening of trabeculations, due to bone marrow hyperplasia in the long bones, meta-

carpals, and metatarsals. Skull bones show “hair-on-end.” The maxilla may overgrow, which results in maxillary overbite, prominence of the upper incisors, and separation of the orbit. These changes contribute to the classic chipmunk facies observed in patients with thalassemia major [21]. Chest radiography is used to evaluate cardiac size and shape. Left ventricular function can be quantified using MRI, MUGA (multiple gated acquisition scan) or echocardiography [22].

Cardiac T2*, a noninvasive procedure involves measuring the cardiac T2 with cardiac magnetic resonance (CMR). This procedure has shown decreased values in cardiac T2 due to iron deposit in the heart. Unlike liver MRI, CMR does not correlate well with the ferritin level, the liver iron level, or echocardiography findings. The liver is clear of iron loading much earlier than the heart, and so the decision to stop or reduce chelation treatment based on liver iron levels is misleading [23].

A poor correlation was noted between cardiac and hepatic iron concentrations as assessed by T2-MRI where approximately 14% of patients with cardiac iron overload were identified who had no matched degree of hepatic hemosiderosis [24].

6.3. Molecular genetic analysis

PCR-based procedures can detect the commonly occurring mutations in β -globin gene. The most commonly used methods are reverse dot blot analysis or primer-specific amplification, with a set of probes complementary to the most common mutations in each population. β -Globin gene sequence analysis is used to detect mutations in the β -globin gene in case of failure of targeted mutation analysis [25].

6.4. Prenatal diagnosis

Prenatal diagnosis is possible through analysis of DNA obtained through chorionic villi sampling at 8–10 weeks' fetal gestation or by amniocentesis at 14–20 weeks' gestation. In most laboratories, the DNA is amplified using the PCR assay test and then is analyzed for the presence of the thalassemia mutation using a panel of oligonucleotide probes corresponding to known thalassemia mutations. Prenatal diagnosis may be performed noninvasively, with the use of maternal blood samples to isolate either fetal cells or fetal DNA for analysis [26].

7. Treatment of thalassemia

7.1. Transfusion therapy

In general, children with β -thalassemia major and hemoglobins of less than 6–7 g/dl should receive chronic transfusions. It is important to start early before the child has a chance to develop splenomegaly and hypersplenism and before skeletal changes and growth retardation. It is also important to establish a reliable, routine transfusion schedule that maintains hemoglobin levels of 9–10 g/dl [27].

Transfusions of washed, leukocyte-depleted RBCs are recommended for all the patients to reduce the incidence of febrile and urticarial reactions as well as infectious cytomegalovirus contamination [28]. Extended red cell antigen typing, including at least the Rh antigens, Duffy, Kidd, and Kell, is recommended before the patient is started on a transfusion regimen [27].

7.2. Iron chelation therapy

Children with thalassemia major should begin therapy at the earliest possible age and certainly by the time they have accumulated more than 7 g of excess iron. In young children, a serum ferritin level much greater than 1,000 µg/l or 1 year of regular transfusions (or both) can be used as surrogate indicators to initiate chelation therapy [27].

7.2.1. Deferoxamine

Deferoxamine (DFO) is a hexadentate iron chelator (deferioxamine mesylate; desferal®). DFO was introduced as parenteral therapy for iron overload associated with β-thalassemia major in 1976 [30]. Plasma half-life of DFO is short (20–30 min). Therefore, standard treatment involves the subcutaneous infusion of 40 mg DFO for 8–12 h nightly for 5–7 nights weekly using a battery-operated infusion pump. Subcutaneous administration is preferred except in patients with severe cardiac iron deposition, for whom continuous intravenous deferoxamine therapy is recommended. Iron excretion occurs through biliary and urinary routes [29].

Adverse events of DFO include growth retardation, skeletal changes, ocular and auditory disturbances, pulmonary, and renal toxicities. They are preventable if proper monitoring is practiced to detect early signs of toxicity. Susceptibility to infection with *Yersinia* and perhaps other Gram-negative bacilli is increased in thalassemia patients who receive DFO therapy. Painful local skin reactions at the infusion site are common. Zinc deficiency can occur [29].

7.2.2. Deferiprone

Deferiprone (DFP) is an orally administered bidentate iron chelator (Ferriprox®, Kelfer®). The usual dose of DFP is 75–100 mg/kg/day taken orally in three divided doses. Plasma half-life of DFP is 2–3 h, and iron is mainly excreted in urine [30]. Adverse effects of DFP include gastrointestinal disturbances, agranulocytosis and neutropenia, arthropathy, increased liver enzyme levels, and low plasma zinc level [29, 30].

7.2.3. Deferasirox

Deferasirox (DFX) is an orally administered tridentate iron chelator that is indicated for the treatment of transfusion iron overload in persons more than 2 years of age. The US Food and Drug Administration approves a recommended daily dose of 20–40 mg/kg body weight, taken once on an empty stomach at least 30 min before food [29]. The most common adverse events with DFX therapy include gastrointestinal disturbances, rash, and mild increases in serum creatinine [31].

7.2.4. Combination therapy

1. "Shuttle hypothesis": combination of DFO and deferiprone

Combined DFO and DFP regimens offer an alternative option for patients with severe heart disease. Deferiprone, though a weaker chelator, is a relatively small uncharged molecule that can enter the cardiac cells more easily than DFO and transfers the chelated iron from the myocardial cells to the stronger chelator, DFO in the plasma [32].

2. Combination of Deferasirox and DFO

The efficacy of combining DFO with Deferasirox has been assessed in a number of studies. This combination has an additive effect, allows decreasing the dose of both chelators and improves the compliance. The "shuttle effect" is also applicable with this combination as Deferasirox acts as an intracellular chelator and DFO as a powerful extracellular chelator [32].

7.3. Splenectomy

Splenectomy is recommended when the calculated annual transfusion requirement is >200 to 220 ml RBCs/kg per year with a hematocrit of 70%. Splenectomy may be necessary to decrease the disabling effects of abdominal pressure and to increase the life span of supplemental RBCs. After splenectomy, children generally require fewer transfusions, although the basic defect in Hb synthesis remains unaffected. Splenectomy is a therapy that should not be considered casually because susceptibility to infection with *Streptococcus pneumoniae*, *Haemophilus influenza*, and *Neisseria meningitidis* increases after splenectomy in children, particularly in those younger than 5 years. Standard therapy for splenectomized individuals includes immunizations, prophylactic penicillin, and a high index of suspicion and aggressive antibiotic therapy for febrile illness. Thromboembolic events and pulmonary hypertension are also increased in splenectomized patients. These complications may be minimized by the routine use of aspirin or low dose anticoagulants [28].

7.4. Stem cell transplantation (SCT)

SCT or bone marrow transplantation (BMT) is the only possible, proven curative treatment for β -thalassemia major. The most important role of SCT is the high chance of cure for individuals who otherwise face a lifetime of invasive, demanding treatment and a reduced life span [33].

7.5. Vitamin supplementation

Vitamin E supplementation in thalassemia major is often suggested, but data demonstrating its efficacy are lacking. Folic acid supplements help to maintain folic acid levels in the face of increased requirements [27].

7.6. Fetal hemoglobin induction

In β -thalassemia, pharmacologically induced increase in γ -globin chains would be expected to decrease globin chain imbalance with consequent amelioration of clinical manifestation. Pharmacologically, three classes of agents have been shown to be capable of inducing HbF to therapeutic levels: erythropoietins, short-chain fatty acid derivatives, and chemotherapeutic agents. Hydroxyurea has been recommended in patients with thalassemia intermedia [28].

7.7. Gene therapy

β -Thalassemia is a potential attractive target for gene therapy. This could be a reality if a functional β -globin gene could be safely and efficiently introduced into the hematopoietic stem cells, and lineage-restricted expression of the β -globin protein exceeding 15% could be achieved in erythroid progenitor cells. Gene therapy for β -thalassemia requires gene transfer into hematopoietic stem cells (HSCs) using integrating vectors that direct the regulated expression of β -globin at therapeutic levels [34].

8. Prevention

Prevention strategies include community education, carrier detection, genetic counseling, and prenatal diagnosis [27].

9. Conclusion

β -Thalassemias are markedly heterogeneous at their molecular level with a clear association between genotype and clinical phenotype. Study of the molecular genetics in patients with β -thalassemia in their early life will serve as a tool to predict clinical disease severity and help in planning of early intervention strategies.

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