Chapter

The Key Genetic Determinants Behind the Phenotypic Heterogeneity of HbE/β-thalassemia Patients and the Probable Management Strategy

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Abstract

HbE/β-thalassemia is the most common severe form of thalassemia which is very prominent in South East Asian countries. It is responsible for nearly one-half of all the severe types of β-thalassemia all over the world. It is also known to represent a wide range of phenotypic diversity which varies from asymptomatic to transfusion-dependent severe phenotype. The most important predictive factor is mutations within the beta-globin gene (*HBB*). Apart from the primary genetic modifiers, there are certain other determinants regulating the phenotypic heterogeneity including, co-inheritance of alpha thalassemia mutations and other secondary modifiers including *Xmn*1 polymorphism, *HBS1L-MYB*, *GATA-1, BCL11A* polymorphism, and presence of HPFH mutations. Although the degree of severity is also determined by other tertiary genetic modifiers like increase in serum erythropoietin due to anemia, previous infection with malaria, environmental factors, splenectomy, etc. This review aimed to reveal the potential genetic predictors of HbE/β-thalassemia patients and the probable management strategy. This also enhances the generation of "personalized medicine" for better patient care. The instability of clinical phenotype and remarkable variation indicate careful monitoring of treatment for each patient and the therapeutic approaches should be monitored over time.

Keywords: HbE/β-thalassemia, genotype, phenotype, genetic modifiers, management strategy

1. Introduction

Thalassemia is a group of congenital anemias which are characterized by deficient synthesis of one or more globin chain of normal hemoglobin molecules.

It is primarily caused due to defective synthesis of globin chain production [1]. It is the most prevalent recessive monogenic disorder and it occurs in about 4.4/10,000 live births all over the world [2]. In European Union, annually 1/10,000 people are symptomatic whereas the global incidence rate is 1/100,000 [3]. It has been found that every year approximately 300,000 and 400,000 babies are born with hemoglobin disorders and most of them are reported from low-income countries [4]. India is now known to possess the largest number of thalassemia major children (150,000) [5]. It has been estimated that approximately 10,000–15,000 new cases are added every year in this country. Moreover, there are 42 million β-thalassemia carriers reported with an average prevalence rate of 3–4% [6].

Hemoglobinopathies are broadly classified into two main groups: thalassemia syndrome and structural hemoglobin variants (abnormal hemoglobins). According to the quantitative reduction in the production of globin chain, thalassemia can be categorised into: 1) thalassemia major (the absence of globin synthesis); 2) thalassemia intermedia (reduced synthesis of globin chain); 3) thalassemia minor (silent type) [7]. The main types of thalassemia include α, β, and δβ thalassemias whereas the clinically important hemoglobin variants include HbS, HbC, HbE, and HbD. So far, >800 different types of mutations and structural variants in the Human beta globin (HBB) gene have been well characterized using the existing genomic protocol. Out of which more than 350 different mutations are known to be associated with β-thalassemia [8, 9]. In the case of α-thalassemia, most of the mutations are deletion type, whereas a wide spectrum of β-thalassemia mutations involved one or a limited number of nucleotides situated within the β-globin gene or its immediate flanking region. Beta-thalassemia has over 200 different point mutations that cause several types of clinical variability due to varying levels of arrangements of compound heterozygous alleles [7, 10]. The structural hemoglobin variants result from the substitution of one or more amino acids in the globin chains of the hemoglobin molecule. The prevalence rate of thalassemia is widely variable depending on the ethnicity of a particular geographical domain. All over the world, HbE/beta-thalassemia represents nearly 50% of all the cases affected from severe beta-thalassemia [11]. It is one of the commonest forms of hemoglobinopathies in many Asian countries including India, Bangladesh, Laos, Indonesia, and Sri Lanka [12]. Moreover, in Southern China, thousands of people are suffering from HbE/beta thalassemia where its gene frequency is about 4% [13]. In certain parts of the world, the number has increased up to 70% like in Thailand and Cambodia [14]. HbE and HbE/β-thalassemia are prominently found in the north-eastern parts of India [15, 16]. The incidence of HbE and beta thalassemia carrier rate is 4.4% and 3.9% correspondingly in this country [17].

HbE/β-thalassemia is formed due to differential interaction between β-thalassemia mutant allele and HbE allele. In many cases, there is interface between α-thalassemia allele with HbE which results in a complex series of phenotypes, although the clinical severity is comparatively milder [18]. It is not always possible to predict the appropriate phenotype from the genotype and it needs adequate genetic counseling. The objective of the present study is to describe the wide range of clinical spectrum of HbE/β-thalassemia and the probable primary and secondary genetic modifiers which influence the variable phenotype. At the same time, the probable management strategy and future therapeutic approach of thalassemia have been focussed.

2. Pathophysiology

HbE is a hemoglobin variant which appears mildly unstable and shows increased sensitivity to oxidants. The blood oxygen dissociation curves of patients with homozygous HbE appear to be normal or very slightly right-shifted. HbE is synthesized at a mildly reduced rate and globin chain imbalance. It is formed due to substitution mutation at codon 26 of the β-globin gene (GAG>AAG) which leads to the substitute of lysine for glutamic acid. This mutation activates the cryptic splice site toward the 3′ end of exon 1, which causes abnormal messenger RNA processing [19]. Therefore, the normally spliced β^E messenger RNA is declined as the normal donor site must complete with the newly formed spliced site (**Figure 1**).

In the case of HbE patients, hemoglobin constitutes about 25–30% of the total hemoglobin and the level widely varies between 3 and 11 g/dl. The HbE patients with β + thalassemia mutations are characterized by low levels of HbA% and elevated HbE%. The blood smear reveals hypochromic, microcytic red cells with considerable morphologically altered blood cells with increased numbers of target cells. HbE (EE) homozygous red blood cells are not very flexible while moving through the blood vessels. These blood cells have a smaller outside surface area to carry oxygen. EE red blood cells have a reduced capacity to hold oxygen. The lifespan of these RBCs is also shorter than that of the normal. They appear mildly anemic and their hematological findings are very similar to that of heterozygous β-thalassemia [20].

3. The interactions of hemoglobin E with different forms of thalassemia

The interaction of HbE with other types of hemoglobinopathies can be complex and puzzling. Due to a lack of proper diagnosis and genetic counseling, the chance of different types of hemoglobinopathies can be enhanced [21]. HbE alone cannot lead to any significant clinical complications, although the co-association with α and β-thalassemia leads to a diverse range of clinical syndromes of varying severity.

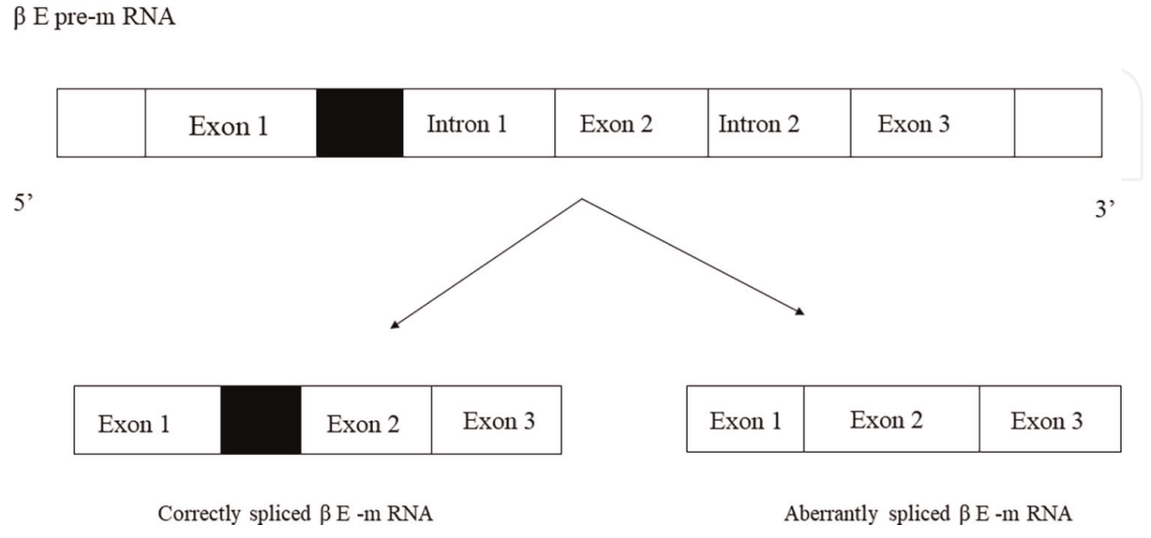

Figure 1.
Simplified schematic representation of abnormal splicing of β^E-globin mRNA. The black box denotes 16 nucleotides at the 3′-end of exon-1 deleted by abnormal splicing mechanism.

Phenotype	Genotype	Anaemia
Asymptomatic		
HbE heterozygote	β^N/β^E	No
HbE heterozygote and α^+-thalassemia heterozygote	β^N/β^E and $\alpha\alpha/\alpha^+$-thal	No
HbE heterozygote and α^0-thalassemia heterozygote	β^N/β^E and $\alpha\alpha/\alpha^0$-thala	No
HbE homozygote	β^E/β^E	No
HbE homozygote and α-thalassemia heterozygote	β^E/β^E and αα/ α^+-thal	No
HbE/HbC	β^E/β^C	No
Symptomatic		
HbE homozygote and Hb CS homozygote	β^E/β^E and Hb CS/ Hb CS	Mild
HbE/β^0-thalassemia	β^0/β^E	Moderate to severe
HbE/β^+-thalassemia	β^E/β^+	Mild

N: normal; CS: constant spring; thal: thalassaemia.

Table 1.
Common HbE syndrome and their respective genotype.

In Thailand and different South East Asian countries, the association between HbE and α-thalassemia (−α/αα) causes a various range of phenotypic diversity. Clinical parameters exhibit that level of HbE is almost similar in the case of HbE heterozygous and compound heterozygous for α^+ − thalassemia (−α/αα), whereas the co-association of α^0-thalassemia (−α/−−) have mild thalassemia like syndrome with HbE ranges between 19 and 21%. In certain extreme cases where HbE is associated with HbH disease (−−/−α) which is characterized by 13–15% of HbE and it is called HbAE Bart's Disease [22]. On other hand, the compound heterozygote condition for HbE and β-thalassemia leads to the formation of HbE/β-thalassemia which exhibits a remarkably heterogenous range of phenotypic variability. The phenotypic variability may be influenced by the inheritance of α^0 and α^+ mutant alleles [23]. Heterogenous types of HbE syndromes are also observed due to interaction with other hemoglobinopathies. The symptomatic and asymptomatic forms of HbE syndrome are summarized in **Table 1**. The blood smears of different types of HbE-thalassemia and its co-association with other types of hemoglobinopathies have been depicted in **Figure 2**.

4. Phenotypic heterogeneity of HbE/β-thalassemia

The clinical heterogeneity of HbE/β-thalassemia is not well understood. The condition may present as mild, asymptomatic anemia or life-threatening disorder that may lead to lethality from anemia in the first years of life. The phenotype of HbE/β-thalassemia seems to be unstable. Scanty reports have been found on the clinical heterogeneity of these patients. At one end of the spectrum, there are patients whose clinical severity is alike to that of β-thalassemia major; whereas at another end there are patients who can lead a normal life without the need for regular blood transfusions.

At the time of birth, infants with severe HbE/β-thalassemia patients are asymptomatic as the HbF level becomes high. As the production of HbF becomes low with increasing age and is replaced by HbE, gradually anemia and splenomegaly develop during the first decade of life [18]. Moreover, deficient blood transfusion can lead to

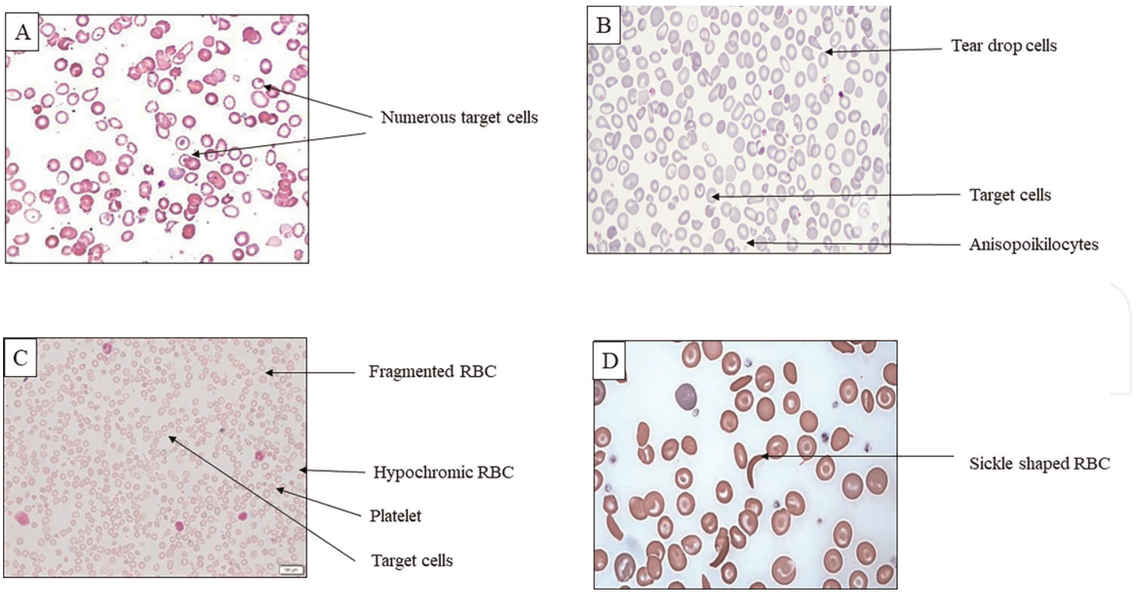

Figure 2.
The peripheral blood film in the (A) homozygous state for hemoglobin E shows large numbers of target cells, (B) HbH disease indicating tear drop cells and anisopoikilocytes, (C) HbE/β-thalassemia contains numerous fragmented RBC, hypochromic RBC, and target cells, (D) HbSE disease having sickle-shaped RBC in blood smear.

anemia, jaundice, hepatomegaly, and growth retardation. Sometimes chronic leg ulcer is also associated with delayed sexual development. Decreased oxygen delivery leads to ineffective erythropoiesis which is like β-thalassemia major. Patients with milder forms of HbE/β-thalassemia tend to grow normally and are generally active. Usually, there is a delayed pubertal growth pattern and under-developed secondary sexual characteristics. Although it is still not distinct whether they further develop complications in the near future. Gradual iron absorption may develop endocrine complications like diabetes. The clinical symptoms of HbE/β-thalassemia has been depicted in **Figure 3**.

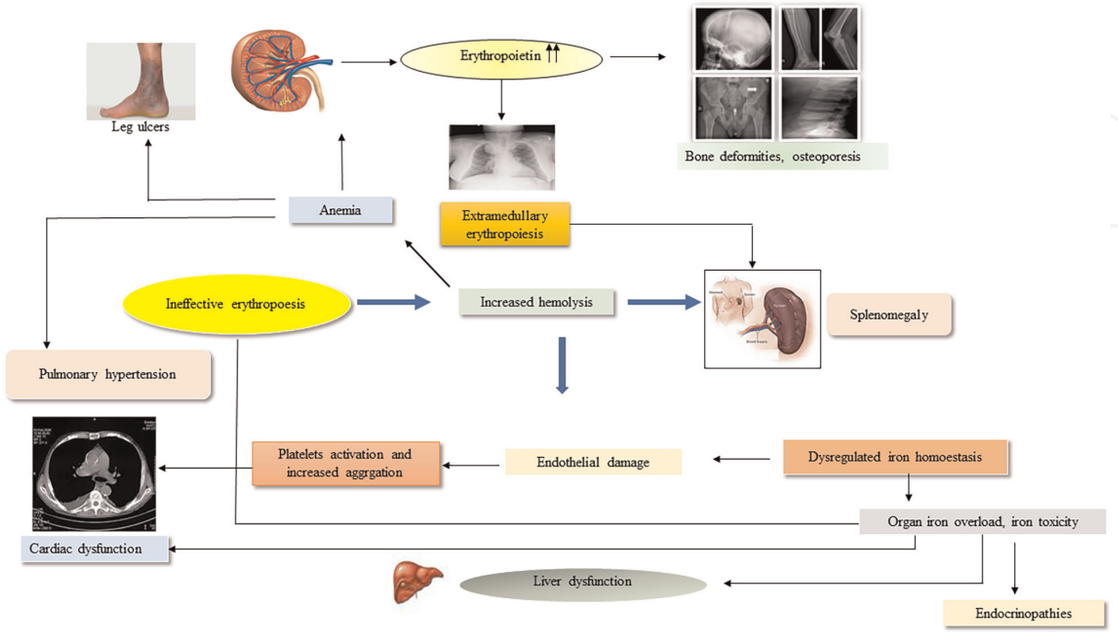

Figure 3.
Clinical heterogeneity of HbE/β-thalassemia patients.

4.1 Asymptomatic forms

HbE trait: Individuals with the HbE trait are clinically normal with minimal changes in blood count and erythrocyte indices. Haemoglobin electrophoresis reveals the presence of HbE is approximately 28.5 ± 1.5%. Likewise, the hemolysate in compound heterozygotes for HbE and α + −thalassemia contains 25–30%. Due to the coinheritance of α^0-thalassemia, HbE levels are reduced up to 19–21% and in the case of HbAE Bart's disease syndrome, there is a markedly reduction in HbE (13–15%). On the other hand, the interaction of β-thalassemia can cause the elevation of HbE (>39%). Iron deficiency also causes lower amounts of HbE and MCV, MCH in the case of HbE trait [24].

Homozygous HbE: The clinical symptoms of HbE homozygous appear as normal individuals except few clinical conditions like jaundice and hepatosplenomegaly; although the reticulocyte count and hemoglobin level appear as normal (>10 g/dl). The hematological profile reveals nearly 85–95% of HbE and about 20–80% target cells with reduced osmotic fragility. In these patients, HbE/A2 level is high (10–90%) with lower HbA and HbF levels [25]. The interaction of HbE with different types of hemoglobinopathies has been depicted in **Figure 4** where chromatograms of high performance liquid chromatography have been described.

4.2 Symptomatic forms

The co-inheritance of the HbE and β^0-thalassemia trait can lead to transfusion-dependent form of thalassemia major. Likewise, the co-association between HbE homozygote and Hb CS causes mild anemic condition. In contrast, the association between HbE and β^0 mutant allele can cause moderate to severe thalassemia. The coinheritance of HbE homozygotes with HbH disease (α^o-Thal/α + −thal and β^E/β^E)

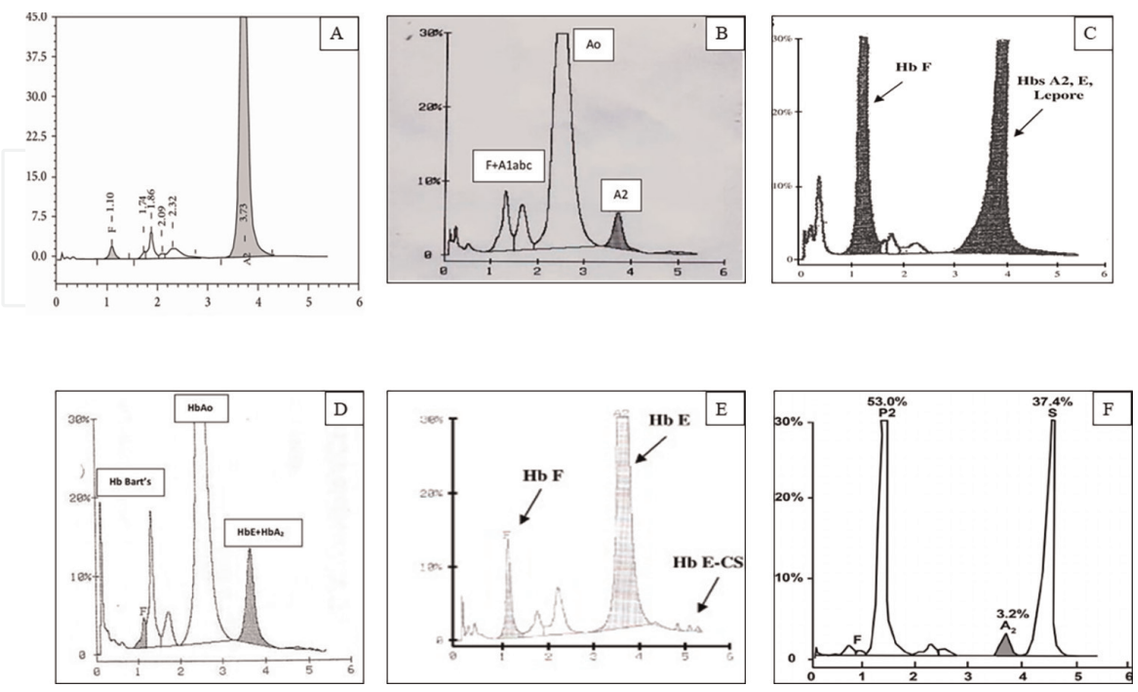

Figure 4.
Chromatograms of HPLC showing interactions of HbE with different hemoglobinopathies (A) HbE homozygous, (B) HbE/β-thalassemia, (C) Hb Lapore, (D) HbAE Bart, (E) HbE-CS, (F) HbSE.

Types of HbE	Clinical symptoms
HbEE	• Hematological findings are similar to that of beta-thalassemia carrier • Hypochromic microcytic red cells with target cells • Reduced mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) • Normal mean corpuscular hemoglobin concentration (MCHC) • Asymptomatic • Enlarged spleen
HbEE and heterozygous alpha-thalassemia	• Phenotype same as that of heterozygous alpha-thalassemia disease • Mild hypochromic anemia • A little elevated HbF levels
HbH (EF Bart disease)	• Moderate anemia and elevated levels of HbF and Bart
HbE beta^{0}-thalassemia	• The most severe form of HbE syndrome • More severe phenotype than HbE beta^{+}-thalassemia • Haemoglobin level is lower than in HbE disease • Phenotype is similar to that of transfusion-dependent beta-thalassemia major including ineffective erythropoiesis, chronic hemolytic anemia, and iron overload
HbE beta^{+}-thalassemia	Clinical severity is less in comparison to HbE beta^{0}-thalassemia
HbAE Bart	Small amounts of Hb Bart are present, but hemolytic crises rare
HbEF Bart	HbH is not present, possibly because beta-E chains do not form tetramers; relatively mild form of thalassemia
HbE Constant Spring	Co-inheritance of Hb Constant Spring ameliorates the disease phenotype
HbE Lepore	Mild phenotype
HbE delta-beta-thalassemia	Mild phenotype
HbSE	• Mild anemia with microcytic red cells • Sickling disorder, clinical phenotype like sickle beta^{+}-thalassemia • Vaso-occlusive episodes rare
HbE trait (HbAE)	• Asymptomatic • Minimal morphological abnormalities of red blood cells including reduced MCV, reduced MCH, mild or no anemia • Peripheral smear is characterized by hypochromia, microcytosis, target cells, and irregularly contracted cells

Table 2.
Different types of HbE thalassemia and their clinical symptoms.

and HbH-CS (α^{o}-Thal/Hb CS and β^{E}/β^{E}) lead to form moderate to severe anemia. Severe anemia may also form due to the presence of HbE/β-thalassemia along with HbH disease (α^{o}-Thal/α^{+}-thal & β^{o}/β^{E}) or HbH-CS disease (α^{o}-Thal/Hb CS and β^{o}/β^{E}) [26].

The different forms of symptomatic and asymptomatic forms of HbE disease have been enlisted in **Table 2**.

5. Genotype-phenotype interaction

The exact reason behind the phenotypic heterogeneity of HbE/β-thalassaemia is not properly understood. For a proper understanding of the clinical severity there is a need for a standardized, robust classification of disease severity; although a lack of

suitable clinical severity scoring may impair deciphering of the proper clinical spectrum of HbE/β-thalassemia. According to the phenotypic heterogeneity, patients have been classified into "severe," "moderate," and "mild." There are considerable number of patients who are transfusion-independent while others are regular transfusion-dependent [27–29]. According to the severity of the disease, patients are classified into five groups. Group 1 included those patients who need minimal transfusion requirements as well as normal growth and sexual maturity. Group 2 comprised patients with similar types of quality of life to that of Group 1 except for transfusion history as these patients usually experience longer history of transfusion. Group 3 includes patients who have undergone splenectomy and have an advantageous response to splenectomy. Group 4 comprises patients who are transfusion-dependent and their secondary sexual characteristics and growth rate are not satisfactory. Likewise, Group 5 includes patients who are unable to maintain their regular lifestyle without transfusion [29].

6. Genetic modifiers of HbE/β-thalassemia

The wide range of clinical phenotypes of HbE/β-thalassemia is believed to be regulated through several genetic as well as environmental factors. There is an emerging understanding of the interaction between genetic and environmental factors which triggers the clinical progression and severity.

According to some previous findings, alterations in the *HBB* gene play a crucial role in modulating phenotypic features of HbE/β-thalassemia. Although *HBB* mutations are not only responsible for modulating phenotypic alterations by changing the patterns of gene expression. Currently, genome-wide association study (GWAS) has shown the linked genetic loci to predict phenotype diversity. The genetic modifiers can be classified into the following three groups: the first one is the primary genetic factors including the β-globin gene mutations which are responsible for the manifestation of β-thalassemia; the second one includes loci involved in globin chain synthesis; and the third one is the tertiary factors which are not involved directly in globin chain synthesis but might modulate the disease severity [30].

6.1 Primary modifier

The primary modifier is one of the most important factors responsible for regulating the phenotype variability in β-thalassemia disorder. Such type of defects occurs in the β-globin gene itself [31]. The mutant allele can reduce the synthesis of the β-globin chain or lead to the complete absence of the β^0-globin chain. The patients who inherit a mild β-thalassemia allele with HbE might exhibit minor disease, whereas patients who co-inherit severe β + or β0-thalassemia alleles might exhibit the severe form of the disease [32]. In addition, the severity of β-mutation is an important parameter for determining the clinical diversity of HbE/β-thalassemia. Most of the mutations in the *HBB* gene are point mutation, deletion, or insertion type and situated in the promoter, exon, intron, or at the junction between the intron-exon boundary, and polyadenylation site. Defects in single base substitution in the coding sequence of β-polypeptides will lead to premature stop codon whereas small insertion or deletion may lead to alteration in the reading form of mRNA. Likewise, β + allele defects are generally caused due to single base substitution which causes alteration of mRNA reading frame. The list of mutations responsible for mild and silent types of thalassemia is enlisted in **Table 3**.

Location of mutation	Mild β+	Silent
Transcriptional mutants in the proximal CACC box	−90 (C-T) −88 (C-T) −87 (C-A) −87 (C-T) −87 (C-A) −86 (C-T) −86 (C-G)	−101 (C-T) −92 (C-T)
TATA box	−31 (A-G) −30 (T-A) −29 (A-G)	
5′ UTR	+22 (G-A) +10 (−T) +33 (C-G)	+1 (A-C)
Alternative splicing	CD 19 (A-C), Malay CD 24 (T-A)	CD 27 (G-T), Hb Knossos
Consensus splicing	IVS 1-6 (T-C)	
Intron		IVS 2-844 (C-G)
3′ UTR		+6 (C-G)
Poly-A site	AACAAA AATGAA	
Mild β°-frameshift	CD 6 (−AA) CD 8 (−AA)	

Table 3.
List of HBB *gene mutations responsible for mild β + and silent type thalassemia.*

In the Indian population, five mutations are the most frequently found β-globin gene including IVS 1-5 (G → C), IVS 1-1 (G → T), Codon 41/42 (− TCTT), Codon 8/9 (+G), and 619 bp deletion which account for over 90% of all the mutations associated with β-thalassemia [33, 34], whereas in Malaysian population, IVS1-5(G > C), IVS1-1 (G > T) mutations and Chinese population, CD41/42 (-TCTT), CD71/72(+A), CD 17 (A-T) and − 28 (A-G) mutations are most commonly found. Among the non-sense mutations CD43(G > T), CD35(C > A), CD15(A > G) mutations lead in formation of nonfunctional mRNA, whereas CD8/9(+G), CD15(−T) are frame shift mutations. Certain mutations cause alterations in RNA processing including splice junction changes [CD27/28 + C, CD14/15 + G, CD 95 + A, CD41(-C), CD26 (G > T), IVS1-1 (G > T)]. There are certain deletion mutations including 619 bp del, 3.5 kb del, 45 kb del, and 105 bp del. [35]. Mutation at nucleotide −28 in the ATA box of the β globin gene, was reported earlier among the HbE/β-thalassemia patients [36]. The interaction of two β + globin alleles which is IVS1-5(G > C) and − 28(A > G) resulting in the mild phenotype The beta-thalassemia alleles in "*trans*"-condition to HbE do not seem to have a significant role in regulating phenotypic variation of HbE/β-thalassemia and there must be some other modifying factors. The position of different *HBB* mutations is schematically represented in **Figure 5.** The co-inheritance of β^0-allele with HbE gives rise to widely variable clinical phenotype. Therefore, β-globin gene mutations alone cannot determine the clinical severity.

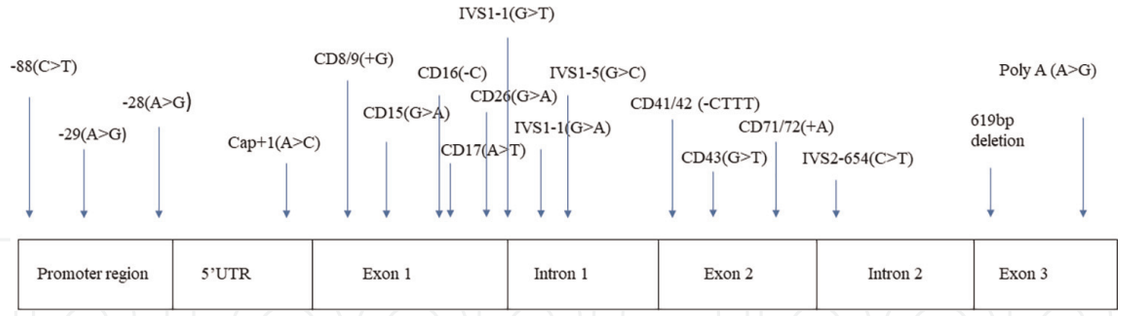

Figure 5.
The position of different HBB *gene mutations within the promoter, 5′ UTR, Exon 1, Intron 1, Exon 2, Intron 2, and Exon 3 region which act as the primary genetic modifiers in HbE/β-thalassemia.*

6.2 Other modifying factors

6.2.1 α-thalassemia

HbE/β-thalassemia patients who co-inherit determinants for α-thalassemia may have some unmatched α-globin chains leading to more balanced globin chain synthesis and resulting in a milder phenotype. According to some previous studies, HbE/β-thalassemia patients with α^{+}-thalassemia allele demonstrate higher state of hemoglobin in comparison to those who do not have α-thalassemia [37, 38]. One of the studies done on Thai HbE patients revealed that the mean age of clinical presentation was more than those patients who did not possess α-thalassemia mutations [39]. Another study done on Indian HbE/β-thalassemia patients presented that coinheritance of the triplicated α-globin gene led to the formation of mild phenotype and their transfusion requirement was also minimal [40]. Therefore, the coinheritance of the alpha thalassemia gene appears as a major genetic factor regulating the clinical phenotype.

6.2.2 Determining factors for regulating increased HbF level

Xmn1 polymorphism: The presence of G to T substitution at −158 position 5′ to Gγ gene (*Xmn*1 polymorphism) is known to be associated with increased HbF production. Earlier investigations showed that patients with the *Xmn*1 (+/+) genotype were identified only in mildly affected patients; in contrast, patients having the *Xmn*1 (−/−) genotype exhibited severe phenotypes including early age of onset and more transfusion dependency [41, 42]. Overall homozygosity for *Xmn*1 appeared as a crucial genetic determinants for HbE/β-thalassemia; although some conflicting data were presented.

Additional genetic factors: Recently, genome-wide association studies elucidate the role of several additional genetic factors in regulating clinical variability. Numerous single nucleotide polymorphisms (SNPs) in the *BCL11A* gene on chromosome 2p16.1 are known to increase the F-cell number [43]. An extensive genetic association study revealed the presence of quantitative trait loci (QTL) on chromosomes 6q23, 8q, and Xp22 may facilitate the amount of HbF production [44]. Apart from these loci, there are certain other protein molecules including erythropoietin, β-protein 1, *EKLF, GATA-1,* and *NF-E2* which have significant role in regulating the HbF level. The strongest correlation was observed in SNPs in the β-globin gene cluster (chr.11p15),

rs2071348 of the *HBBP*1 and intergenic region between the *HBS1L* and *MYB* genes (chr.6q23) which had important significant role in modulating the clinical heterogeneity.

α-hemoglobin stabilizing protein (AHSP) gene: The alpha hemoglobin stabilizing protein (AHSP) is an erythroid-specific protein which has a potential role as a molecular chaperone for binding with the free α-chains of hemoglobin molecule. AHSP participated in the hemoglobin synthesis and reduced the cytotoxic effect of excessive α-globin chain accumulation [45]. Since these proteins are essential for conformational change in many essential proteins required for erythropoiesis, it might have a certain important role in Hb E/β-thalassaemia.

6.2.3 Bilirubin metabolism

The phenotype of HbE disease is also altered by the presence of chronic hyperbilirubinemia, and gallstone formation. The increased level of bilirubin is associated with the polymorphism of the promoter of the UDP-glucuronosyltransferase-1 (UGT1) gene [46]. The UGT1 gene polymorphism is also important in the genesis of gall stone. Investigators found significantly higher bilirubin levels in HbE/β-thalassemia patients [47].

6.2.4 Coinheritance of other hematologic disorders

Coinheritance of other hematologic aberrations may play important role in phenotypic alterations within the patients with HbE/β-thalassemia. The deficiency of pyrimidine 5 nucleotidase 1 (P5N-I) is found to be resulting in hemolytic anemia while co-associated with homozygous HbE [48]. Therefore, the inhibition of P5N-I activity may lead to severe hemolysis related to HbE.

6.2.5 Variation in iron overload

Poor growth and delayed sexual maturation are the common complications found in a majority of HbE/β-thalassemia and it may be resulted in chronic anemia, iron overload, or a combination of these. Many patients with a transfusion history have substantial iron overload and end-organ damage. Some of the previous studies reported that mutations within the *SLC40A1*, hepcidin, and hemojuvelin might play a crucial role in iron overload [49–51]. Although the complete profile of iron overload among the HbE/β-thalassemia patients is not completely understood.

7. Environmental influences on the phenotype of HbE/beta-thalassemia

There are scanty reports available on the environmental influence of HbE/beta-thalassemia. In tropical regions and developing countries, there is a higher incidence of malaria. It is one of the major health issues in many Asian countries and mostly the transmission occurs through *P. falciparum* and *P. vivax*. A population-based study among the HbE/β-thalassemia revealed the level of malarial antibody is significantly high, especially among splenectomized patients rather than those with the intact spleen. Overall, the quantity of malarial antibodies was quite higher in HbE/β-thalassemia patients rather than control population [52]. Studies reported

that the growth of malarial parasites was inhibited in HbE cells [53]. Children with HbE/β-thalassemia are more prone to *P. vivax* due to the production of an increased amount of young red cell population. *P. vivax* has high potentiality for invasion within the young red blood cell [54]. Although, the biological pathways linking HbE thalassemia and malarial infection are not completely still understood and yet not investigated so much. Further research is required to elucidate whether there is a positive selection for HbE due to protection against malarial infection. The finding is very important for the treatment purpose of malaria, especially in developing countries like India and other South-East Asian countries where HbE/β-thalassemia is very common. Patients with HbE/β-thalassemia should be provided with prophylaxis against common forms of malaria.

7.1 Treatment strategy

The time of diagnosis, physical examination, and clinical history may give proper information for providing potentially effective treatment strategies. The proper history of appetite, weight gain, energy level, irritability, different major and minor infections, and daily functioning details may help in defining the proper clinical status of a child with thalassemia. Moreover, the genotype of the patients, the presence of α-thalassemia, and polymorphism associated with increased HbF production should also be investigated intermittently. Among the pathological features, Hb concentration, and platelet count should be determined in each visit. Serum EPO level, ferritin, or transferrin saturation should also be considered in the regular interval [55]. In the case of HbE/β-thalassemia patients, there is a significant decrease in the oxygen affinity of hemoglobin in comparison to other types of β-thalassemia diseases which indicates that HbE thalassemia might adapt better to anemia; therefore, proper treatment guidelines should be followed which depends on the clinical severity score of the patient.

7.2 Transfusions

Patients with severe forms of HbE need lifelong RBC transfusions, iron chelation therapy, and management of complications. In contrast, patients with milder forms of disease severity only require occasional blood transfusions. The pre-transfusion hemoglobin concentration of 9–10 g/dl is recommended as this is required for preventing ineffective erythropoiesis. The hemoglobin threshold for determining the increased frequency of disease complications was 7–8 g/dl [56]. The optimal quantity of hemoglobin should be maintained with accessibility to iron chelation therapy. Some patients may need regular transfusion as it is essential to identify phenotypic heterogeneity between "mild" and "severe" within a narrow range of stable stage hemoglobin values [27, 28]. Generally, transfusion-dependent patients should be monitored carefully especially their quality of life, spleen size, signs, and symptoms of anemia. Sometimes, many HbE/β-thalassemia children suffer from different complications due to unnecessary administration of regular transfusion therapy for several years.

7.3 Splenectomy

One of the treatment strategies for transfusion-dependent thalassemia patients is splenectomy which is believed to ameliorate red blood cell transfusion. Although not

all patients respond equally to splenectomy. Moreover, splenectomy might facilitate many unfavorable consequences like postoperative infections and thromboembolic events [57]. In recent years, the use of splenectomy is characteristically reduced due to an adequate amount of blood transfusion. When the annual blood transfusion volume exceeds 225–250 ml/kg, splenectomy is prescribed among patients with increased demand for transfusion, hypersplenism, or splenomegaly due to severe hemolysis [58]. Splenectomy might have some benefits in HbE/β-thalassemia patients. Although it is not clear that morbidity and mortality due to infection after splenectomy are higher in all groups of patients.

7.4 Iron chelation therapy

Regular transfusion-dependent patients require iron chelation therapy. Even in the absence of transfusions, chelation therapy may be required in some patients with HbE thalassemia because of excess intestinal iron absorption. In the case of regular blood transfusion, each RBC contains 200 mg of iron which leads to nearly 0.3–0.6 mg/kg per day iron accumulation. Iron chelators are classified into three classes: Deferasirox (DFX), Deferiprone (DFP), and Deferoxamine (DFO) [59]. DFX (Exjade) is recommended as an iron chelator after 2005 for transfusion overloaded [60, 61]. It is found to be effective both in the case of offspring and grownups. Generally, 20–30 mg/kg daily dose is recommended; although in certain cases, the dose is recommended to enhance up to 40 mg/kg daily. DFP is generally absorbed by the gastrointestinal tract and the half-life period of DFP is 1.5–4 hours in plasma. The dose is recommended as 75 mg/kg daily. This dose might be elevated to 100 mg/kg daily [62]. It has efficiency for improving cardiac function by removing iron from cardiac muscle [63]. Deferoxamine (DFO) enters the parenchymal cells of the liver where it chelates the iron as the iron chelator DFO in plasma and bile. The dose and duration of administration differ from patient to patient and depend on how much amount of iron is accumulated after transfusion [64]. Chelation therapy is generally initiated after 20–25 RBC units are transfused between 2 and 4 years of age [65].

Patients should be prescribed iron chelation treatment according to proper guidelines. Quantitative assessment of iron is now recommended among patients before starting chelation therapy [37]. In patients who fail to respond sufficiently to a single iron-chelating drug, the dose can be augmented for betterment [66, 67]. The low molecular weight orally absorbed DFP and DFX quickly access intracellular iron in cytosol and organelles whereas larger DFO molecule contacts with these intracellular iron pools relatively slowly; although it interacts more effectively with lysosomal ferritin iron [68].

7.5 Bone marrow transplantation (BMT)

The bone marrow transplantation is regarded as the main conclusive treatment approach for thalassemia patients [69]. The most effective transplantation was completed in the 1980s. The thalassemia-free survival rate is 70% in very young individuals after satisfactory BMT whereas the rejection rate is 23%, and the mortality rate is 7% [70]. In this therapy, hematopoietic stem cells (HSC) from the bone marrow of healthy individuals are collected and transmitted to thalassemia patients [71]. Although BMT has a few disadvantages like human leukocyte antigen-matched compatible donor is required for the successful attempt. Graft versus host disease (GVHD) is the most clinically important problem associated with bone marrow transplantation

which may lead to lethality [72]. In low socioeconomic developing countries, treatment through BMT is still not accessible for all patients and the accessible treatment includes chelation therapy and transfusion of packed red cells.

7.6 Gene therapy

Gene transfer therapy helps in introducing genetic materials into the cells. If the altered gene leads to forming the necessary protein being defective, gene transfer therapy can bring about a normal copy of the gene to regain the proper function of the targeted protein. In gene therapy initially, a hematopoietic stem and progenitor cells (HSPCs) of patients are harvested from the peripheral blood, bone marrow, and umbilical cord blood. Through a lentiviral vector, normal β or γ-gene is transferred into the genome of host cells. Hemoglobin genome is transferred into pluripotent hematopoietic cells and is also performed carefully in humans [73]. The cells that contain expected genes are again implanted into patients where they multiply and proliferate in the bone marrow.

Induced pluripotent stem cells (iPSCs) are also used in future gene therapies. Recently, iPSCs are used as in vitro models to reveal the pathophysiological mechanisms of human diseases. In this technique, somatic cells are first isolated from the patients and then remodeled into a pluripotent form [74]. Induced pluripotent stem cells (iPSCs) are susceptible to acchieve alterations in the gene. Then after, these cells are distinguished into hematopoietic stem and progenitor cells and then transferred back into the individuals.

Gene editing is another approach to future gene therapy. According to this method, human DNA can be cut at specific nucleases, like CRISPR/Cas9 and zinc-finger nucleases [75]. They can either enhance the production of HbF, by reorienting the mutations seen in the hereditary persistence of fetal hemoglobin or act specifically on the erythroid enhancer region that regulates the switch from the γ-globin gene to β-globin gene. Recently, gene editing was done by treating with CRISPR/Cas9 gene-editing method in a thalassemia patient with β0/IVS-1-110 genotype. By editing *BCL11A* gene (chromosome 2) the HbF level was elevated and the patient was transfusion-independent at 12-months follow-up [76].

8. Induction of fetal hemoglobin production

Various drugs are used to induce the production of HbF including Hydroxyurea (HU). It is used for the treatment of sickle cell anemia as well as thalassemia. HU enhances the production of gamma-globin gene and improves the hematological profile of thalassemia patients. It increases the expression of fetal hemoglobin by regulating the expression of GATA-2 (fetal hemoglobin regulating gene) which is related to the cell cycle and apoptosis. It may also facilitate the propagation of progenitor cells and enhance the quantity of erythropoietin [77]. At the same time, 5-azacytidine and butyrate analogs have also been used most frequently to elevate the HbF level [78, 79].

Several HbF inducers inhibit the histone deacetylase (HDAC) activity [80] and can stimulate the HbF level without disturbing the growth and proliferation of other cells. The combined use of HbF inducers may improve the result. Presently, different oligonucleotide (ODN)-based approaches might help design specific treatment strategies for different types of β-thalassemia [81].

9. Molecular therapy for HbE/β-thalassemia

The severity of β-thalassemia, as well as HbE/β-thalassemia, can be reduced by regulating the amount of free α-globin chain synthesis by the coinheritance of α-thalassemia which may reduce the disease severity. The upregulation of AHSP protein or synthesis of such type of similar agent can limit the formation of α inclusion bodies and ineffective erythropoiesis [45].

Moreover, there are some additional prognostic indicators including *Xmn*1 polymorphism, co-existence of Alpha globin gene mutations, and age of onset [82]. Ethnicity and environment are also significant parameters for the analysis of genotype and phenotype correlation. Genetic modifiers that enhance the secondary complications resulting from severe anemia or excessive iron overload due to frequent transfusion are also considered for determining the disease's severity and progression [83]. Therapeutic antisense m RNA is used for correcting aberrent RNA splicing. The use of morpholino oligonucleotides has the ability for high level correction of transcribed mutant β-globin m RNA. These oligonucleotides have been shown to correct the aberrant splice site in a HeLa cell line bearing β^E/IVS1–6 mutations. The repaired β^E-mRNA was stable and translated into mature β^E globin polypeptide [84]. Another approach for molecular therapy of HbE/β -thalassemia in the Ubiquitin-dependent α-chain proteolysis. The excess α-globin chains in β -thalassemia and HbE/β -thalassemia erythrocytes are degraded by ATP and ubiquitin-dependent mechanisms. The cytosolic α-chain precipitation and subsequent cellular damage and haemolysis may be reduced due to efficient proteolysis. Previous experiments demonstrated that radiolabelled α-chains in hemolysates obtained from β-thalassaemia patients were degraded in increased level due to hydrolysis through Ubiquitin aldehyde [85]. *Role of antioxidants in cellular damage* several studies have demonstrated that reactive oxygen species (ROS) play a crucial role in the pathophysiology of thalassemia. Thalassemia patients have very high level of malonyldialdehyde, a biproduct of lipid peroxidation. Transfusion-dependent HbE/β-thalassemia patients have very high level of serum iron and correlates positively with levels of malonyldialdehyde [86]. Treatment with Vitamin C and E is well known to improve the oxidative profile of thalassemia patients [87].

The phenotype heterogeneity of HbE/β-thalassemia causes difficulty in the proper management and classification of the disease. Although some of the genetic factors have been recognized as possible modifiers, the wide range of phenotypic alterations cannot be well understood and it requires thorough investigation from early childhood before substantial medical intervention [88, 89]. With the advancement of molecular technologies, the association studies between genetic polymorphism and thalassemia may help explore potential clinical applications by providing possible risk markers and therapeutic targets. The development of personalized medicine is the main objective and genetic counseling should be included in providing patient care programs in the proper management of HbE/β thalassemia patients in the future.

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Author contributions

Conceptualization, Supervision and Editing: TKD and SMC.
Conceptualization, Original Draft writing formatting and Editing: AP.
Writing and formatting: BD.

Conflict of interest

The authors declare that they have no competing interests.

Appendix A: Mutations in the human beta globin gene cluster-responsible for β0 or β+ type of thalassemias

Mutation position in *HBB* gene	Name of the mutations	HGVS nomenclature	Phenotype
(A) G-gamma, A-gamma, ψβ, δ and Locus control region (hypersensitive sites) of HBB gene	Turkish G gamma (Agamma delta beta)	NG_000007.3:g.45410_81665del36256	β0
	HPFH-6	NG_000007.3:g.45595_124872del79278	β0
	German G gamma (Agamma delta beta)	NG_000007.3:g.(45922_46319)_(98640_99640)del	β0
	Malay-2 Ggamma (Agamma delta beta)	NG_000007.3:g.(47376_47553)_(89149_90149)del	β0
	Italian (Agamma-delta-beta)	NG_000007.3:g.(48103_48100)_(103161_103158) del55059	β0
	Algerian HPFH deletion	U01317.1:g.[(48747_72606)del23860;(72609_72611)delA]	HPFH
	French West-Indies HPFH deletion	U01317.1: g.48762_72489del23728insCAGCAGGAAAGTGTGAGAAG	HPFH
	Chinese Ggamma (A gamma deltabeta)	NG_000007.3:g.48795_127698del78904	β0
	Black Ggamma (Agammadeltabeta)	NG_000007.3:g.49040_84889del35850	β0
	Indian Ggamma Agamma(deltabeta)	NG_000007.3:g.50509_83170del32662	β0
	Japanese (deltabeta)	NG_000007.3:g.51483_165148del113666	β0
	French HPFH deletion	U01317.1:g.[53013_72746del19734;53009_53010insC].	HPFH
	HPFH-3; Indian	NG_000007.3:g.53390_103157del49768	HPFH
	HPFH-2; Ghanaian	NG_000007.3:g.54867_139178del84312	HPFH
	HPFH-1; Black	NG_000007.3:g.59478_144395del84918	HPFH
	Spanish (deltabeta)0-Thal	NG_000007.3:g.60375_153285del92911	(δβ)0
	Black (deltabeta)0-Thal	NG_000007.3:g.(60530_60730)_(72351_72551)del11822	β0
	-125 bp deletion	U01317:g.61953_62077del125	β0
	Kabylian deletion-insertion	U01317.1:g.62009_64049del2040insATAAG	β0
	NG_000007.3:g.(63154-63209)-(70570-70625) del7417	NG_000007.3:g.(63154_63209)_(70570_70625)del7417	(δβ)0

Mutation position in *HBB* gene	Name of the mutations	HGVS nomenclature	Phenotype
	Sicilian (deltabeta)0-Thal	NG_000007.3:g.64336_77738del13403	β0
	Thai (deltabeta)0-Thal	NG_000007.3:g.64384_76993del12610	β0
	~45 kb deletion; the Filipino deletion	NG_000007.3:g.66258_184734del118477	β0
II. 5'-UTR: CAP site	CAP +1 (A->C)	HBB:c.-50A>C	β+
	5'UTR; +10 (-T)	HBB:c.-41delT	β+(Silent)
	5'UTR; +22 (G->A)	HBB:c.-29G>A	β+
	5'UTR; +33 (C->G)	HBB:c.-18C>G	β+ (silent)
	5'UTR; +43 to +40 (-AAAC)	HBB:c.-11_-8delAAAC	β+
(B) Codon ***I. Exon -1***	Initiation codon ATG->GTG	HBB:c.1A>G	β0
	Initiation codon T>A	HBB:c.2T>A	β (0 or + unclear)
	Initiation codon ATG->ACG beta0	HBB:c.2T>C	β0
	Initiation codon ATG->AGG	HBB:c.2T>G	β0
	Initiation codon ATG->ATA	HBB:c.3G>A	β0
	Initiation codon ATG->ATT	HBB:c.3G>T	β0
	Initiation codon ATG->ATC	HBB:c.3G>C	β0
	Codon 1 (-G); GTG(Val)->-TG	HBB:c.4delG	β0
	Codons 2/3/4 (-9 bp; +31 bp)	HBB: c.7_15delinsCCTGAGGTGAAGTCTGCCTGAGGAGAAGTCT	β0
	Codon 5 (-CT); CCT(Pro)->C–	HBB:c.17_18delCT	β0
	Codon 5/6 (-TG)	HBB:c.18_19delTG	β (0 or + unclear)
	HBB:c.20_45del26	HBB:c.20_45del	β0
	Codon 6 (-A); GAG(Glu)->G-G	HBB:c.20delA	β0

Mutation position in *HBB* gene	Name of the mutations	HGVS nomenclature	Phenotype
	HBB:c.8delA	HBB:c.8delA	β0
	Codon 8 (-AA); AAG(Lys)->–G	HBB:c.25_26delAA	β0
	Codons 8/9 (+AGAA)	HBB:c.23_26dup	β0
	Codons 7/8 (+G); GAG AAG(Glu;Lys)->GAG G AAG	HBB:c.24_25insG	β0
	Codons 8/9 (+G); AAG TCT(Lys;Ser)->AAG G TCT	HBB:c.27_28insG	β0
	Codons 9/10 (+T); TCT GCC(Ser;Ala)->TCT T GCC	HBB:c.30_31insT	β0
	Codon 10 (C->A); GCC(Ala)->GCA(Ala)	HBB:c.33C>A	β+
	Codon 11 (-T); GTT(Val)->GT	HBB:c.36delT	β0
	Codons 14/15 (+G); CTG TGG(Leu;Trp)->CTG G TGG	HBB:c.45_46insG	β0
	Codon 15 (-T); TGG(Trp)->-GG	HBB:c.46delT	β0
	Codon 15 (G->A); TGG(Trp)->TAG(stop codon) beta0	HBB:c.47G>A	β0
	Codon 15 (G->A); TGG(Trp)->TGA(stop codon)	HBB:c.48G>A	β0
	Codon 16 (-C); GGC(Gly)->GG	HBB:c.51delC	β0
	Codon 17 (A->T); AAG(Lys)->TAG(stop codon)	HBB:c.52A>T	β0
	Codon 22 (G->T); GAA(Glu)->TAA(stop codon)	HBB:c.67G>T	β0
	Codons 22/23/24 (GAA GTT GGT; Glu Val Gly); deletion of -AAGTTGG	HBB:c.68_74delAAGTTGG	β0
	Codon 24 (T->A); GGT(Gly)->GGA(Gly)	HBB:c.75T>A	β+

Mutation position in *HBB* gene	Name of the mutations	HGVS nomenclature	Phenotype
	44 bp deletion	HBB:c.76_92+27del	β0
	Codon 24; GGT(Gly); (-G; +CAC)	HBB:c.74delinsCAC	β0
	Codons 25/26 (+T); GGT GAG(Gly-Glu)->GGT T GAG(Gly-Term)	HBB:c.78_79insT	β0
	Codon 26 (+T); GAG(Glu)->GTAG	HBB:c.79_80insT	β0
	Codon 26 (G->T); GAG(Glu)->TAG(stop codon)	HBB:c.79G>T	β0
	Codons 27/28 (+C); GCC CTG(Ala Ser)->GCC C CTG	HBB:c.84_85insC	β0
	Codon 28 (-C); CTG(Leu)->-TG	HBB:c.85delC	β0
	Codons 28/29 (-G); CTG GGC(Leu Gly)->CTG -GC	HBB:c.89delG	β0
	25 bp deletion	HBB:c.93-22_95del	β0
II. Exon-2	Codons 36/37 (-T); CCT TGG(Pro-Trp)->CCT -GG	HBB:c.112delT	β0
	Codon 37 (TGG>TAG)	HBB:c.113G>A	β0
	Codon 37 (G->A); TGG(Trp)->TGA(stop codon)	HBB:c.114G>A	β0
	Codons 37/38/39 (-7 nts)	HBB:c.114_120delGACCCAG	β0
	Codons 38/39 (-C); ACC CAG(Thr Gln)->ACC -AG	HBB:c.118delC	β0
	Codon 39 (C->T); CAG(Gln)->TAG(stop codon)	HBB:c.118C>T	β0
	Codon 39 (-A)	HBB:c.119delA	β0
	Codon 40 (-G); AGG(Arg)->AG	HBB:c.123delG	β0
	Codons 40/41 (+T); AGG TTC(Arg-Phe)->AGG T TTC	HBB:c.123_124insT	β0

Mutation position in *HBB* gene	Name of the mutations	HGVS nomenclature	Phenotype
	Codons 41/42 (-TTCT); TTCTTT(Phe-Phe)->- - - -TT	HBB:c.126_129delCTTT	β0
	Codon 41 (-C); TTC(Phe)->TT	HBB:c.126delC	β0
	Codons 42/43 (+G); TTT GAG(Phe Glu)->TTT G GAG	HBB:c.129_130insG	β0
	Codon 43 (G->T); GAG(Glu)->TAG (stop codon)	HBB:c.130G>T	β0
	Codon 44 (-C); TCC(Ser)->TC	HBB:c.135delC	β0
	Codon 45 (-T); TTT(Phe)->-TT	HBB:c.138delT	β0
	Codon 46/47 (+G); GGG GAT(Gly; Asp))->GGG G GAT	HBB:c.142_142dup	β0
	Codon 47 (+A); GAT(Asp)->GAA(Glu) T	HBB:c.143_144insA	β0
	Codons 47/48 (+ATCT); GAT CTG(Asp Leu)->GAT CTATCTG	HBB:c.146_147insATCT	β0
	Codon 51 (-C); CCT(Pro)->-CT	HBB:c.155delC	β0
	Codon 52 (-A)	HBB:c.158delA	β0
	53/54 (+G); GCT GTT(Ala-Val)->GCT G GTT	HBB:c.162_163insG	β0
	Codon 54 (-T); GTT(Val)->GT	HBB:c.165delT	β0
	Codons 54/55 (+A); GTT ATG(Val Met)->GTT A ATG	HBB:c.165_166insA	β0
	HBB:c.166_178del13	HBB:c.166_178delATGGGCAACCCTA	β0
	Codons 56/57/58/59/60 (GGC AAC CCT AAG GTG); duplication of 14 bp	HBB:c.170_183dup	β0
	Codons 57/58 (+C); AAC CCT(Asn Pro)->AAC C CCT	HBB:c.174_175insC	β0
	Codon 59 (AAG>TAG)	HBB:c.178A>T	β0

Mutation position in *HBB* gene	Name of the mutations	HGVS nomenclature	Phenotype
	Codon 59 (-A); AAG(Lys)->-AG beta0	HBB:c.179delA	β0
	Codon 61 (A->T); AAG(Lys)->TAG(stop codon)	HBB:c.184A>T	β0
	HBB:c.187_251dup	HBB:c.187_251dup	β0
	Codon 64 (-G); GGC(Gly)->-GC	HBB:c.194delG	β0
	Codon 67 (-TG); GTG(Val)->–G	HBB:c.203_204delTG	β0
	HBB:c.216delT	HBB:c.216delT	β0
	Codons 71/72 (+A); TTT AGT(Phe Ser)->TTT A AGT	HBB:c.216_217insA	β0
	Codon 72/73 (+T)	HBB:c.219_220insT	β (0 or + unclear)
	Codons 74/75 (-C); GGC CTG(Gly Leu)->GG-CTG	HBB:c.226delC	β0
	Cd76 (-GC)	HBB:c.229_230delGC	β0
	Codon 76 (-C); GCT(Ala)->G-T beta0	HBB:c.230delC	β0
	Codon 77/78 (-C)	HBB:c.232delC	β (0 or + unclear)
	Codons 82/83 (-G); AAG GGC(Lys Gly)->AAG -GC	HBB:c.251delG	β0
	Codon 84 (65bp duplication)	HBB:c.252_253insNG_000007.3:g.70911_70975	β0
	Codons 84/85 (+C); ACC TTT(Thr Phe)->ACC C TTT	HBB:c.255_256insC	β0
	Codons 84/85/86 (+T); ACC TTT GCC(Thr Phe Ala)->ACC TTT T GCC	HBB:c.258_259insT	β0
	Codon 88 (+T); CTG(Leu)->CTTG	HBB:c.266_267insT	β0
	Codons 89/90 (-GT); AGT GAG(Ser Glu)->A–GAG	HBB:c.270_271delTG	β0

Mutation position in *HBB* gene	Name of the mutations	HGVS nomenclature	Phenotype
	Codon 90 (G->T); GAG(Glu)->TAG(stop codon)	HBB:c.271G>T	β0
	Codon 95 (+A); AAG(Lys)->AAAG	HBB:c.287_288insA	β0
	Codon 100; -CTT, +TCTGAGAACTT	HBB:c.301_303delinsTCTGAGAACTT	β0
III. Exon-3	Codon 104 (-G)	HBB:c.314delG	β0
	Codon 106 (-CTGGGCAACGTG)	HBB:c.319_330delCTGGGCAACGTG	β (0 or + unclear)
	Codons 106/107 (+G); CTG GGC(Leu Gly)->CTG G GC	HBB:c.321_322insG	β0
	Codons 108/109/110/111/112 (-12 bp)	HBB:c.326_337delACGTGCTGGTCT	β0
	Codon 112 (T->A); TGT(Cys)->TGA(stop codon)	HBB:c.339T>A	β0
	Codon 114 (+TGTGCTG)	HBB:c.345_346insTGTGCTG	β (0 or + unclear)
	Codon 116 (+TGAT)	HBB:c.349_350insTGAT	β0
	Codon 117 (-C)	HBB:c.354delC	β (0 or + unclear)
	Codons 120/121 (+A); AAA GAA(Lys-Glu)->AAA A GAA	HBB:c.363_364insA	β0 (mild/ dominant β)
	Codon 121 (G->T); GAA(Glu)->TAA(stop codon) beta0 (dominant beta-thal trait)	HBB:c.364G>T	β0
	Codon 123/124/125 (-ACCCCACC)	HBB:c.370_378delACCCCACCA	β0
	Codon 124 (-A)	HBB:c.375delA	β0
	Codon 124/125(+A)	HBB:c.375_376insA	β (0 or + unclear)
	Codons 124-126 (+CCA)	HBB:c.378_379insCCA	β0

Mutation position in *HBB* gene	Name of the mutations	HGVS nomenclature	Phenotype
	Codon 125 (-CCAGTG)	HBB:c.376_381delCCAGTG	β (0 or + unclear)
	Codon 125 (-A); CCA(Pro)->CC-	HBB:c.378delA	β0
	Codons 126-131 (Val-Gln-Ala-Ala-Thr-Gln) (-17 bp); GTG CAG GCT GCC TAT CAG->G	HBB:c.380_396delTGCAGGCTGCCTATCAG	β0
	Codon 127 (C->T); CAG(Gln)->TAG(stop codon)	HBB:c.382C>T	β0
	Codon 127 (A->C); CAG(Gln)->CCG(Pro)	HBB:c.383A>C	β+
	Codon 127 (A->G); CAG(Gln)->CGG(Arg)	HBB:c.383A>G	β+
	Codons 127/128 (-AGG); CAG GCT(Gln Ala)->C— CT(Pro)	HBB:c.383_385delAGG	β0
	Codons 128/129 (-4, -GCTG; +5, +CCACA) Codons 132-135 (-11, -AAAGTGGTGGC)	HBB:c.[385_388delinsCCACA;397_407delAAAGTGGTGGC]	β0
	Codon 130 (TAT→TAA)	HBB:c.393T>A	β (0 or + unclear)
	Codon 131 (+A)	HBB:c.394_395insA	β (0 or + unclear)
	Codons 131/132 (-GA)	HBB:c.396_397delGA	β0
	Codon 132A>T	HBB:c.397A>T	β0
	Codons 134-137 [-(G)TGGCTGGTGT(G) and +(G)GCAG(G)]	HBB:c.404_413delinsGCAG	β0
	-7719 bp del	U01317:g.71551_79269del7719	β0
	619 bp deletion	NG_000007.3:g.71609_72227del619	β0
IVS-I	IVS-I (-1) or codon 30 (G->A) AG^GTTGGT->AA^GTTGGT	HBB:c.92G>A	β0
	IVS1-1(G>A); AG^GTTGGT->AGATTGGT	HBB:c.92+1G>A	β0

Mutation position in *HBB* gene	Name of the mutations	HGVS nomenclature	Phenotype
	IVS-I-1 (G->T); AG^GTTGGT->AGTTTGGT	HBB:c.92+1G>T	β0
	IVS-I-2(T>C); AG^GTTGGT->AGACTGGT	HBB:c.92+2T>C	β0
	IVS-I-2 (T->A); AG^GTTGGT->AGGATGGT	HBB:c.92+2T>A	β0
	IVS-I-2 (T->G); AG^GTTGGT->AGGGTGGT	HBB:c.92+2T>G	β0
	IVS-I-5 (G->T)	HBB:c.92+5G>T	β+(severe)
	IVS-I-5 (G->A) plus the Corfu deletion	HBB:c.92+5G>A	β+
	IVS-I-5 (G->A)	HBB:c.92+5G>A	β+(severe)
	IVS-I-5 (G->C)	HBB:c.92+5G>C	β+(severe)
	IVS-I-6 (T->C); the Portuguese	HBB:c.92+6T>C	β+
	IVS I-7 (A>T)	HBB:c.92+7A>T	β+
	IVS-I-110 (G->A) beta+; the mutation is 21 nucleotides 5' to the acceptor splice site AG^GC	HBB:c.93-21G>A	β+
	IVS-I-116 (T->G)	HBB:c.93-15T>G	β0
	IVS-I-128 (T->G); TTAG^GCTG->TGAG^GCTG	HBB:c.93-3T>G	β+
	IVS-I-129 (A>G)	HBB:c.93-2A>G	β0
	IVSI-129 (A>C)	HBB:c.93-2A>C	β (0 or + unclear)
	IVS-I-130 (G->A); TTAG^GCTG->TTAA GCTG	HBB:c.93-1G>A	β0
	IVS-I-130 (G->C); TTAG^GCTG->TTAC GCTG	HBB:c.93-1G>C	β0
IVS-II	IVS II-1 (G>T)	HBB:c.315+1G>T	β0
	IVS-II-1 (G->C)	HBB:c.315+1G>C	β0
	IVS-II-1 (G->A)	HBB:c.315+1G>A	β0

Mutation position in *HBB* gene	Name of the mutations	HGVS nomenclature	Phenotype
	IVS II-2 (T>C)	HBB:c.315+2T>C	β0
	IVS-II-2 (T>A)	HBB:c.315+2T>A	β (0 or + unclear)
	IVS-II-2 (-TGAGTCTATGGG)	HBB:c.315+2_315+13delTGAGTCTATGGG	β (0 or + unclear)
	IVS-II-4,5 (-AG)	HBB:c.315+4_315+5delAG	β (0 or + unclear)
	IVS-II-5 (G->C)	HBB:c.315+5G>C	β+(severe)
	IVS-II-654 (C->T); AAGGCAATA->AAG^GTAATA	HBB:c.316-197C>T	β+(severe)
	IVS-II-705 (T->G); GATGTAAGA->GAG^GTAAGA	HBB:c.316-146T>G	β+
	IVS II-726 (A>G)	HBB:c.316-125A>G	β+
	IVS-II-745 (C->G); CAGCTACCAT->CAG^GTACCAT	HBB:c.316-106C>G	β+
	IVS II-761 A>G	HBB:c.316-90A>G	β (0 or + unclear)
	IVS2-781 C>G	HBB:c.316-70C>G	β (0 or + unclear)
	IVS-II-837 (T->G)	HBB:c.316-14T>G	β+/β0
	IVS-II-843 (T->G)	HBB:c.316-8T>G	β+
	IVS-II-844 (C->G)	HBB:c.316-7C>G	β+
	IVS-II-848 (C->G)	HBB:c.316-3C>G	β+
	IVS-II-848 (C->A)	HBB:c.316-3C>A	β+
	IVS-II-849 (A->G)	HBB:c.316-2A>G	β0
	IVS-II-849 (A->C)	HBB:c.316-2A>C	β0
	IVS-II-850 (G->A)	HBB:c.316-1G>A	β0

Mutation position in *HBB* gene	Name of the mutations	HGVS nomenclature	Phenotype
	IVS-II-850 (G->T)	HBB:c.316-1G>T	β0
	IVS-II-850 (G->C)	HBB:c.316-1G>C	β0
	IVS-II-2,3 (+11, -2)	HBB:c.315+2_315+3delinsACGTTCTCTGA	β0
	IVS-II-765 L1	HBB:c.316-86_316-85insCTGCTTTTATTTT	β+
	IVS-I, 3' end; -17 bp	HBB:c.93-17_93-1delTATTTTCCCACCCTTAG	β0
	IVS-II-850 (-G)	HBB:c.316-1delG	β0
	+1480 (C->G)	HBB:c.*6C>G	β+
(E) 3'- UTR region and poly A signalling	3'UTR (-GCATCTGGATTCT)	HBB:c.*91_*103delGCATCTGGATTCT	β (0 or + unclear)
	Poly A (T->C) AATAAA->AACAAA	HBB:c.*110T>C	β+
	Poly A (A->G) AATAAA->AATGAA	HBB:c.*111A>G	β+
	Poly A (A→T) AATAAA>AATATA	HBB:c.*112A>T	β (0 or + unclear)
	Poly A (A->G); AATAAA->AATAGA	HBB:c.*112A>G	β+
	Poly A (A->G); AATAAA->AATAAG	HBB:c.*113A>G	β+
	Poly A (-AATAA); AATAAA->———A	HBB:c.*108_*112delAATAA	β+
	Poly A (-AT or -TA); AATAAA->A–AAA	HBB:c.[*109_*110delAT or *110_*111delTA]	β+

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