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REVIEW ARTICLE

Hydroxyurea treatment in β-thalassemia patients: to respond or not to respond?

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Abstract Hydroxyurea (HU) is a drug that induces fetal hemoglobin production. As a result, HU is widely used to treat β-thalassemia (β-thal) patients. However, the response of these patients to HU varies. Some β-thal patients respond favorably to treatment while others do not respond at all. HU has a number of side-effects and therefore its targeted prescription is beneficial. Hence, identifying the genetic determinants which lead to the differential HU response is important. This review summarizes recent findings which have shed light on this topic. Special emphasis is given to the mechanisms and genetic loci which may govern these differences. These findings have helped identify several single nucleotide polymorphisms which associate with the response to HU in both β-thal and sickle cell disease patients.

Keywords β-thalassemia · Hydroxyurea · Pharmacogenomics · HbF

Abbreviations

HU	Hydroxyurea
β-thal	β-thalassemia
SNP	Single nucleotide polymorphism
SCD	Sickle cell disease
Hb	Hemoglobin
HbF	Fetal hemoglobin
HbA	Adult hemoglobin
β-TI	β-thalassemia intermedia
β-TM	β-thalassemia major
HPFH	Hereditary persistence of fetal hemoglobin
GR	Good responder
MR	Minor responder
NR	Nonresponder
GWAS	Genome-wide association study
QTL	Quantitative trait loci

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Molecular basis of β-thalassemia

The hemoglobin (Hb) molecule is composed of two α-globin chains and two β-like globin chains [1]. Expression of the human α-globin gene, located on chromosome 16, begins shortly after life and persists throughout adulthood [2–4]. In contrast, the β-like globin genes are expressed in a spatially and temporally restricted manner (Fig. 1). The human β-like globin genes are composed of five structural genes (*ε*, *Gγ*, *Aγ*, *δ*, and *β*) located on the β-locus of chromosome 11 [2–4]. The ε-globin gene is expressed during the first month postgestation in the yolk sac. Afterwards, ε-globin is silenced and expression of the fetal γ-globin genes (Gγ and Aγ) commences in the fetal liver and spleen to produce the fetal hemoglobin or HbF ($\alpha_2\gamma_2$). A second switch occurs shortly after birth. At this time, the γ-globin genes are silenced and expression of the adult δ- and β-globin genes begins in the bone marrow. At this stage, the predominant hemoglobin is the adult hemoglobin or HbA ($\alpha_2\beta_2$) [2–4].

β-thalassemia (β-thal) is an autosomal recessive disorder that is caused by mutations in the β-globin gene [5, 6]. Persons heterozygous for β-globin mutations are merely carriers (termed β-thal trait). However, patients homozygous for β-globin mutations develop anemia and iron

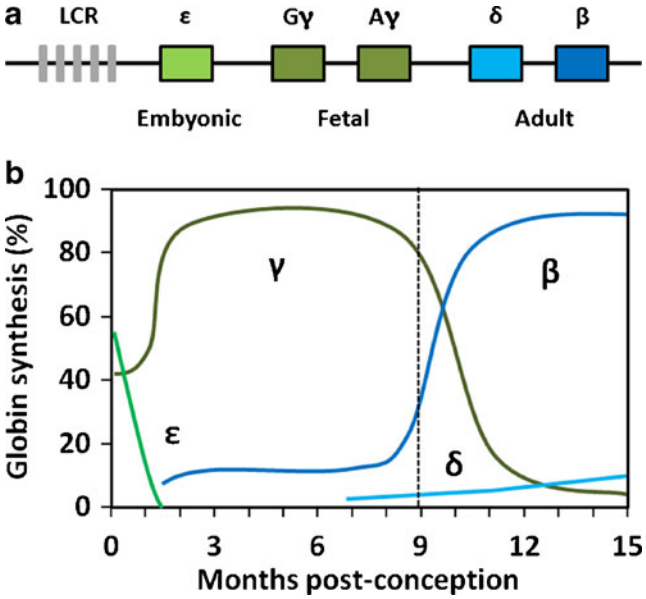

Fig. 1 The β-like globin genes are expressed in a developmentally restricted manner. Schematic of the human β-globin-like genes (*ε*, *Gγ*, *Aγ*, *δ*, and *β*) located on chromosome 11 is depicted (*LCR* locus control region). Also shown is the temporal expression of these genes. The graph is adapted from [4]

overload, followed by complications such as bone deformities, splenomegaly, and growth retardation [5].

To date, over 200 mutations in the β-globin gene have been identified [7, 8]. These mutations may reside in the β-globin promoter, untranslated region (UTRs), exons, or splice sites. As a result of these mutations, β-globin transcription, translation, or RNA processing becomes impaired. Subsequently, β-globin expression becomes abolished (β°), severly reduced (β^{+}), or slightly diminished (β^{++}). Deletions in the β-globin gene, on the other hand, are relatively infrequent [9]. These deletions range from 290 bp to >80 kb. The larger deletions may result in removal of the δ- and β-globin genes. A complete list of the β-globin mutations and deletions can be found in the HbVar database (http://globin.cse.psu.edu/) [10].

In β-thal patients, reduction in β-globin expression causes an imbalance in the α- to β-globin chain ratios in the red blood cells (RBCs) [5, 9]. This imbalance, in essence, leads to the pathophysiology of the disease. The excess α-globin chains precipitate and form inclusion bodies, damaging and destroying the RBCs through apoptosis. Depending on the extent of the α/β chain imbalance, patients may develop a mild or severe anemia leading to conditions referred to as β-thal intermedia (β-TI) and β-thal major (β-TM), respectively.

Reactivation of HbF as a therapeutic strategy

Reactivation of the fetal γ-globin genes in adults may serve as a therapeutic strategy for the treatment of β-thal patients [11]. An early indication for this premise came from a condition known as the hereditary persistence of fetal hemoglobin (HPFH). Persons with HPFH have high HbF levels (10–30 %) even as adults [2, 3, 7]. In one variety, HPFH is caused by deletions in the β-locus (termed deletion-type HPFH). These deletions, which range from 7 kb (e.g., Corfu deletion) to >80 kb (e.g., Black deletions), result in the reactivation of γ-globin expression.

Two mechanisms may account for the upregulation of γ-globin expression by HPFH deletions. Certain HPFH deletions may result in juxtaposition of a distant enhancer next to the γ-globin genes. Experiments using transgenic mice carrying the human β-locus with HPFH-like deletions provide evidence for this model [12–14]. Alternatively, these deletions may lead to the removal of a γ-globin silencer element. Recently, it has been determined that in persons with HPFH, the common truncated region contains binding sites for the γ-globin repressor, BCL11A [15, 16].

Certain HPFH deletions (e.g., HPFH-1 and HPFH-2) begin just 3′ of the *Aγ* gene and lead to the removal of the δ- and β-globin genes. In spite of this, individuals bearing these deletions are clinically normal [3, 7]. Persons homozygous for these truncations have high Hb levels (15–18 g/dL) and their Hb is entirely comprised of HbF. Moreover, patients who are compound heterozygous for β-globin mutations and these HPFH deletions show clinically mild symptoms [7]. These observations imply that reactivation of γ-globin expression to levels matching the HPFH deletions may serve as a therapeutic strategy for treating β-thal patients.

In part as a result of these observations, a number of chemicals have been identified to reactivate HbF expression in patients with Hb disorders [17–20]. Prominent examples include sodium butyrate (a histone deacetylase inhibitor), 5-azacytidine (a methyl-transferase inhibitor), and hydroxyurea (HU; a DNA replication inhibitor). However, HU is currently the only drug that is prescribed for the treatment of anemia in β-thal patients.

Response of β-thal patients to HU

HU (or hydroxycarbamide) is a chemotherapeutic agent, which was initially used to treat patients with myeloproliferative disorders [17, 18, 21]. In the 1980s, it was discovered that HU can induce HbF expression in sickle cell disease (SCD) patients. The observation followed experiments aimed at understanding the mechanism of HbF induction by the nucleoside analogue, 5-azacytidine (5-Aza) [21]. At the time, it was debated as to whether 5-Aza was inducing HbF production in SCD patients through DNA hypomethylation or via inhibition of DNA synthesis. HU was a well-known ribonucleotide reductase inhibitor (an

enzyme that is required for the generation of deoxyribonucleotides and DNA replication) with no known DNA methyltransferase inhibitor activity [22] (Fig. 2). Thus, to provide insight, the effect of HU on HbF production was tested in monkeys [21]. Results of these experiments showed that HU can induce HbF expression in baboons [21]. More importantly, ensuing clinical trials demonstrated that HU treatment can induce HbF expression in SCD patients [23–26]. HU also reduced the incidence of painful crises and alleviated the acute chest syndrome in these patients [23–27]. As a result, HU has been approved by the US Food and Drug Administration (FDA) for the treatment of SCD patients.

At present, HU is also widely used to treat β-thal patients [28–41]. In a subset of these patients, HU treatment leads to the improvement of hematological parameters (HbF%, Hb, mean corpuscular volume, and mean corpuscular hemoglobin). These responder patients can generally be divided into two groups: good responders (GR) and minor responders (MR) [32, 33, 36, 41]. In transfusion-dependent patients, the donor blood is mixed with that of the recipient and thus perturbs the Hb values. Therefore in these patients, HU response is measured through increased blood transfusion intervals (note: transfusion is generally initiated when Hb <8 g/dL). As such, the transfusion-dependent GR patients (either β-TM or β-TI) show a significant increase in their blood transfusion intervals post-HU treatment (>6 months) [32, 36, 41]. The transfusion-independent GR patients (β-TI), on the other hand, show a significant increase in their Hb levels (≥2 g/dL) posttreatment [33].

However, not all β-thal patients respond favorably to the drug. While some transfusion-dependent MR patients shift from regular to sporadic blood transfusions posttreatment, others show a modest 2–3 fold increase in their blood transfusion intervals [33, 36, 41]. Furthermore, some transfusion-independent MR patients merely exhibit a 1 g/dL increase in their Hb levels posttreatment [33]. More importantly, approximately 20–30 % of β-thal patients do not respond to HU treatment at all (termed nonresponders (NR)) [28–41].

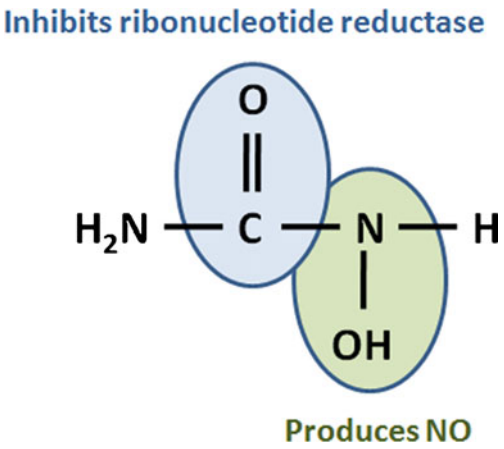

Fig. 2 The chemical structure of HU is shown. The regions of HU involved in ribonucleotide reductase inhibition (*blue-shaded area*) and nitric oxide generation (*green-shaded area*) are highlighted. The figure is adapted from [26]

HU has a number of benefits, which has resulted in its widespread use. In particular, HU can be taken orally, is inexpensive, and is considered to be safe [26, 42, 43]. In the long-term, however, HU may not be as safe as is generally perceived. Several reports suggest that HU treatment in SCD patients may lead to leukemia, impaired spermatogenesis, and leg ulcers [44, 45]. HU may also produce somatic mutations in children with SCD [46]. In addition, HU treatment may cause adverse side-effects in β-thal patients. These side-effects include headaches, hyperpigmentation, nausea, and dizziness [40].

Because of the potential adverse effects and lack of response in a subset of patients, targeted prescription of HU is preferable. Targeted prescription, however, requires an understanding of the reasons behind the differential response of patients to the drug.

Explanations for the differential HU response

Two mechanisms may account for differences in the response of β-thal patients to HU. In one possibility, the erythroid cells of responders and NR may react differently to HU treatment. The erythroid cells of NR may, for instance, upregulate HbF less vigorously due to a deficiency in the γ-globin induction pathways. Or else, the cells of NR may be more susceptible to the cytotoxic effects of HU. Here, this has been termed the differential susceptibility model. Alternatively, HU treatment may augment HbF production in both the responder and NR patients. The HU response, however, may only become manifested in patients who have higher cellular HbF levels. Here, this has been labeled the differential baseline HbF model.

Several lines of evidence support the differential baseline HbF model. Recent findings suggest that HU treatment can induce HbF production from the erythroid progenitor cells of both the responder and NR β-thal patients (15 GRs and 12 NRs). The baseline HbF levels, however, seem to be significantly higher (i.e., 20-fold) in erythroid progenitors of the responders [47].

In addition, several cohort studies suggest that the pretreatment peripheral blood HbF levels (Hb and HbF%) are higher in the responder patients [33, 39, 41]. In one study involving 79 Indian β-thal patients (41 β-TM and 38 β-TI), for example, the baseline HbF levels in the β-TI patients were as follows: GR (Hb, 7.6 g/dL; HbF, 51.8 %)>NR (Hb, 6.3 g/dL; HbF, 25.9 %) [39]. In another cohort of 37 Indian β-TI patients, the pretreatment HbF levels were as follows: GR (Hb, 6.5 g/dL; HbF, 67.0 %)>NR (Hb, 6.5 g/dL; HbF, 40.9 %) [33]. Of note, transfusion-dependent patients have also been included in these cohorts. Unfortunately, Hb levels

in transfusion-dependent patients are influenced by the donor blood and may not be completely reliable. Nevertheless, some of the reported differences between the responder and NR β-thal patients are too high to overlook [39].

On the other hand, there is also evidence to support the differential susceptibility model. Several cohort studies suggest that HbF and HbF producing cell (termed F cells) levels are increased posttreatment only in the responder patients. In one study, for instance, Hb and HbF% levels were significantly increased only in the GR patients (Hb, ↑1.5 g/dL; HbF, ↑35 %) [39]. Similar results were obtained in another study (Hb, ↑2.6 g/dL; HbF, ↑9 %) [33]. Furthermore, a significant increase (>30 %) in F cell levels of GR patients has also been reported after HU treatment [39].

In further support of this model, expression microarray experiments show that the erythroid progenitors of responders, in contrast to that of NR, have an activated stress response program (see section below). High-expression levels of several such genes (specifically *ARG1*, *ARG2*, and $BCLX_L$) may protect the erythroid cells of responders from cell stress and apoptosis, thus allowing them to expand in the presence of HU [47]. As a result, the erythroid cells of responders may become immune to the cytotoxic effects of HU.

In summary, HU response in β-thal patients may be determined by differences in (1) pretreatment HbF levels of the erythroid cells and (2) the response of erythroid cells to HU treatment.

The mechanism of HbF induction by HU

As noted above, the erythroid cells of responders and NR may react differently to HU treatment (differential susceptibility model). Here, a summary of the mechanisms by which HU may induce γ-globin expression is provided. A better understanding of these mechanisms may offer further insight into the differential response of β-thal patients to HU.

Two mechanisms have been proposed to explain HbF induction following HU treatment [48]. In one, HU may promote stress erythropoiesis to increase the number of F cells. Alternatively, HU may activate signaling pathways which lead to γ-globin upregulation. These two courses of action are not mutually exclusive [48]. In particular, HU may upregulate the expression of both γ-globin and genes which promote stress erythropoiesis. In concert, these events could lead to the production of high HbF levels posttreatment.

Stress erythropoiesis and HbF induction

It has been proposed that inhibition of DNA synthesis by HU may lead to stress erythropoiesis, similar to what occurs during conditions of low oxygen [49]. In particular, it is hypothesized that HU may block DNA synthesis in the rapidly dividing erythroid precursors which produce HbA. This may, in turn, lead to a selective advantage for expansion of the less mature HbF producing F cells [50, 51]. As a result, this so-called "stress erythropoiesis" would lead to the induction of HbF production. In support of this notion, a reduction in burst forming unit-erythroid cell levels and an increase in F cell levels have been reported following HU treatment in SCD and β-thal patients [39, 52, 53].

Further support for this model comes from several expression profiling studies. In one study, using reticulocytes from children with SCD, HU treatment downregulated the expression levels of genes involved in translation, ribosome assembly, and chromosome organization [54]. These results suggest that HU may alter the kinetics of erythropoiesis by inhibiting protein (rather than DNA) synthesis. Another report has shown that GATA-1 levels in the erythroid progenitor cells of healthy donors decrease following HU treatment [55]. Reduced GATA-1 expression can delay the maturation of erythroblasts [56]. This delay may, in turn, alter the kinetics of erythropoiesis to favor HbF production. In addition, HU may induce the expression of a number of apoptosis-related genes (e.g., *DR5, caspase-3*, and *BCL6*) in the erythroid precursor cells of both healthy adults and β-thal patients [47, 56]. As noted above, selective activation of several such genes (e.g., $BCLX_L$ and *BCL6*) may protect the erythroid precursors of NR from apoptosis [47].

The signaling model

Two cell types have been widely utilized to investigate the HU/γ-globin induction pathways. One model system is the K562 erythroleukemia cell line [57]. K562 cells express the γ-globin gene and more importantly, γ-globin expression in these cells is induced following HU treatment [58, 59]. The more biologically relevant cells are the erythroid progenitors of healthy persons and patients with Hb disorders, which also upregulate γ-globin expression in response to HU treatment [60, 61]. Using these cell types, a number of the HU/γ-globin induction pathways have been deciphered (Fig. 3).

In one pathway, induction of γ-globin may occur through the generation of nitric oxide (NO). Once taken orally, HU can rapidly spread from the intestine to blood cells through facilitated uptake by solute carrier transporters [62]. HU can then react with heme to produce NO [63]. The generated NO can then nitrosylate (and activate) the soluble guanylate cyclases to produce cGMP [64, 65]. Subsequently, the generated cGMPs can lead to the induction of γ-globin expression [66]. The downstream activator(s) of this pathway have not been identified. However, possibilities include the AP-1 (c-fos/jun) and the Sp1 transcription factors [67].

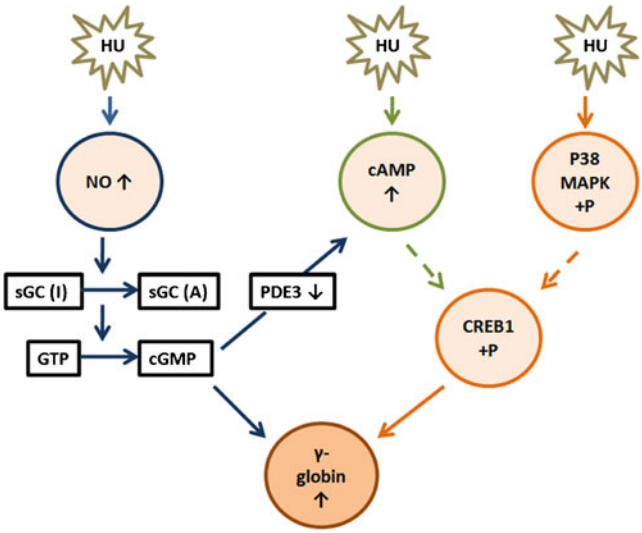

Fig. 3 Summary of the signaling pathways leading to γ-globin induction in erythroid progenitors and K562 cells is depicted. HU can induce γ-globin expression by increasing nitric oxide (*NO*) and cAMP levels or through phosphorylation of p38 MAPK and CREB1. *sGC* soluble guanylate cyclase, *pDE3* phosphodiesterase 3

In addition, cGMP can downregulate phosphodiesterase 3 expression to activate the cAMP pathway [68]. Activation of the cAMP pathway results in upregulation of γ-globin expression in erythroid progenitor cells [69]. However, the opposite effect is seen in K562 cells, a phenomenon that has been linked to induction of the already high MYB levels in these cells (MYB may act as a γ-globin repressor) [70].

HU can also upregulate γ-globin expression through the p38 MAPK/CREB1 pathway. Firstly, both p38 MAPK and CREB1 seem to be important in maintaining steady-state γ-globin expression levels [71]. Furthermore, HU treatment (like sodium butyrate) can lead to the phosphorylation of p38 MAPK in K562 cells [72–74]. We have further established that in K562 cells, CREB1 is phosphorylated following HU treatment, and its knockdown by RNA interference blocks γ-globin induction [75, 76]. Collectively, these results underscore the importance of this signaling pathway in γ-globin induction.

In addition, specific miRNAs may be involved in the upregulation of γ-globin expression by HU. In particular, the expression levels of two miRNAs (miR-26b and miR-151-3p) seem to be increased in the reticulocytes of SCD patients following HU-treatment [77]. Furthermore, this upregulation has been associated with increased HbF levels. Whether these miRNAs play a direct role in γ-globin upregulation, however, has yet to be determined.

QTLs that affect baseline HbF levels

As discussed above, genomic loci which affect baseline HbF levels may also influence the response of β-thal patients to HU (differential baseline HbF model). Genome-wide association studies (GWAS) have identified three quantitative trait loci (QTLs) that affect baseline HbF levels in healthy persons and in patients with Hb disorders (Table 1). Several single nucleotide polymorphisms (SNPs) in these loci may account for 20–50 % of the HbF variance [78, 79]. Notably, minor alleles of these SNPs also associate with a milder anemia in β-thal patients.

The *Xmn*I polymorphism

A well-known HbF QTL is the *Xmn*I polymorphism (rs7482144), a C → T SNP at position -158 of the Gγ promoter [80]. Early reports and recent GWA studies show that presence of the *Xmn*I T allele correlates with higher HbF levels in β-thal and SCD patients [81–83]. In addition, a large twin study suggests that this SNP can influence F cell levels in healthy adults [84].

The *Xmn*I polymorphism has also been correlated with reduced disease severity in β-thal patients. In particular, a report shows that frequency of the *Xmn*I T allele is higher in the French β-TI patients compared with patients having β-TM [85, 86]. In support, we have observed a significant correlation between the *Xmn*I T/T genotype and β-TI in a cohort of >300 Iranian patients [87]. Despite these association data, no function for the *Xmn*I SNP has been established. Therefore, it has been postulated that linked elements in the β-locus rather than the *Xmn*I polymorphism itself may affect γ-globin expression [88].

BCL11A

Another HbF QTL lies in the *BCL11A* gene. BCL11A is a developmental repressor of the γ-globin gene [89, 90]. In particular, knockdown of BCL11A in human erythroid progenitor cells can result in a significant increase in γ-globin expression [89]. In addition, BCL11A knockout induces γ-globin expression in human β-locus transgenic mice [91, 92].

Several GWAS and replication studies have associated SNPs in intron 2 of the *BCL11A* gene (e.g., rs11886868, rs4671393, and rs766432) with HbF levels in healthy persons and in patients with hemoglobinopathies [83, 93–96]. Furthermore, a GWAS has correlated one of these SNPs (rs766432) with F cell levels in SCD patients [97]. The minor alleles of these SNPs have also been correlated with a milder disease phenotype (i.e., β-TI) in French, Italian, and Iranian patients [85–87, 93, 98]. Preliminary data by Orkin and colleagues suggest that this *BCL11A* intronic region may contain an erythroid-specific enhancer [99].

HBS1L-MYB

A third HbF QTL has been located between the *HBS1L* and *MYB* genes. Several SNPs in this intergenic region (e.g.,

Table 1 Summary of the HbF QTLs which influence HbF and F cell levels is shown

Locus	Chromosome	Key SNPs	Populations	HbF/F cell	Associated with β-TI	References
β-globin	11	rs7482144	Healthy adults, SCD, and β-thal	HbF and F cells	Yes	[82–87]
BCL11A	2	rs766432, rs11886868, and rs4671393	Healthy adults, SCD, and β-thal	HbF and F cells	Yes	[83, 85, 93–98]
HBS1L-MYB	6	rs9399137 and rs4895441	Healthy adults, SCD, and β-thal	HbF and F cells	Yes	[83, 85, 93–98]

Also indicated is the association of these SNPs with β-TI

rs9399137 and rs4895441) have been associated with HbF and F cell levels in healthy persons and in patients with hemoglobinopathies [83, 93, 94, 100, 101]. The MYB oncogene can act as a γ-globin repressor in erythroid cells [102, 103]. Thus, the *HBS1L-MYB* intergenic region is likely involved in regulation of *MYB* expression. In support of this notion, this region contains erythroid-specific DNase I hypersensitive sites and histone acetylation patterns [104]. Furthermore, a number of sites within this region have enhancer activity and form long-range interactions with the *MYB* promoter during mouse erythroid cell development [104, 105].

Markers that predict the HU response

Predictive markers in SCD patients

Several SNPs have been identified which associate with the HU response in SCD patients. In a cohort of 386 adult SCD patients, a number of SNPs in the promoter and 5′UTR of the *SAR* gene associated with the HU response [106]. *SAR* is a gene that is involved in protein trafficking [59]. SAR expression is induced by HU and its over-expression leads to γ-globin upregulation in $CD34^+$ cells [106]. It has therefore been suggested that SAR may increase the transport of γ-globin transcription factor precursors from the ER to the Golgi [59].

Furthermore in a cohort of 137 SCD patients, an association study of 29 candidate genes has shown a correlation between the HU response and SNPs in several stress response genes (e.g., *FLT1*, *NOS1*, *TOX*, *ARG1*, and *ARG2*) [107]. Interestingly, a recent study shows that ARG1 and ARG2 are upregulated in the erythroid cells of β-thal Responder patients after HU treatment [46]. However, the associations of these SNPs with the response to HU have not been investigated in β-thal patients.

In a study involving 93 SCD children, minor alleles of two *BCL11A* SNPs (rs4671393 and rs1427407) associated with reduced pretreatment BCL11A levels and increased baseline HbF% levels in the patients' reticulocytes [54].

In another prospective association study of 174 SCD children under HU treatment, the *Xmn*I polymorphism and several SNPs in the *BCL11A* gene correlated with baseline HbF% levels [108]. In addition, SNPs in the *ARG1* and *ARG2* genes associated with increased HbF% levels post-treatment. However none of the 70 selected SNPs, which were in the ribonucleotide reductase, HU transporter, and HbF modifier genes, correlated with HbF% levels at the maximum tolerated dose of HU [108].

Predictive markers in β-thal patients

An association has been established between the *Xmn*I polymorphism with the response to HU in β-thal patients (Table 2). Several studies have shown an association between the *Xmn*I T/T genotype with a robust HU response and the *Xmn*I C/C genotype with a lack of response [31, 32, 36, 39, 41]. However, other studies have failed to detect such a correlation [33]. These studies have used different inclusion criteria (e.g., number of β-TM and β-TI patients) and have not utilized a uniform definition of HU response (Table 2). Some of these inclusion criteria (e.g., β-thal type and co-inheritance of α-thal) may affect the response to HU [31, 38, 39, 109]. Therefore, a direct comparison of these results is not possible.

In addition, we have shown an association between minor alleles of two linked *BCL11A* SNPs (rs766432 and rs4671393) with the response to HU in transfusion-dependent β-thal patients (Table 2) [41]. By using both the *Xmn*I T/T and the *BCL11A* rs766432 markers, we were able to predict the HU response in >85 % of the β-thal patients.

Interestingly, a recent report shows that HU treatment alone does not trans-activate reporter gene expression from mouse erythroid leukemia (MEL) cells stably transfected with a dual-reporter modified human β-globin locus construct (MEL^{A}γRedβEGFP). However, HU treatment preceded by BCL11A knockdown led to a synergistic upregulation of reporter gene (^{A}γRed) expression [110]. These results provide insight into how BCL11A expression may influence the HU response in β-thal patients [54].

Future directions

Insights into the mechanisms of HU-mediated γ-globin induction have led to the identification of several SNPs which

Table 2 Summary of association studies which have correlated candidate SNPs with the HU response in β-thal patients

Cohort location	Cohort size	Thal type	Definition of HU response	Number of responders					Predictive marker(s)	References
				β-TM	β-TI	GR	MR	NR		
Iran	45	β-TM=36 and β-TI=9	β-TM patients=becoming transfusion independent after treatment; β-TI patients =an increase in Hb levels by >1 g/dL	25/36 (70 %)	9/9 (100 %)				*Xmn*I T/T associated with a response	[31]
Iran	133	β-TM	Good response (GR)=regular transfusions→transfusion independent; minor response (MR)=regular transfusions→>6 months transfusion intervals; and no response (NR)=no change in transfusion requirements			81/133 (61 %)	31/133 (23 %)	21/133 (16 %)	*Xmn*I T/T associated with a good response	[32]
India	37	β-TI	GR=patients becoming transfusion independent or Hb increases by >2 g/dL; MR≥50 % decrease in transfusion requirements or Hb increases by 1–2 g/dL; and NR=no change in transfusion requirements			17/26 (65 %)	9/26 (35 %)	11/37 (30 %)	*Xmn*I showed no correlation with response	[33]
Algeria	54	β-TM=45 and β-TI=9	GR=decrease in annual transfusion requirements by >70 %; MR=decrease by 40–70 %; and NR=no change in transfusion requirements			28/54 (52 %)	9/54 (17 %)	17/54 (31 %)	*Xmn*I C/C associated with a worse response	[36]
Israel	18	TM=11 and β-TI=7	For transfusion-dependent patients, response=patients becoming transfusion independent and for transfusion-independent patients, response= Hb increases by ≥2 g/dL	9/11 (82 %)	2/7 (29 %)				The *Xmn*I T allele associated with a response in the β-TM patients	[38]
India	79	β-TM=41 and β-TI=38	GR=patients becoming transfusion independent; MR=50 % reduction in transfusion requirements; and NR=no change in transfusion requirements			22/79 (28 %)	19/81 (23 %)	38/81 (47 %)	The *Xmn*I T/T genotype associated with GR in β-TI patients	[39]
Iran	81	β-TM=54 and β-TI=27	GR=transfusion dependent→transfusion independent; MR=≥2-fold increase in transfusion intervals; and NR=no change in transfusion intervals			37/81 (46 %)	24/81 (30 %)	20/81 (25 %)	*Xmn*I T/T and *BCL11A* rs766432 C and rs4671393 A alleles associated with response	[41]

associate with the HU response in β-thal patients. In several retrospective association studies, the *Xmn*I and *BCL11A* SNPs have been correlated with the HU response in β-thal patients [31, 32, 36, 39, 41]. As a first step, it is necessary to verify the predictive ability of these markers in prospective association studies by using large cohorts from different populations.

In addition, associations of the *Xmn*I and *BCL11A* markers with the HU response have not been established in SCD patients [108]. Conversely, associations of SNPs in several stress response genes, which correlate with the HU response in SCD patients, have not been tested in β-thal patients [107]. Of special interest are the *ARG1* and *ARG2* genes, which also show differential expression levels posttreatment in β-thal responder and NR patients [47]. In order to form a unified scheme of the HU response, associations of these markers needs to be cross-checked between the two disease types.

Finally, it would be interesting to determine the entire set of SNPs which correlate with the HU response in both β-thal and SCD patients. Such GWA studies would require a large number patients, which necessitates a collaborative multicenter approach to the problem [83, 93, 94]. In all likelihood, several such SNPs should fall in loci known to affect γ-globin expression. However in the process, new loci modulating the HU response may also be discovered.

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