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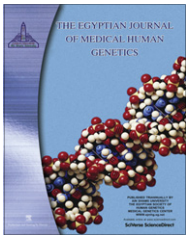

REVIEW

Thalassemia intermedia: An overview

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Abstract β-Thalassemia is considered the most common chronic hemolytic anemia in Egypt. Patients with β-thalassemia whose anemia is not so severe as to necessitate regular transfusions are said to have thalassemia intermedia. It is characterized by a significant genetic and clinical heterogeneity. The clinical phenotype ranges between the severe, transfusion-dependent thalassemia major and the asymptomatic carrier state. Thalassemia intermedia represents up to one-fourth of β-thalassemia patients. It is essential to discuss the reasons for the remarkable heterogeneity and natural history of thalassemia intermedia so that an effective method for the control and management of thalassemia intermedia can be established. This review will outline the genetics of hemoglobin biosynthesis as well as an overview on the pathogenesis, molecular basis, hematologic and clinical features of thalassemia intermedia, in addition to management of complications affecting patients with such disorder.

Contents

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1. Introduction

The thalassemia syndromes constitute the commonest of all single gene disorders [1]. They are considered as the world's most widespread genetic diseases [2]. Beta thalassemia comprises a heterogenous group of hemoglobin disorders characterized by a reduction or a complete absence of β-globin gene expression [3] and is inherited as an autosomal recessive disease [4]. The total annual incidence of symptomatic individuals is estimated at 1 in 100,000 throughout the world [5].

In Egypt, homozygous beta (β) thalassemia was first described by Diwany in the year 1944. It is a significant public health problem in Egypt where over 1–5 million newborns are expected to be affected with this disorder, and it is considered the most common chronic hemolytic anemia (85.1%) [6]. A high rate of carriers has been reported in Egypt ranging from 4–5% [7] reaching up to 9–10% [8]. There is a high rate of consanguineous marriage in Egypt which helps to accumulate deleterious genes in families, reaching 35.3% with an average inbreeding coefficient of 0.019 which could be considered high [9]. Hemoglobin is a tetramer with a molecular weight of 64.5 kDa [10]. It consists of two α and two non-α globin polypeptide chain, each of which has a single covalently bound heme group [11]. Each of the four heme groups is made up of an iron atom bound within a protoporphyrin IX ring [12].In humans there are six known polypeptide chains designated α, β, γ, δ, ε, and ζ. The ε, γ and δ chains are more similar to β chains than to α chain and are designated β-like chains. HbA ($\alpha_2\beta_2$) is the major component of hemoglobin in normal adults, usually comprising about 97% of the total hemoglobin. The remainder is HbA_2 ($\alpha_2\delta_2$) which usually constitutes 2–3% in normal individuals. HbF ($\alpha_2\gamma_2$), fetal hemoglobin, comprises the bulk of hemoglobin (50–85%) in human newborns, but declines rapidly after birth, reaching concentrations of 10–15% by four months of age. Subsequently the decline is slower and adult levels of less than 1% are reached by 3–4 years. Hb Gower I ($\zeta_2\varepsilon_2$), Gower II ($\alpha_2\varepsilon_2$) and Portland ($\zeta_2\gamma_2$) are embryonic hemoglobins synthesized in the yolk sac before eight weeks of gestation [10]. The temporal switches of globin synthesis are accompanied by changes in the major site of erythropoiesis. Embryonic globin synthesis occurs in the yolk sac from the third to eighth week of gestation [13] with a decrease in ζ- and ε-chain production together with a compensating increase in α- and γ-chain production. At this stage of gestation, β-chain synthesis in reticulocytes accounts for 4% of non-α chain synthesis and gradually increases thereafter, but about the fifth week of gestation, the major site of hematopoiesis begins to move from the yolk sac to the liver. At around 8 weeks of gestation the fetal liver takes over, synthesizing HbF plus a small amount of HbA. At 18 weeks gestation the liver is progressively replaced by bone marrow as the major site of red cell production, accompanied by a gradual switch from HbF to HbA synthesis. Synthesis of the δ chain also continues after birth, but HbA_2 ($\alpha_2\delta_2$) never accounts for more than about 2% of adult hemoglobin. Unfortunately, the small amounts of δ-globin (and therefore HbA_2) and γ-globin (and therefore HbF) that are found normally in adult blood are insufficient to compensate for the reduced amounts of β-globin (and therefore HbA) that are found in diseases such as β-thalassemia [10,11,13].

2. Genetics of hemoglobin biosynthesis

The genetic control of globin chain synthesis is primarily achieved by two developmentally regulated gene clusters (Fig. 1a–c) located on chromosome band 16p13.3 and 11p15.5 [for alpha (α)- and non-alpha(α)-gene cluster, respectively] [14]. In humans there are eight different genetic loci that code for the six types of globin chains. In addition there are at least four pseudogenes that have sequences similar to other globin genes but are not expressed into globin proteins. In the β-globin cluster, sequences around the gene appear to carry some developmental specificity and the genes on the human β-globin cluster are arranged in their temporal expression during development [15]. The genes in each globin cluster are arranged $5' \rightarrow 3'$ on the coding strands in the order of their developmental expression during embryonic, fetal and postnatal life [12]. The β-like globin genes spread over a region of approximately 60 kb. There are five functional genes: $5'$–ε–$^{G}\gamma$–$^{A}\gamma$–ψβ–δ–β-$3'$. The globin gene sequences comprise only 7 kb of the 60 kb of DNA in the β-gene region, and the remaining 53 kb are flanking sequences that contain sequences with specific regulatory roles. These include the locus control region, enhancer sequences and the promoter regions of the globin genes. The β-locus control region (LCR-β) is found in a cluster of DNAse I-hypersensitive sites 6–18 kb $5'$ to the ε-globin gene. Erythroid cells contain a variety of erythroid-specific transcription factors, the most important being GATA-1 and NF-E with binding sites both at the locus control regions and in the promoters [10]. The β-globin promoter consists of a series of short functional elements interacting with transcription factors that restrict expression of these genes to erythroid cells. One important promoter sequence is the TATA box, a conserved region rich in adenines and thymines, appears to be important for determining the position of start of transcription, which is approximately 50 base pairs upstream from the translation initiation site. A second conserved region, the CAT box (actually CCAAT), is located further upstream [16–18]. Many mutations in these regulatory elements have been identified in patients with β-thalassemia. Enhancers are sequence elements that act at a distance from a gene to stimulate transcription. They appear to be involved in establishing the tissue specificity or level of expression of genes. Unlike promoters, enhancers are both position and orientation independent and can be located either $5'$ or $3'$ of the transcription start site [17]. Mutations that disrupt or delete either enhancer or LCR sequences interfere with

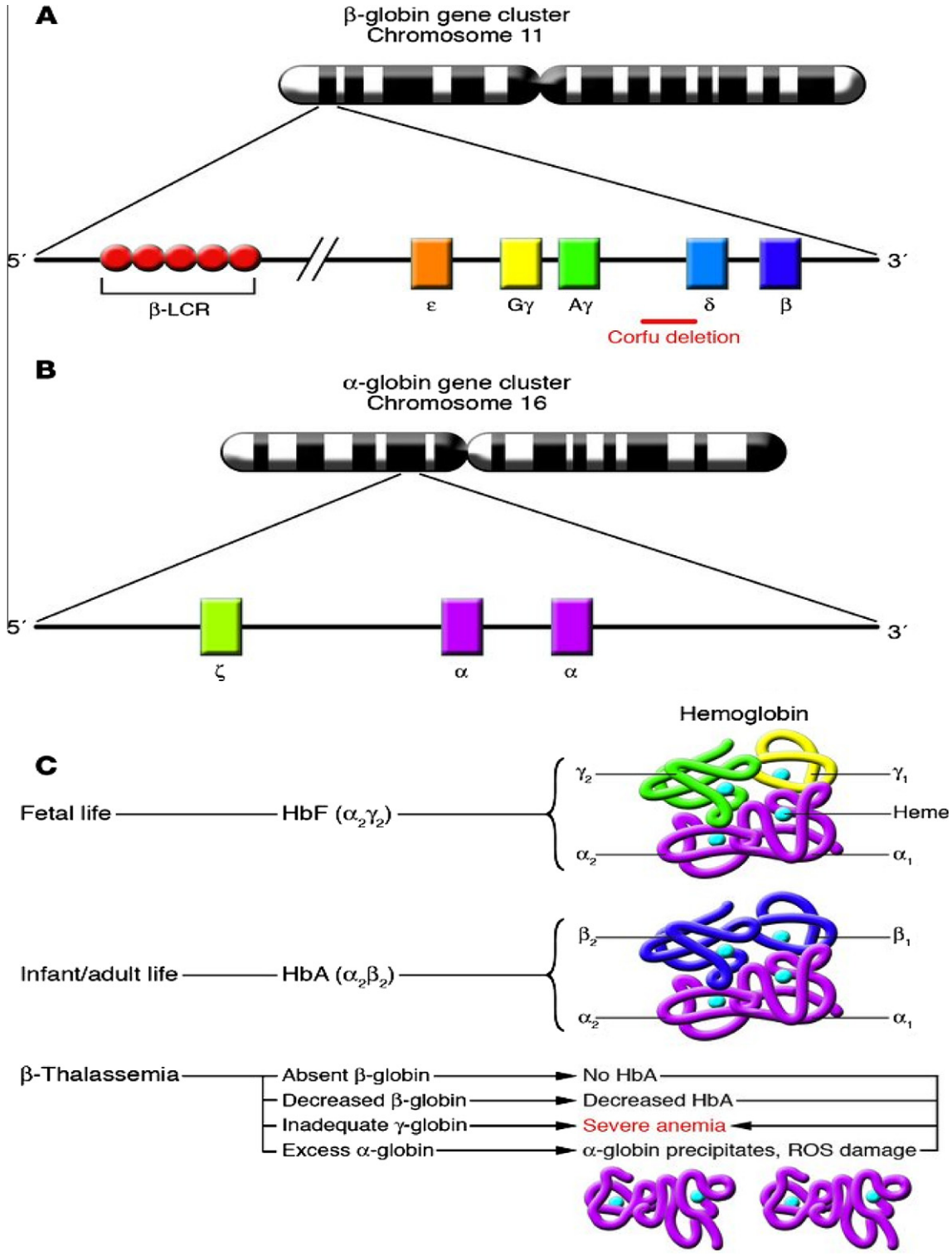

Figure 1 β-Globin gene cluster and α globin-gene cluster [10, 11 and 13].

or prevent β-globin gene expression. Patients with εγδβ-thalassemia demonstrated that the LCR is required for the expression of all the genes in the β-globin cluster on chromosome 11 [19]. The LCR, along with associated DNA-binding proteins, interacts with the genes of the locus to form a nuclear compartment called the active chromatin hub, the compartment where β-globin gene expression takes place [14]. It is involved in the sequential switching of gene from the embryonically expressed ε-globin gene to the δ- and β-globin genes in adults [20]. β-globin mutations have no prenatal consequences because γ-globin is the major β-like globin before birth [13,16].

3. Definition of thalassemia intermedia

Patients whose anemia is not so severe as to necessitate regular transfusions are said to have thalassemia intermedia [11]. Thalassemia intermedia is characterized by a significant genetic and clinical heterogeneity. A wide spectrum of different genotypes – homozygous, heterozygous and compound heterozygous – have been thought to be responsible for thalassemia intermedia. The clinical phenotype ranges between the severe, transfusion-dependent thalassemia major and the asymptomatic carrier state [21]. It represents up to one-fourth of β-thalassemia patients [22].

4. Clinical features

- Some thalassemia intermedia patients are asymptomatic until adult life and may present with moderate anemia and do not require regular transfusions, whereas others are symptomatic from as young as 2 years of age [23,24]. Red cell production in patients with β-thalassemia intermedia may be adequate to sustain hemoglobin of 6–7 g/dl [25] without RBC transfusion [26].
- Main clinical features that may be present in patients with thalassemia intermedia are hypertrophy of erythroid marrow with medullary and extramedullary hematopoiesis with its complications (osteoporosis, masses of erythropoietic tissue that primarily affect the spleen, liver, lymph nodes, chest, spine with bone deformities and typical facial changes) and gallstones[5]. Pathologic fractures and skeletal abnormalities occur due to massive expansion of erythroid bone marrow activity and hepatosplenomegaly due to hemolysis or extramedullary hematopoiesis [26].
- Although chronically anemic [11,27] the need for transfusion support might be eliminated or delayed thereby minimizing iron overload at the expense of severe anemia, with consequent cardiomegaly, splenic enlargement, osteoporosis and bony deformities [25]. Transfusion may become necessary with advancing age, during infection and pregnancy, and when hypersplenism develops [28].
- Iron overload in non-transfused patients is due to augmented gastrointestinal absorption and involves mainly the liver [28]. The hepatic peptide hepcidin, normally regulated by marrow activity and by iron load, is disproportionately low in individuals with thalassemia, allowing iron to be absorbed from the gut even in the presence of severe overload [29]. Iron-induced peroxidative injury to the phospholipids of lysosomes and mitochondria, produced by free hydroxyl radicals, is believed to be the most important pathogenetic factor for tissue damage. The estimated amount of iron that subjects with thalassemia intermedia absorbed on a standard diet is 3–10 times more than is normal. By the third or fourth decade, the iron load may be similar in magnitude to that of transfusion-dependent thalassemic patients in their teens [11].
- Although individuals with thalassemia intermedia are at risk of iron overload, hypogonadism, hypothyroidism and diabetes are not common [30].
- Although patients with thalassemia intermedia (TI) are capable of surviving without regular blood transfusion, growth and development are retarded [5].
- Pubescence takes place normally, and fertility is preserved [31]. Women may have successful spontaneous pregnancies. However, if blood transfusions are necessary during pregnancy, those never or minimally transfused are at risk of developing hemolytic alloantibodies and erythrocyte autoantibodies. Intrauterine growth retardation, despite a regular transfusion regimen, has been reported due to hypoxia [32]. Splenomegaly can interfere with the enlargement of uterus and can be complicated by hypersplenism [33].
- Pallor, intermittent icterus, and facial bony changes similar to those of thalassemia major are observed regularly [23].
- Survival into adulthood is the rule [27].

5. Complications affecting patients with thalassemia intermedia

- Pulmonary hypertension and thromboembolic events [23,28]. Hemolysis per se has been linked to the development of a hypercoagulable state and pulmonary hypertension (PHT). The pathogenesis may include: activation of platelets, endothelial cells and monocytes [34] with increased both thromboxane, and endothelin, decreased prostacycline [33] along with dysfunction of the coagulation system. These have been associated with thromboembolic phenomenon, especially in splenectomised thalassemia intermedia patients. Moreover, hemolysis carries a role in the dysregulation of nitric oxide (NO) homeostasis which is correlated with PHT and probably thrombotic phenomena [34]. Such events include deep vein thrombosis, portal vein thrombosis, stroke and pulmonary embolism [35].
- In both patients with beta thalassemia major and beta thalassemia intermedia, total cholesterol and LDL-cholesterol are decreased. The mechanisms that may account for these findings are increased erythropoiesis and cholesterol consumption in beta thalassemia intermedia, and iron overload and oxidative stress in beta thalassemia major [36].
- Cardiac disease in thalassemia intermedia is determined by two main factors. One is the high output state that results from chronic tissue hypoxia and from hypoxia-induced compensatory reactions. The other is the vascular involvement that leads to an increased pulmonary vascular resistance and an increased systemic vascular stiffness. Valvular abnormalities and iron overload also contribute to a less extent. Right heart involvement with age-related pulmonary hypertension followed by congestive heart failure dominates the clinical picture. Although the left heart is also affected, systolic left ventricular function is usually preserved but this may also be decompensated under conditions characterized by excessive cardiac work load [34]. Patients with thalassemia intermedia who do not usually have severe hemosiderosis are less prone to cardiac problems [37].
- Pseudoxanthoma elasticum, a diffuse connective tissue disorder with vascular manifestation: caused by degeneration of the elastic lamina of the arterial wall and calcium deposition, has been described in such patients [38]. The pathophysiology has been attributed to iron-induced oxidative damage. Its progression is complicated by thrombotic and hemorrhagic events similar to those of the inherited form [39].

- Erythropoietic masses formation: more commonly intrathoracic and sometime accompanied by hemothorax. It is due to the chronic anemia and the associated hypoxia with the frequent extramedullary erythropoiesis [40]. Spinal cord compression may cause paraparesis and cauda equina syndrome [41].
- Leg ulcers over the medial malleolus are common in thalassemia intermedia. Their pathogenesis has been related to: hypoxia caused by chronic anemia, abnormal rheology of the thalassemic red cells, venous stasis and high HbF concentration [33].
- Hepatocellular carcinoma represents a frequent complication in patients with liver cirrhosis either secondary to genetic hemochromatosis or to chronic viral hepatitis. As a consequence of the numerous risk factors present in thalassemia patients, the development of the tumor is to be expected [42].
- The primary cause of premature death is myocardial hemosiderosis [11].

6. Management

Currently there are no clear guidelines for managing patients with thalassemia intermedia [43]. There are a number of options currently available for managing patients with thalassemia intermedia including:

- *Transfusion therapy*: increasing evidence is delineating the benefit of transfusion therapy in decreasing the incidence of complications as pulmonary hypertension and thromboembolic events. Thus although the common practice was to initiate transfusion when complications ensue, it may be worthwhile to start transfusion therapy earlier as a preventive approach which will also help alleviate the increased risk of alloimmunization with delayed initiation of transfusion. Although earlier introduction of blood transfusions will increase the rate of iron accumulation, effective methods of iron chelation are now available [44,34]. Transfusion becomes necessary when the sense of well being of the patient decreases to a level inadequate to the activities of a normal life. Problems usually develop, in patients used to chronic hypoxia, for levels of Hb below 70 g/l. The patient's general condition must be considered in terms of regular growth, growth velocity, bone age, bone deformities, size of the spleen, periods of rapid growth and pregnancy. Sometimes transfusion becomes necessary during infection-induced aplastic crises. Heart disease is also an indication to transfusion therapy. When the decision to transfuse is made, the transfusion regimen should be similar to the one generally adopted for thalassemia major [34].
- *Splenectomy*: The current indications for splenectomy in TI include growth retardation, leukopenia, thrombocytopenia, increased transfusion demand and symptomatic splenomegaly [45]. Splenectomy, however, can contribute to an increased susceptibility to thromboembolic events and pulmonary hypertension in TI [23].The presence of a chronic hypercoagulable state could be due to the procoagulant effect of the anionic phospholipids exposed on the surface of the damaged circulating red blood cells [46]. Overwhelming postsplenectomy sepsis is an abrupt event that can be fatal. The most frequent bacteria are *Streptococcus pneumoniae, Hemophilus influenzae, Neisseria meningitidis, Klebsiella, Escherichia coli* and *Staphylococcus aureus* [34]. Iron-loaded macrophages lose the ability to kill intracellular pathogens via the interferon-γ-mediated pathways. Some of this loss of ability is related to the reduced formation of nitric oxide in the presence of iron [47]. Splenectomy usually allows discontinuation of transfusion in the majority of the patients. However, it does not usually modify the high output state and the increased pulmonary artery pressure that often characterizes thalassemia intermedia [48]. If gallstones are present, cholecystectomy should also be performed at the time of splenectomy [34]. Partial splenectomy and partial dearterialization of the spleen have an immediate beneficial effect, but are not long-lasting [49].
- *Iron chelation therapy*: Iron chelation, when begun after age of 10 years may not prevent the development of overt cardiac disease [50]. A direct assessment of liver iron concentration is recommended, either by biopsy or by a non-invasive method such as R2 MRI. Chelation therapy should generally be initiated if liver iron concentration exceeds 7 mg/g dry weight of liver tissue. Threshold serum ferritin values of 400–500 ng/ml could be considered as an indicator for initiation of iron chelation therapy [51]. The orally active chelators seem to be more efficient in gaining access to the chelatable iron pools of cardiomyocytes, binding labile iron, and attenuating reactive oxygen species formation [52].
- *Modulation of fetal hemoglobin production*: It has been suggested that increasing levels of hemoglobin F ($\alpha_2\gamma_2$) by pharmaceutical agents could be of benefit in patients with β -thalassemia intermedia because of an improvement in the balance off globin synthesis [53]. Hydroxycarbamide, also known as hydroxyurea is capable of inducing HbF synthesis and stimulating γ-globin production. It may have a more general role in augmenting globin synthesis, including β-globin in some thalassemia intermedia patients who maintain the capacity to express normal β-globin chains [25]. Both hydroxyurea and butyrate derivatives have shown only modest increases in hemoglobin [54]. Some patients with β-thalassemia intermedia, who are not transfusion dependent, may respond to hydroxyurea treatment, albeit transiently [55] by increasing their packed cell volumes [56]. Hydroxycarbamide has been administered to thalassemia patients according to many different regimens, alone or in combination with other drugs [33]. A significant decrease in the need for blood transfusions was observed in many patients; the need was completely obviated in some patients [57]. α-deletions, the XmnI polymorphism [58] and Hb E/β-thalassemia [59] may be predictive of a good response to hydroxycarbamide. The improved sense of well-being, almost universally reported, may reflect the significant decrease of ineffective erythropoiesis [60]. Trials of recombinant human erythropoietin (rHuEPO) for the treatment of thalassemia in humans showed a significant, dose-dependent increase in thalassemic erythropoiesis, without an increase in HbF, mean corpuscular volume and mean hemoglobin content, and without a change in the α/non-α ratio has been observed, mainly in splenectomized patients with thalassemia intermedia [61]. Combination therapy with erythropoietin and hydroxyurea in thalassemia

patients appears to be more advantageous than either therapy alone with respect to HbF augmentation and an increased packed cell volume [62,56]. Haemin, the ferric chloride salt of heme, when combined with erythropoietin, preferentially increased the production of HbF in human erythroid cells [60].

- *Antioxidants*: Oxidative damage may be generated by the presence on the cells of free globin chains and labile plasma iron (LPI), a chelatable component of non-transferrin-bound iron [63] and can be reduced by treatment with iron chelating agents. Reactive oxygen species play an important role also in the oxidative state of platelets potentially favoring thromboembolism. The chronic stress on neutrophils can reduce their antibacterial capacity and their respiratory burst response [64]. Combined use of a lipid antioxidant (vitamin E) with a protein antioxidant (*N*-acetylcysteine) and iron chelators could be more effective than the administration of a single antioxidant [65].
- *Folic acid supplementation*: Folic acid deficiency is common in TI and occurs due to poor absorption, low dietary intake, or, most significantly, an increased demand for folic acid from active bone marrow with increased erythropoiesis. This is a particular concern in pregnancy since deficiency can cause neural tube defects, such as spina bifida, in the growing fetus [33,34]. Daily supplementation with 1 mg of folic acid is advised for patients with thalassemia intermedia [66].
- *Zinc supplements*: Zinc supplementation may become necessary during intense chelation [33].
- *Hematopoietic stem cell transplantation*: Hematopoietic stem cell transplantation (HSCT) is an established treatment for beta-thalassemia major [34], but it has rarely been used in patients with thalassemia intermedia [33]. The decision as to which patients are eligible for transplantation is complex and is related to both the quality of life and expected survival-time of the transplanted patient, when compared with supportive care only [34].
- *Molecular therapies and future perspectives*: The transfer of a globin gene in autologous haematopoietic stem cells poses challenges in terms of controlling transgene expression, which ideally should be erythroid-specific, differentiation- and stage-restricted, position independent and sustained over time [67]. Considerable progress has now been made using *lentiviral vectors* [68]. *Globin gene transfer* by homologous recombination in ex vivo embryonic stem cells is a possibility for the hemoglobinopathies [69]. Therapeutic *antisense mRNA* using antisense oligonucleotides targeted at aberrant splice sites can restore correct splicing in erythroleukaemic cell lines. These studies have implications for the treatment of HbE/β-thalassaemia [70]. *α-hemoglobin stabilizing protein* (AHSP) binds free α-globin chains, limiting the oxidative effects of α-Hb and prevents its precipitation. In humans, it is directly regulated by GATA-1[33].
- *Treatment of complications*
  - The development of pulmonary hypertension could be prevented by starting transfusion and chelation therapy early in life for patients with thalassemia intermedia [33]. Several therapies have been available for the treatment of pulmonary hypertension including oxygen therapy, diuretics, digoxin, anticoagulants and calcium channel blockers. In addition, Prostacyclin analogs, endothelin receptor antagonists and phosphodiesterase 5 inhibitors have been introduced for the treatment of pulmonary hypertension [71,72]. Treatment of extramedullary erythropoietic masses includes hypertransfusion [41], radiotherapy, hydroxycarbamide [73] and surgery in selected cases [74].
  - Leg ulcers: The treatment is difficult and includes pressure dressing, skin grafting, blood transfusion, hydroxycarbamide, arginine butyrate, granulocyte colony-stimulating factor, rHuEPO, platelet growth factor and local hyperbaric oxygen chamber [75].
  - Fractures due to minor traumas may suggest the need for transfusion [76]. 25-OH vitamin D deficiency has been reported [77]. Vitamin D may be given in a dose of 700–800 IU [78] and calcium should be given at 1200–1500 mg/day [79]. Additional therapy may be necessary including biphosphonates, potent inhibitors of osteoclast activation [80]. However, care should be taken because several cases of jaw necrosis have been described [81].
  - Prevention and therapy of thromboembolic events [5]. Ischemic strokes have been described in combination with cardiac valvular lesions – a consequence of the elastic tissue defect and/or atrial fibrillations on a background of hypercoagulability. At the same time, thrombosis may be a silent or subclinical process [82]. Early transfusion therapy, combined with iron chelation, may prevent heart damage by ameliorating factors that cause cardiac deterioration, such as high output state, hemolysis and hypercoagulability [83]. Blood transfusion might reduce the thrombotic risk by diluting the procoagulant thalassemic red cells. Platelet anti-aggregation agents and low molecular weight heparin followed by long-term oral anticoagulants in patients with a history of thrombosis seem reasonable choices [33].

7. Molecular basis of β-thalassemia intermedia

Beta-thalassemias are caused by point mutations or, more rarely, deletions in the beta globin gene on chromosome 11, leading to reduced ($beta^{+}$) or absent ($beta^{0}$) synthesis of the beta chains of hemoglobin (Hb). Transmission is autosomal recessive; however, dominant mutations have also been reported [5]. More than 200 mutations have been reported that result in beta-thalassemia in the β-thalassemia globin gene [10,84], together with a much smaller number of gene deletions ranging from 25 bp to 67 kb [85]. Comparison between the prevalence of β-thalassemia mutations in thalassemia major and thalassemia intermedia in the Mediterranean region is shown in Table 1. The clinical severity of β-thalassemia depends mainly on the degree of β chain deficiency [86]. The mutations either affect globin gene transcription, RNA processing or translation, RNA cleavage and polyadenylation, or result in a highly unstable globin chain. Frameshift and nonsense codon mutations have been observed in all three exons, and RNA processing mutations have been found in both introns and the four splice junctions. Transcription mutations are all relatively mild and are commonly observed in β-thalassemia intermedia; they are single nucleotide substitutions in the TATA box at −30 from the transcription start site and in the ACACCO distal promoter region at −90 [10].

Table 1 Comparison between prevalence of β-thalassemia mutations in thalassemia major and thalassemia intermedia in the Mediterranean region [117,34].

Mutation	Thalassemia intermedia *N* (%)	Thalassemia major *N* (%)
cd39 C->T	72(24)	136(53.5)
IVSI-110 G->T	52(17)	58(23)
IVSI-6 T->C	94(31.5)	16(6.3)
IVSI-1 G->A	8(2.7)	19(7.4)
IVSII-1G->A	14(4.7)	10(3.9)
IVSII-745 C->G	11(3.7)	9(3.5)
−101 C->T	10(3.3)	–
cd6-A	10(3.3)	3(1.2)
−87 C->G	8(2.7)	–
δβ Siciliana	13(4.3)	–
Lepore Boston	2(0.7)	–
IVSI-5 G->A	–	1(0.4)
IVSI-5 G->C	1(0.3)	–
IVSII-844 G->C	1(0.3)	–
IVSI-2 T->A	1(0.3)	–
cd 44-C	–	1(0.4)
cd 8-AA	1(0.3)	1(0.4)

'Thalassemia intermedia' is a heterogenous group with interplay of several genetic factors. It is essential to determine the reasons (or genetic modifiers) for the remarkable phenotypic heterogeneity and natural history of these disorders so that the most cost-effective methods for their control and management can be established [87].

Modifier genes are defined as inherited genetic variation that leads to a qualitative or quantitative difference in disease phenotype. This has made the prediction of the phenotype based upon the genotype more difficult. Beta-thalassemia phenotype is modified by co-existent other genetic alterations. Beta-thalassemia has been found in association with other hemoglobins: Hb D, Hb G, Hb J-Baltimore and Hb KÖln. Hemolytic anemia, if present, is of mild to moderate severity. In combination with Hb Crete, a β-chain variant with high oxygen affinity, β^0- and δβ-thalassemia produce an unusual clinical picture of an overcompensated hemolytic state, with erythrocytosis, splenomegaly, abnormal red cell morphology, and marked erythroid hyperplasia [11].

Genetic modifiers of β-thalassemia have been classified into:

(1) Primary modifiers, which describe the different β alleles of varying severity. They primarily affect the clinical presentation including inheritance of a mild β-thalassemia mutation, alpha gene changes, *Xmn*I polymorphism and hereditary persistence of fetal hemoglobin (HPFH) variants [88,89]. In general, any factor able to reduce the globin chain imbalance results in a milder form of thalassemia including the presence of silent β-thalassemia allele, associated with a high residual β-globin production [90]. It is not possible to consistently predict phenotype from α and β genotypes alone, due to the influence of modulating factors [91,92]. The syndrome of intermediate severity between thalassemia major and thalassemia minor may be produced by homozygous state of some β-thalassemia alleles [88]. A few mutations have been described, in the promoter or in the 5′ untranslated regions, of the β-globin gene leading to a slight decrease of β-globin chain synthesis [85]. Carriers of the silent mutations show normal or slightly decreased hematologic parameters and a normal or slightly elevated Hb A_2 level; the co-inheritance of another β-thalassemia mutation in trans gives rise to a thalassemia intermedia phenotype. Silent thalassemia mutations are most often identified among thalassemia intermedia patients who have one parent with a normal phenotype [93–95]. The doubly heterozygous state for the β^0- and $(\delta\beta)^0$-genes has been described in Greek, Italian, Oriental and black patients with thalassemia intermedia [11]. Hemoglobin Lepore in association with either the β^0- the β^+-gene also produces a thalassemia of intermediate severity [2]. The homozygous state for the $(\delta\beta)^0$-thalassemia gene regularly produces thalassemia intermedia [96]. Hb A and Hb A_2 are absent owing to deletion of the β- and δ-structural genes. The relatively benign nature of homozygous δβ-thalassemia relates to greater preservation of γ-chain synthesis than is the case in the usual forms of homozygous β-thalassemia. Individuals with a high γ/α-ratio have a smaller free α-chain pool and the clinical syndrome of thalassemia intermedia rather than thalassemia major [11].

(2) Secondary modifiers, which describe the co-inheritance α-thalassemias or genetic determinants which increase the level of Hb F production, both of which reduce the degree of globin-chain imbalance. The secondary modifiers affect the severity of jaundice, bone disease, cardiac and thrombotic complications [88]. The interaction of α- and β-thalassemias gives rise to a chronic hemolytic anemia of only intermediate severity. Coinheritance of α-thalassemia in homozygotes or in double heterozygotes for severe and mild β-thalassemia mutations have repeatedly been found in long-living patients with thalassemia intermedia [97]. Alpha-hemoglobin-stabilizing protein (AHSP) is an erythroid-specific protein that acts as a molecular chaperone for the free α chains of hemoglobin. Evidence strongly suggests that AHSP participates in hemoglobin synthesis and may act to neutralize the cytotoxic effects of excess free alpha-globin subunits that accumulate both in normal and beta-thalassemic erythroid precursor cells. As such, AHSP seems to be essential for normal erythropoiesis, and impaired upregulation of AHSP may lead to premature erythroid cell death, resulting in ineffective erythropoiesis. Reduced *AHSP* mRNA expression has been associated with clinical variability in some cases of β-thalassemia [98]. Galanello et al. [99] provided the first evidence that AHSP could modulate β-thalassemia. They reported the association of reduced *AHSP* mRNA expression and a more severe phenotype among individuals with identical β-thalassemia genotypes in two unrelated Sardinian families. Several different genotypes have been defined: homozygous α^0-thalassemia with heterozygous β^0- or β^+-thalassemia, heterozygous α^+-thalassemia with heterozygous β-thalassemia, Hb H disease with heterozygous β-thalassemia, and heterozygous α^0-thalassemia with homozygous β-thalassemia [100–102]. The combination of the triple α-globin gene and the heterozygous state for β^0-thalassemia also is characterized clinically by mild thalassemia intermedia [6]. Coinheritance of α-thalassemia with heterozygous β-thalassemia does not usually result in reduction of the increased Hb A_2 level typical of β-thalassemia. On the contrary, different δ-globin gene defects in cis and in trans to high Hb A_2 β-thalassemia mutations constantly reduce the Hb A_2 level [103,104]. Increased Hb F levels have been associated with certain

β-globin gene promoter mutations such as those in proximal CACCC box [105]. The striking inverse correlation between the Hb F and Hb A_2 levels is interesting, as it could reflect competition between adult and fetal globin genes for cis-acting regulatory elements such as the LCR [86]. Sequence variations located in the LCR 5′ HS-2 [106], the $^{A}\gamma$ IVS-II [107] and the 5′ flanking region of the β-globin gene [108] have also been implicated in the regulation of Hb F expression, mainly in patients with sickle cell anemia (SS) and β-thalassemia. Analysis of the possible sequence variations within the β-globin gene cluster that could have an effect on the γ chain production concerned first the LCR 5′ HS-4, HS-3 and HS-2 regulatory segments because of their role in conferring high level erythroid-specific expression on the β-like globin genes, and because of the finding of gene and developmental stage specificity for the HS-4 and HS-3 elements [109]. Additional studies included the γ-globin gene promoters, the 5′ flanking sequences and the IVS-II, since these regions also contain sequences involved in modulating the globin gene expression [110]. The 5′ flanking regions of the β-globin genes were investigated because of the presence of polymorphic AT repeats from position −540 which have been implicated in regulation of globin gene expression through differential binding of the negative transacting factor BP-1 [111]. An X-linked determinant has also been associated with elevated Hb F levels and F-cell production in normal adults and SS patients [112].

The inheritance of a specific arrangement of restriction fragment length polymorphism (β RFLP haplotype $-+--++$ 5′) associated with a high Hb F determinant, and the inheritance of a mild β-thalassemia gene are the major factors that modify the severity of the disease in Asian Indians. The inheritance of a β chromosome with the *Xmn* I-γ (+) haplotype is clearly associated with a milder phenotype. However, a single copy of this haplotype-associated determinant may not be enough to affect a sufficient increase in Hb F to modify β^0-thalassemia. In contrast, patients who were *Xmn* I-γ (±) all had a mild disease [89]. Homozygote of the *Xmn*I site, +/+, was found to have a strong linkage with high Hb F levels and high hemoglobin production in two patients who had mild clinical symptoms. However, some patients who had *Xmn*I site −/− also had mild clinical symptoms because the *Xmn*I- was found to be associated with beta$^+$-thalassemia mutation [113,57]. Two common underlying mechanisms include co-inheritance of alpha globin gene deletions in homozygous thalassemia intermedia and presence of *Xmn*I polymorphism. The newly described mechanisms include unstable hemoglobin disorders and somatic deletions in beta-globin gene [114]. Papachatzopoulou et al. [115] established that (i) The combination of T haplotype of the $^{A}\gamma$–δ-globin intergenic region, the motif $(TA)_9N_{10}$ in HS2 site of locus control region (LCR), and TAG pre-$^{G}\gamma$ haplotype is sufficient but not necessary for high Hb F [116]. (ii) The genetic determinant(s) for high Hb F involves an element associated with this combination and must be present in the specific R haplotype occurring in β-thalassemia intermedia. (iii) The genetic determinants for high Hb F does not involve the abolition of intergenic transcription in the $^{A}\gamma$–δ-globin intergenic region.

(3) Tertiary determinants, which are applied to modifiers that, although not involved in hemoglobin synthesis, cause variation in the degree of severity of many complications of β-thalassemia [16].

7.1. Pathophysiology and hematologic features of the main types of globin gene disorders

Decreased β-globin production causes hypochromic, microcytic anemia and imbalance in globin synthesis leading to precipitation of α chains. The excess α chains are insoluble which in turn leads to damage of the red cell membrane and ineffective erythropoiesis. Because δ gene is intact, HbA_2 production continues. Elevation of HbA_2 level is unique to β-thalassemia heterozygotes. The level of Hb F is also increased, not because of a reactivation of the γ-globin gene expression that was switched off at birth, but because of selective survival and perhaps increased production of the minor population of adult red blood cells that contain Hb F [13]. The severity of thalassemia intermedia depends on the degree of imbalance between alpha and non-alpha chains as well as other genetic and environmental factors that modify the natural history of the disease. By definition, the patients spontaneously maintain hemoglobin at or above 7 g/dL, sometimes at the price of intense hyperplasia of the bone marrow that is in turn responsible for bone deformities, osteoporosis, and extramedullary erythropoietic masses that often characterize thalassemia intermedia [54].

Peripheral blood erythrocytes show significant anisocytosis, hypochromia, target cells, basophilic stippling, and nucleated forms. Bone marrow hyperplasia is prominent with inclusion of denatured α-chains can be demonstrated in late normoblasts. The hemoglobin electrophoretic pattern is highly variable reflecting the heterogeneity of genotypes [117]. Δβ-thalassemia is a mild disorder and one of the types of thalassemia intermedia. Homozygotes have 100% HbF and lack HbA and HbA_2. The mild anemia and hemolysis are due to increased γ-chain synthesis, which makes imbalance between synthesis of α chains and non-α chains less than that seen in other β-thalassemias [85]. They are classified into two groups, the $(\delta\beta)^0$-thalassemia, in which the HbF is composed of both $^{G}\gamma$ and $^{A}\gamma$ chains, and the $(^{A}\gamma\delta\beta)^0$-thalassemias, in which the HbF contains only $^{G}\gamma$ chains [10]. In the homozygous state, the hematologic features of Hb Lepore show thalassemia major/intermedia with HbF about 80% and Hb Lepore 20%. The HbE/β-thalassemia exhibits a varied clinical expression ranging from severe transfusion dependence to relatively mild thalassemia intermedia [92]. The severest conditions are found in individuals with β^0-thalassemia who usually have about 50–70% HbF, the remainder being HbE. Compound heterozygotes for HbE and β^+-thalassemia have a milder disorder [10]. Coinheritance of alpha-thalassemia with beta^0-thalassemia/Hb E produces a milder clinical phenotype in contrast to an interaction of alpha-globin gene triplication in severe thalassemia [118]. Infants heterozygous for the rare condition of εγδβ-thalassemia are born with a hemolytic, hypochromic anemia and microcytosis. The condition improves at 3–6 months after birth to become similar to heterozygous β-thalassemia, with normal HbA_2 level [85].

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