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Fetal hemoglobin regulation in β-thalassemia: heterogeneity, modifiers and therapeutic approaches

Orapan Sripichai and Suthat Fucharoen

Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, Nakhonpathom, Thailand

ABSTRACT

Introduction: Stress erythropoiesis induces fetal hemoglobin (HbF) expression in β-thalassemias, however the level of expression is highly variable. The last decade has seen dramatic advances in our understanding of the molecular regulators of HbF production and the genetic factors associated with HbF levels, leading to the promise of new methods of the clinical induction of HbF. Areas covered: This article will review the heterogeneity and genetic modifiers of HbF and HbF induction therapy in β-thalassemia. Expert commentary: One promising curative β-thalassemia therapy is to induce HbF synthesis in β-thalassemic erythrocytes to therapeutic levels before clinical symptom occurs. Further understanding of HbF level variation and regulation is needed in order to predict the response from HbF-inducing approaches.

1. Introduction

β-Thalassemias and related disorders are among the most common inherited monogenic diseases [1]. Some of these disorders are associated with severe morbidity and mortality, including a lower-than-average life expectancy and some are associated with serious long-term disability. Although genetic screening and prenatal diagnosis successfully reduced the incidence of β-thalassemia in some areas, β-thalassemias remain common in Asian countries with limited resources for patient managements. Most affected patients who received inadequate treatments die in early childhood, whereas most of the affected individuals under specific early care survive for several decades, albeit with chronic and severe complications. With adequate transfusion and the administration of the chelating agent, children may grow and develop well and survive into adult life [2,3]. However, as more of these children survive and more effective treatment is developed, the population of β-thalassemia patients on long-term therapy will steadily increase.

β-Thalassemia is classified by a reduction in the synthesis of β-globin chains (β-thalassemia) or the absence of the synthesis (β0-thalassemia). To date, it is known that there are nearly 300 β-globin gene mutations underlying β-thalassemia [4]. Homozygous β0-thalassemia (β/β0-thalasemia) has clinical features of β-thalassemia major or Cooley’s anemia, which is a severe form of β-thalassemia and most of the patients die in the pediatric age group. Although β+thalassemia is a milder form of β-thalassemia since the patients can produce a certain amount of HbA, compound heterozygous β−/β0-thalassemia and β0/β0-thalassemia (β+/β−) diseases display a severe form of β-thalassemia. Homozygous β−/β−thalassemia (β+/β+) has a variable clinical presentation, depending on the specific interaction of either mild- or severe-type β−/β−thalassemia mutations. Among the Asian population, although the frequency of β-thalassemia is relatively low (varying between 3% and 9%), its interaction with hemoglobin E (HbE; the most common structural hemoglobin variant globally) makes β-thalassemia/HbE disease the most common thalassemia syndrome in this region [5,6], and it accounts for half of the severe β-thalassemia patients worldwide. In Thailand alone, about 3000 children are estimated to be born with β-thalassemia/HbE each year and there are about 100,000 living patients in the Thai population with an average life expectancy of 30 years [5,7]. HbE results from the substitution of guanine by adenine (GAG → AAG) in the triplet codon 26 of β-globin gene, which creates an additional aberrant 5′ donor splice site at codon 25 [8]. Aberrantly spliced mRNAs lead to the loss of a certain part of exon 1 and the out-of-frame translation of an abnormal β-globin protein which cannot function. Consequently, the synthesis of βE-globin chains is reduced and the βE-globin gene behaves like a β−-thalassemia. Normally, heterozygous and homozygous HbE individuals have no symptoms, but β-thalassemia/HbE disease can be as severe as Cooley’s anemia or as mild as non-transfusion-dependent thalassemia (NTDT).

2. Heterogeneity of β-thalassemias

In β-thalassemias, defective synthesis of the β-globin chain leads to the imbalance of α- to non-α-globin chain production and consequently the precipitation of the excess unmatched α-globin chains (Figure 1). The precipitation of α-hemoglobin leads to erythroid membrane rigidity and accelerated apoptosis and premature destruction of the erythroid precursors in the bone marrow (ineffective erythropoiesis), and premature red blood cell destruction [2,9–12]. The increased iron absorption, together with regular blood transfusion, can result in

CONTACT Suthat Fucharoen  suthat.fuc@mahidol.ac.th  Thalassemia Research Center, Institute of Molecular Biosciences, Mahidol University, 25 Phutthamonthon 4 Road, Phutthamonthon, Nakhonpathom 73170, Thailand

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chronic iron overload and death in β-thalassemia patients. However, β-thalassemia intermediate and β-thalassemia/HbE patients show remarkable variability in clinical expression, ranging from asymptomatic or mild clinical symptoms with normal growth development and survival without transfusions, to transfusion-dependent thalassemia major with marked anemia, growth retardation, severe bone changes, hepatosplenomegaly, and heavy iron overload [13–15]. Hemoglobin levels in homozygous β^0-thalassemia can be as low as 3–5 g/dl, while patients with β-thalassemia intermedia maintain hemoglobin values between 7 and 9 g/dl, without the need for a regular transfusion regimen [16]. Hemoglobin levels in β-thalassemia/HbE range from 3 to 12 g/dl, with an average level of 7 g/dl [17].

Although β-thalassemia occurs due to mutation in the β-globin gene, the disease phenotype is the result of multi-gene interactions. The severity of anemia in β-thalassemia reflects the degree of α- and non-α-globin chain imbalance and the excess of unmatched α-globin chains with all their deleterious effects on the erythroid cell. Therefore, any factors that can reduce the degree of globin chain imbalance and the size of the free α-globin chain pool could moderate the clinical features of the β-thalassemias. The primary modifying factor is the nature of the underlying β-thalassemia mutation itself. Generally, the interaction of a mild β^+ -thalassemia allele results in a milder disease. Hemoglobin levels in β^+ -thalassemia/HbE are between 9 and 11 g/dl and usually do not require any treatment [18]. Furthermore, in β-thalassemia/HbE patients, it is proposed that the amount of alternative spliced β^E-globin mRNA may play a role in the variability of disease severity [19]. β-Thalassemia patients who co-inherit α-thalassemia have less redundant α-globin chains and tend to have less severe symptoms [17,20]. A single α-globin gene defect is sufficient to improve the clinical phenotype of homozygous β^+ -thalassemia and β-thalassemia/HbE patients, whereas impairment of the expression of two α-globin genes is necessary in β^0-thalassemia. However, several studies in different cohorts have shown that many patients who are β-thalassemia intermediate or β^0-thalassemia/HbE and do not have any α-thalassemia co-inheritance still have a mild clinical course that is correlated with a higher level of HbF [17]. In addition, the group of homozygous β^0-thalassemia patients showing a mild disease produced a reasonable level of HbF [21]. Furthermore, the interaction of β-thalassemia with hereditary persistence of fetal hemoglobin (HPFH), where HbF levels are steadily high, presents as clinically asymptomatic [14]. To date, the role of increased HbF as an ameliorating factor of β-thalassemias has become more evident, although the extent to which variation of HbF levels in individual patients contributes to the disease heterogeneity has not been clearly evaluated.

3. Fetal hemoglobin in β-thalassemia

HbF is tetramer of two α-globin and two γ-globin chains. The γ-globin chains are encoded by two nearly identical genes (HBG2 and HBG1) within the β-like globin gene cluster on chromosome 11p. HbF is the predominant hemoglobin from early gestation until 1 to 2 months after birth, after which adult HbA predominates. The average level of HbF at birth is around 80%, and the variation depends on the age of gestation. HbF levels steadily decrease after birth and reach adult levels within the second year of life. In normal adults, HbF is less than 1% of total hemoglobin and is distributed unevenly among erythrocytes. There is a delayed switch from γ-globin to β-globin expression in β-thalassemia, and subsequently HbF levels remain above normal in most patients. Patients with β-thalassemia usually present clinically after 2 years of age. Production of HbF after the neonatal period in β-thalassemia is an extremely complex process and is still poorly understood. Table 1 shows the HbF levels in β-thalassemia and its related conditions. About half of the β-thalassemia heterozygotes have slightly increased HbF levels while others have normal levels with a mean HbF of 1.4% ± 1.0% [22]. Similarly, HbE heterozygotes most often have HbF levels near normal or levels that are only slightly increased [18], while HbF levels in HbE homozygotes range

<table>
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<tr>
<th>Conditions</th>
<th>HbF level (%)</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Heterozygous β-thalassemia</td>
<td>1.4% ± 1.0</td>
<td>[22]</td>
</tr>
<tr>
<td>Heterozygous HbE</td>
<td>0.4% ± 0.6</td>
<td>[18]</td>
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<tr>
<td>Homozygous HbE</td>
<td>1–8</td>
<td>[18,23]</td>
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<tr>
<td>Homozygous β^0-thalassemia</td>
<td>95–98</td>
<td>[14]</td>
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<tr>
<td>Homozygous β^-thalassemia</td>
<td>70–90</td>
<td>[14]</td>
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<tr>
<td>Compound heterozygous β^-β^-thalassemia</td>
<td>70–90</td>
<td>[14]</td>
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<tr>
<td>β^-thalassemia/HbE</td>
<td>2–76</td>
<td>[17,24]</td>
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<tr>
<td>Mildly affected</td>
<td>42.5% ± 11.6</td>
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<tr>
<td>Severely affected</td>
<td>31.9% ± 11.7</td>
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between 1% and 8%, with a mean of 4% [23]. In non-transfused β-thalassemia major, Hba is absent while the Hbf level is 95–98% with small amount of HbA2 [14]. In β-thalassemia homozygotes or β/β compound heterozygotes, the hemoglobin pattern shows Hbf level between 70% and 90%, while the Hbf level in β-thalassemia/Hbe ranges from 2% to 76% [17]. Compound heterozygous β/β † thalassemia patients who have increased Hbf level usually have milder clinical symptoms. Mildly affected β-thalassemia/Hbe patients have a mean Hbf of 42.5% ± 11.6% (absolute Hbf 3.35 ± 1.20 g/dl) compared with 31.9% ± 11.7% (absolute Hbf 1.95 ± 0.88 g/dl) in severely affected β-thalassemia/Hbe patients [24]. However, the regulation of diversity of Hbf levels in β-thalassemia is still not clear and requires further investigation.

The increased Hbf level in β-thalassemias is associated with stress erythropoiesis, which may result from the recruitment of erythroid progenitor cells that prematurely undergo terminal differentiation and are committed to producing γ-globin chains [25–27]. Chronic severe anemia in β-thalassemia results in increased erythropoietin (EPO) levels, leading to bone marrow expansion and possibly increased F-cell production. Plasma EPO levels correlate with Hbf levels in β-thalassemia intermedia [28]. The stress signal transduction model suggests that cytokines, such as EPO, stem cell factor (SCF), and transforming growth factor-β (TGF-β), initiate downstream intracellular signaling pathways that activate Hbg expression [29]. Both SCF and TGF-β strongly induce Hbf reactivation in normal adult erythroid cells [30]. Moreover, increased levels of SCF and TGF-β are also found in β-thalassemia patients [31]. In addition, the cAMP signaling pathway is commonly activated in primary erythroid cells isolated from cord blood and β-thalassemia patients who express high levels of Hbf, suggesting that activation of the cAMP signaling pathway may re activate Hbf expression in erythroid cells [32]. Furthermore, Hbf expression can be regulated by posttranscriptional mechanisms [33].

4. Genetic basis of Hbf regulation

Genetic linkage analysis and genome-wide association approaches have identified susceptibility loci for the persistence of Hbf in adulthood or quantitative trait loci (QTLs) controlling Hbf levels, including the β-like globin gene cluster (11p locus), HBS1L/MYB (6q locus), and BCL11A (2p locus). Each of these genetic determinants may themselves show considerable heterogeneity, and the frequency of different alleles may vary widely in different populations. Hence, the various interactions of these modifiers may result in considerable variation in Hbf levels and the wide clinical diversity of β-thalassemias.

4.1. β-like globin gene cluster

Sequencing of approximately 80 kb within the β-like globin gene cluster suggested that polymorphisms comprise two distinct linkage disequilibrium (LD) blocks, one containing the β-globin gene and the other extending from the locus control region (LCR) to the δ-globin gene [34]. Several studies have shown that the C-T polymorphism at position 158 bp upstream of HBG2 (rs7482144; γ-globin XmnI restriction site polymorphism) is associated with high Hbf levels and the clinical severity of β-thalassemia [35–37]. However, other SNPs in LD with rs7482144 were also associated with Hbf levels in β-thalassemia [35]. Moreover, SNP rs10128556 located in the HBBP1 (ω-globin) gene was more strongly associated with Hbf than rs7482144 in β-thalassemia/Hbe [38]. The mechanism whereby this region influences Hbf is still unclear, but it is believed that those SNPs are in LD with the functional elements regulating globin expression. The coordinated function of specific transcription factors such as Kruppel-like factor 1 (KLF1) [39], B-cell lymphoma/leukemia 11A (BCL11A) [40], GATA binding protein 1 (GATA1) [41], zinc finger protein, FOG family member 1 (ZFPFM1/FOG1) [41] and LIM domain binding 1 (LDB1) [42], and the LCR required for correct activation of globin gene expression and hemoglobin switching. Although homozygotes for the Hbf-associated β-like globin gene cluster haplotype, including the T allele of rs7482144, have substantially high Hbf levels, some patients who have other haplotypes, including the C allele of rs7482144, also have very high Hbf, suggesting the importance of other QTLs modulating Hbg expression. In addition, more than 50% of the genetic variance in levels of F-cells is caused by factors not linked to the β-globin gene cluster. While a strong association between Hbf production and β-like globin gene cluster haplotypes is found in β-thalassemia intermedia and β-thalassemia/Hbe, no association was found in homozygous Hbe [43,44], suggesting that the influence of cis-regulatory elements of Hbg expression might not be strong enough to be detected under conditions with slight hematopoietic stress and globin imbalance.

Several mutations in the promoter region of HBG1 and HBG2 are known to modulate Hbf levels and are associated with Hbf values of 2–40% in the heterozygous state. Point mutations associated with non-deletional HPFH were found in regions around positions 114, 117, 175, and 200 in the promoter regions of HBG. The sequences around these variants have been shown to be binding sites for various erythroid-specific and ubiquitous transcription factors such as GATA1, POU class 2 homeobox 1 (POU2F1/Oct-1), and nuclear receptor subfamily 2 group F member 2 (NR2F2/NF-E3) [45]; hence, the variants may modulate the binding of transcription factors to HBG promoter and could result in decreased binding of negative regulators or, vice versa, increased interactions with positive regulators. However, none of these variants was present in the screening of 500 Thai β-thalassemia/Hbe patients [34], suggesting that Hbg promoter variants must be an uncommon cause of high Hbf in populations. The comparison of deletional HPFH with (δβ)′-thalassemia has identified an intergenic region between the γ-globin and δ-globin genes as cis-regulatory elements of the β-like globin gene locus required for Hbf silencing [46,47]. Of note, this region includes polymorphisms that are strongly associated with Hbf levels in β-thalassemia/Hbe patients [34,35].
4.2. **BCL11A (2p15)**

Polymorphisms within intron 2 of BCL11A gene are the major modifiers of F-cell numbers in healthy Northern Europeans [48]. GWASs discovered a strong association of BCL11A polymorphisms with HbF concentrations and clinical features of patients with β-thalassemia [49], β-thalassemia/HbE [35], and sickle cell anemia [50]. In addition, BCL11A polymorphisms were also associated with HbF levels in β-thalassemia heterozygote [51], HbE heterozygote [51], and HbE homozygote [23]. BCL11A is a zinc finger transcription factor that had been largely studied in B-lymphocyte development. However, several lines of evidence suggest that BCL11A is a repressor of γ-globin expression. Knockdown of BCL11A in adult human erythroblasts resulted in increased expression of HbF [52]. The silencing of γ-globin expression is impaired in BCL11A knockout mouse embryos harboring an entire human β-globin locus (β-YAC) transgene [53]. Chromosome immunoprecipitation assays have shown BCL11A binding sites on β-like globin gene cluster, including HS3 of LCR, the γ-δ intergenic region, and a GGCCGG motif in the proximal promoter of HbG. Complexes of BCL11A and other proteins might mediate the suppressive effects of BCL11A on HBG expression [54]. A GWAS-identified SNP-dense region in intron 2 of BCL11A contains an erythroid-specific enhancer element [55]; hence, this region is a potential genomic target for reactivation of HbF via reduction of BCL11A expression.

Several studies have shown that inactivation of BCL11A fails to completely reverse the γ→β-globin switch in adult erythroid cells, suggesting additional hemoglobin switching regulators. Recently, the zinc finger and BTB domain containing 7A (ZBTB7A/LRF/Pokemon) was identified as a potent silencer [56]. The double knockout of ZBTB7A and BCL11A in human immortalized erythroid cell line (HUDEP-2) resulted in increased HbF levels to greater than 95%, whereas lack of either ZBTB7A or BCL11A alone resulted in HbF levels of approximately 50–70% of the total hemoglobin. The initial findings indicated that ZBTB7A occupies the γ-globin genes and physically associates with the NuRD repressor complex independent of BCL11A [56]. In addition, the putative ZBTB7A binding motifs have been identified in the LCR of β-like globin gene cluster, suggesting that ZBTB7A may affect globin gene expression through its binding in the β-like globin gene cluster [57]. To date, none of the rare variants or polymorphisms in ZBTB7A have been found to be associated with elevated HbF levels [57].

4.3. **HBS1L-MYB intergenic region (HMIR, 6q23)**

HBS1L-MYB intergenic polymorphisms (HMIPs) are strongly associated with HbF levels and disease severity among β-thalassemias [49], β-thalassemia/HbE patients [35], sickle cell anemia [50], and are highly associated with HbF expression in β-thalassemia heterozygotes [58]. In addition, HMIP has been consistently identified as highly associated with human erythroid traits such as RBC, MCV, MCH, and others [59,60]. A clear molecular mechanism underlying the association between variants in the intergenic region and erythroid phenotypes remains elusive. The candidate target genes in HMIR are the HBS1L (GTP-binding elongation factor) and MYB (myeloblastosis oncogene encoding the c-MYB transcription factor). c-MYB plays an essential role in regulating hematopoiesis and erythropoiesis [61], whereas the function of HBS1L in RBC development is still uncharacterized. Several HMIPs reduce transcription factor binding affinity, affecting long-range interactions with MYB and MYB expression levels [62]. The 3-bp (TAC) deletion polymorphism located near erythroid-specific DNase I hypersensitive site at 42.6 kb upstream of HBS1L and 83.8 kb upstream of MYB is the most significant functional motif accounting for HMIP modulation of HbF [58]. This polymorphism is in LD with the HbF-QTL SNP rs9399137 identified by GWAS. This region contains the binding sites of erythropoiesis-related transcription factors and appears to have enhancer-like activity. A study in a mouse model has shown that disruption of the Hbs1l-Myb locus causes HPFH in mice [63]. In adult humans with non-deletion HPFH, the expression of MYB and HBS1L was downregulated [64], but only HBS1L expression was correlated with elevated HbF levels in cultured erythroid cells [65]. To date, no expression study has been performed in β-thalassemias. Low levels of MYB were associated with reduced cell expansion and accelerated erythroid differentiation, suggesting that MYB might affect HbF synthesis through its effect on the cell cycle. Overexpression of MYB in KS62 cells inhibited γ-globin expression [65] while downregulated MYB resulting from overexpression of microRNA-15a and -16-1 in human erythroid progenitors increased HbF [66]. Current information suggests that MYB is a promising molecular target for therapeutic induction of HbF level, but HBS1L requires further investigation.

4.4. **KLF1**

KLF1 (formerly known as EKLF) is an erythroid-specific transcription factor critical for erythropoiesis and hemoglobin switching [67,68]. Reduced expression of KLF1 results in downregulation of BCL11A and an increased γ→β-globin ratio [69]. Targeted sequencing has identified rare variants in KLF1 associated with relatively elevated HbF levels in normal controls [70,71], α-thalassemia heterozygotes [72], HbE heterozygotes, and HbE homozygotes [73]. KLF1 mutations causing haploinsufficiency for KLF1 are associated with increased HbF synthesis [69,74] and the amelioration of severity of homozygous β0-thalassemia [75]; hence, manipulating KLF1 expression is possibly another approach for activating HbF in β-thalassemias.

4.5. **Others**

In addition to the well-known HbF-QTLs, other genetic loci have been proposed as modulators of HbF, such as polymorphisms on 8q12, Xp22, and SAR1A genes, but these are less well established in β-thalassemia. The QTL at chromosome 8q appeared to interact with the Xmn1 polymorphism of HBG2 to modulate HbF levels [76]. Polymorphisms in the SAR1A promoter were associated with differences in HbF levels and HbF response to hydroxyurea treatment in sickle cell anemia [77]. The Xp22.3 locus was hypothesized to account for the higher HbF levels in females as compared with males [78].
Although these loci were associated with HbF level or F-cell production in normal subjects and sickle cell anemia patients, they have not been specifically investigated in β-thalassemia. Moreover, these loci did not appear to be associated with HbF in GWASs of β-thalassemia and β-thalassemia/HbE patients.

5. Induction of HbF for treatment of β-thalassemias

Treatment of thalassemia is often undertaken with transfusion therapy, which allows for normal growth and development and suppresses ineffective erythropoiesis, but can result in iron overload. Hence, chelation therapy is provided along with the transfusions, but over the years iron still accumulates in the system. Ultimately, a continuing challenge in thalassemia research is to find new ways to ameliorate the disease severity. The development of an effective therapy to increase hemoglobin levels in β-thalassemia patients, without the use of transfusions, could allow normal growth and development while decreasing the iron overload. Since reactivation of HbF in β-thalassemias can compensate for defective HbA production and reduce imbalanced globin chain synthesis, the goal of HbF-inducing treatments is to reactivate the γ-globin expression to the highest possible degree.

5.1. HbF-inducing agents

Several pharmacologic compounds such as 5-azacytidine, hydroxyurea, decytabine, short-chain fatty acid derivatives, and histone deacetylase inhibitors have been studied, and the clinical trials in β-thalassemia patients have been carried out to evaluate the effects on HbF production [79]. Their potential in the management of β-thalassemia syndromes is still under investigation, since the production of HbF has been disappointing and the exact mechanisms by which these agents induce HbF production are still undefined. Moreover, there is a wide variability in the degree of response, and toxicity as a consequence of long-term use is a point of concern.

Hydroxyurea acts as a potent inhibitor of ribonucleotide reductase, which functions in DNA synthesis and repair. How hydroxyurea induces HbF is not fully understood, but it is believed that the cytotoxic effects of hydroxyurea cause stress erythropoiesis, resulting in an increased HbF level through the cGMP signaling pathway [80]. Hydroxyurea induces γ-globin expression up to two- to nine-fold in cultured primary erythroid cells derived from β-thalassemia [81] and β-thalassemia/HbE patients [82]. The increment of HbF in vivo is largely variable, ranging from 1% to 90% with an average of 20% [79]. Hydroxyurea therapy is also associated with elevation of the total hemoglobin levels and hence reduced transfusion requirement in patients. Although hydroxyurea provides significant benefit to many patients, not all patients respond to hydroxyurea treatment, and approximately 30–70% of β-thalassemia major and at least 50% of β-thalassemia/HbE patients are nonresponders. Discrepancies in the prediction of a good response are noted for β-like globin gene haplotypes, the XmnI polymorphism on HBG2, and co-inheritance of α-thalassemia. Hydroxyurea is also used in β-thalassemia intermedia patients to reduce extramedullary masses [83] and improve leg ulcers in some cases [84]. Long-term follow-up studies have suggested that the side effects of hydroxyurea therapy in β-thalassemia were minimal at the dose used in clinical trials.

5-Azacytidine is a DNA-hypomethylating agent observed to induce HbF in anemic baboons [85] and was used in severe β-thalassemia with initially promising results in increasing γ-globin synthesis [86]; however, continued trials were abandoned because of safety concerns. Decitabine (5-aza-2′-deoxycytidine) was shown to induce DNA methylation inhibition with less toxicity and to increase HbF and total hemoglobin levels in patients with sickle cell anemia [87] and β-thalassemia [88], but phase 2 or 3 studies have not been done to date. Arginine butyrate, a short-chain fatty acid with histone deacetylase (HDAC) inhibitory activity, has been associated with increases in HbF and hemoglobin concentration in sickle cell anemia and β-thalassemia patients [89]. However, a pulsed or intermittent dosing regimen was necessary to avoid anti-proliferative effects on the bone marrow [90] and response to butyrate treatment was relatively low in β-thalassemia patients. Sodium-2,2-dimethylbutyrate, an orally administrable short-chain fatty acid derivative, showed HbF induction in β-thalassemia in early-phase clinical trials with no significant adverse events [91]. Other HDAC inhibitors such as trichostatin A and suberoylanilide hydroxamic acid induced γ-globin expression in K562 cells [92]. In addition, strong candidate inhibitors of HDAC1 and HDAC2, identified through high-throughput screening studies, were associated with substantial increases in γ-globin and HbF expression in vitro [93]. Furthermore, tranylcypromine (TCP), an LSD1 inhibitor, induced HbF production in cultured human hematopoietic erythroid progenitors and transgenic mice containing β-YAC [94]. Lysine-specific demethylase 1A (LSD1/KDM1A) is the histone demethylase component of co-repressor complexes [95] and it regulates hematopoiesis [96]. Recently, a more potent and specific LSD1 inhibitor, RN-1, induced HbF synthesis in sickle cell mouse model and baboons [97–99].

5.2. Gene therapy and genome editing

As several pharmacologic HbF-inducing agents have had disappointing results in clinical trials, genome modification approaches have been investigated [100]. Lentiviral vectors encoding the human γ-globin gene resulted in expression of exogenous γ-globin and high levels of HbF production in normal primary erythroid cells [101], suggesting that lentiviral-mediated treatments have the potential to provide therapeutic HbF levels to β-thalassemia patients. Zinc finger proteins can also be designed to interact with candidate target regulatory regions to reactivate γ-globin expression. The transfection of a lentiviral vector carrying a zinc finger protein that could interact with the -117 region of HBG1 promoter increased HbF levels in human erythroid cells derived from normal controls and β-thalassemia patients [102]. These studies on promising molecular regulators of HbF pave the way to develop therapies to modulate the expression of targeted genes, which may lead to an increase of HbF level, thereby reducing the severity of β-thalassemia disease. The insertion of a lentiviral vector carrying a fusion
zinc finger protein recognizing the γ-globin promoter and LDB1 into human primary erythroid cells can induce loop formation of the LCR to γ-globin and activate γ-globin gene expression [103]. Alternatively, specific knockdown of γ-globin repressors or their regulators without affecting non-erythroid functions is among the most promising genome editing strategies. The possibility of editing the targeted genome in hematopoietic stem cells by a specific engineered DNA-binding domain fused to a nuclease via a suitable zinc-finger nuclease (ZFN), transcription activator-like effector nucleases, or by clustered regularly interspaced short palindromic repeats-associated protein-9 nuclease is being actively investigated.

6. Expert commentary
HbF is among the major modulators of the clinical features of β-thalassemia patients. An ameliorating effect mediated by its forbidden from the imbalance α-to non-α-globin chain synthesis. One of the goals of β-thalassemia patient management has been to develop the efficient therapeutic agents or approaches for the induction of HbF. The ideal agents are capable of inducing therapeutic levels of HbF, a high response rate and they demonstrate long-term safety. Globin genes are genetically regulated; the recent discovery of HbF regulators has triggered new therapeutic approaches that reactivate HbF production beyond those achievable with the current HbF-inducing agents. In addition, various promising HbF-inducing agents were found to induce HbF production by decreasing levels of those key γ-globin transcriptional repressors. Although many of these active agents have been tested in the erythroblasts derived from healthy normal subjects and patients with sickle cell anemia, only few studies have been carried out in either β-thalassemic erythroid cells or mouse model of β-thalassemia disease. It is well known that except for the similarity in defective globin chain production, sickle cell anemia and β-thalassemia are totally different. Thus, any approach with great promise in sickle cell anemia may be effective or have limited success in some cases of β-thalassemia. The good example in this issue is the experience from hydroxyurea treatment that benefit in increasing HbF levels and improving clinical outcomes in sickle cell anemia, but hydroxyurea is effective only in fewer patient with β-thalassemia. The progress in HbF induction therapy in β-thalassemia, therefore, requires sufficient evidence from the in vitro experiments and in vivo studies performed in β-thalassemia. Additional concerning issue is the heterogeneity of the β-thalassemia clinical manifestations and HbF production including response to HbF-inducing agents. Although the mechanisms underlying the disease process have been studied extensively, our understanding of the risk factors that govern the clinical heterogeneity remains limited, and only a few genetic HbF modifiers have been elucidated. Furthermore, a few studies have been conducted to investigate the variation in response to HbF-inducing agents. The interaction of various transcription factors regulating globin gene expression leads us to a better understanding of the interaction and regulation of the globin gene. Recently, several studies in adult human normal erythroblasts showed that knockdown of γ-globin repressors or interfere its molecular pathway able to increase HbF to levels predicted to be therapeutic in β-thalassemia patients. However, besides regulating globin expression, those factors also play an important role in normal erythropoiesis or have distinct functions in non-erythroid cells; hence, interrupting their functions could result in numerous adverse effects. A full understanding of these regulators is highly recommended to maximize their benefits and avoid the off-target effects. With our improved understanding of the HbF regulation along with the innovative technologies, a molecular therapy targeted to the specific molecules or mechanisms is becoming a realistic and promising approach to develop a better treatment for β-thalassemia patients.

7. Five-year view
Current knowledge has shown that multiple genes and regions in the genome are involved in the regulation of γ-globin gene expression and HbF levels in both normal and β-hemoglobinopathies. Based on the advanced technology development, it is expected that new regulators of globin gene expression or erythropoiesis will be identified. The recent studies of HbF regulators, including BCL11A, KLF1, MYB, and LSD1, have stimulated a search for new HbF inducers that might act by modulating the expression of these genes or their related signaling pathways to reactivate HbF in the β-hemoglobinopathies. This expanded understanding of HbF molecular regulators will also help to enable us to develop gene base therapy or identify the mechanism of current promising HbF-inducing agents. It is also expected to get a better understanding of ‘responders’ and ‘nonresponders’ to these agents as well. This will give us better information for the delicate design of a combination therapy to enhance HbF production in the near future. The molecular targets for HbF induction will be tried using the newly developed gene therapy and genome editing technologies. However, a better cellular and animal model for HbF inducers is yet to be developed. Moreover, good preclinical data with a large cohort study are needed before therapeutics are used. It is expected that during this era of post-genomic medicine we will have some alternative treatment for β-thalassemia by the manipulation of γ-globin gene expression and HbF production.

Key issues
- The major mechanism underlying the pathophysiology of the β-thalassemias results from the absence or inadequate β-globin chain production, and can be related to the deleterious effects of imbalanced globin chain synthesis on erythroid maturation and survival.
- Clinical feature of β-thalassemia ranging from mildly affected as non-transfusion dependent thalassemia (NTDT) to severely affected as Cooley’s anemia.
- HbF level in β-thalassemia is very heterogeneous and associated with the disease severity.
- Induction of HbF synthesis can ameliorate the clinical severity of β-thalassemia by reducing the degree of imbalance α-to non-α-globin chains.
The level of HbF in β-thalassemias is genetically regulated, as the level of technology advances it is suspected that additional modifiers will be identified.

The hemoglobin switching is regulated by several key transcription factors that are considered the promising molecular target for inducing HbF expression in β-thalassemia.

The efficacy of HbF-inducing agents treatment in patients with β-thalassemia is still unclear.

More research in β-thalassemia is needed to understand the regulatory mechanisms underlying the variation in clinical outcomes, HbF production and response to HbF induction therapy to improve the management of patients.

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Declaration of interest

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Papers of special note have been highlighted as either of interest (∗) or of considerable interest (++) to readers.


**Association of the three major Hbf-QTLs with the disease severity and level of Hbf in patients with β-thalassemia/HbE.**


**Association of the three major Hbf-QTLs with the level of Hbf in patients with β-thalassemia.**


**Characterization of the erythroid specific enhancer of BCL11A, suggesting a novel Hbf-inducing target for genome editing.**


Summarizing the genetic manipulation approaches for β-thalassemia therapy

