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REVIEW ARTICLE

Hydroxyurea in the management of sickle cell disease: pharmacogenomics and enzymatic metabolism

Sètondji Cocou Modeste Alexandre Yahouédéhou[1,2] · Elisângela Vitória Adorno[2] · Caroline Conceição da Guarda[1] · Uche Samuel Ndidi[1] · Suellen Pinheiro Carvalho[1,2] · Rayra Pereira Santiago[1] · Milena Magalhães Aleluia[1] · Rodrigo Mota de Oliveira[1,2] · Marilda de Souza Gonçalves[1,2]

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Abstract

Hydroxyurea (HU) was approved to be used in the treatment of sickle cell disease (SCD) because of its anti-sickling potential. However, there is variability in HU response among SCD patients and this can be due to physiological, socioeconomic, environmental, metabolic and/or genetic factors. The present review focuses on the latter two. Three quantitative trait loci, *HBG2*, *BCL11A* and *HMIP*, have been suggested as important markers for HU response. Other genes (*ASS1, KLF10, HAO2, MAP3K5, PDE7B, TOX, NOS1, NOS2A*, *FLT1, ARG1, ARG2, UGT1A1, OR51B5/6*, *SIN3A*, *SALL2*, *SAR1A*, *UTB*, *OCTN1*, *CYP2C9*, *AQP9*, *MPO*, *CYP2E1*, and *GSTT1*) have also been considered. Studies implicate catalase, urease, horseradish peroxidase and enzymes of CYP450 family in HU metabolism. However, little is known about these enzymes. Therefore, further studies are needed to elucidate the metabolic pathway of HU, which will facilitate pharmacogenomic studies and help in identification of candidate genes for predicting HU response.

Introduction

Hydroxyurea (HU), or hydroxycarbamide, is a hydroxylated analogue of urea (Fig. 1; CAS Registry Number, 127-07-1) [1, 2], first synthesized in 1869 by Dresler and Stein and later tested in an experimental model in 1928 by Rosenthal, who suggested its myelosuppressive potential [3, 4]. HU has been used to treat myeloproliferative syndromes, particularly chronic myeloid leukemia, polycythemia vera and psoriasis [5, 6], as well as AIDS, since it inhibits DNA synthesis in human immunodeficiency virus type I (HIV-I) by reducing intracellular dNTP levels in activated lymphocytes [7, 8].

HU, due to its anti-sickling potential, was approved in 1999 by the U.S. Food and Drug Administration for the treatment of sickle cell disease (SCD) in patients with severe clinical profiles [9–11]. The benefits of HU in SCD patients have been attributed to increasing fetal hemoglobin (HbF) levels, which inhibits the polymerization of the variant hemoglobin S, leading to a reduction in the incidence of painful crises, as well as decreased rates of hospitalization, acute chest syndrome, blood transfusion and mortality among SCD patients [6, 9]. HU is also associated with increasing hemoglobin and mean cell volume of red cells; reducing white cell, platelet and reticulocyte counts; in addition to reducing expression of adhesion molecules and release of nitric oxide (NO) [12, 13]. However, increase in HbF levels and the clinical response induced by HU have been variable among different patients, necessitating elevated dosages and increasing toxicity [9, 14]. Differences in responses can be attributed to various factors, including physiological, socioeconomic and environmental factors. However, genetic factors have been considered as some of the most important determinants of variations in drug therapy response and tolerance [15]. Recently, studies in SCD patients showed that in addition to genomic variations within the β-globin gene (*HBB*), variants in modifier genes outside *HBB* are also significantly associated with increase in HbF levels, and, consequently, HU treatment response [15].

✉ Marilda de Souza Gonçalves
mari@bahia.fiocruz.br

[1] Laboratório de Investigação em Genética e Hematologia Translacional – LIGHT, Instituto Gonçalo Moniz - IGM/FIOCRUZ, Salvador, Brazil

[2] Laboratório de Pesquisa em Anemia – LPA, Departamento de Análises Clínicas, Faculdade de Farmácia, Universidade Federal da Bahia, Salvador, Brazil

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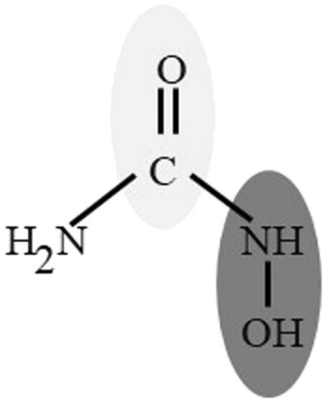

Fig. 1 Chemical structure of hydroxyurea. The areas of hydroxyurea involved in ribonucleotide reductase inhibition and cycling cell killing (grey-shaded area) as well as in nitric oxide production and thereby soluble guanylate cyclase stimulation (black-shaded area) are highlighted. Figure adapted from [17]

Some studies that investigated the enzymatic metabolism of HU, to better understand its mechanism of action, presented experimental evidence suggesting the participation of catalase, urease, horseradish peroxidase and enzymes of the CYP450 family [10, 16]. Pharmacogenomic studies performed later focused on quantitative trait loci (QTL) and modifier genes outside *HBB*, but paid little attention to the genes encoding enzymes that metabolize HU. The present review attempts to summarize findings concerning the genetic factors that influence HU response, such as QTL and the genes that influence HbF levels, in addition to placing a particular emphasis on genes encoding drug-metabolizing enzymes (DME) and transporters, as well as HU metabolism.

Three models attempting to clarify inter-individual variability in SCD patients on HU

In 2013, based on some evidence, Banan proposed two models in an attempt to explain the differential HU responses seen in SCD patients: (1) the differential susceptibility model, in which erythroid cells supposedly react differently to HU in responders and non-responders; (2) the differential baseline HbF model, in which HU increases HbF production in responders and in non-responders, yet only patients with high cellular levels of HbF respond to HU therapy [17]. In the present review, we propose a third model termed the differential HU metabolism model, in which SCD patients, regardless of their baseline HbF%, metabolize HU differently irrespective of whether they respond to HU treatment or not (Fig. 2). It is our belief that differences in HU metabolism may provide further insight into the variability observed in patient responses to HU, since it is known that single nucleotide polymorphisms (SNPs) in the genes encoding DME can lead to poor, intermediate, rapid or ultra-rapid HU metabolism, and, consequently, to differential pharmacokinetics and/or pharmacodynamics [18]. In addition, it was also reported that SNPs within genes encoding DME, transporters or targets could affect therapeutic response in SCD patients, thereby leading to efficacy or toxicity [19].

Biomarkers that affect baseline HbF levels and influence HU response

QTL

According to the differential baseline HbF model, genome-wide association studies (GWAS) have identified three important QTL associated with baseline HbF levels in patients with sickle cell anemia (SCA) and β-thalassemia, as well as in individuals with a normal hemoglobin pattern (HbAA): *HBG2*, *BCL11A* (developmental repressor of the γ-globin gene) and *HBS1L-MYB* [20–22]. The XmnIγ^G polymorphism (rs7482144) −158C > T of the γ^G-globin (*HBG2*) gene, located on chromosome 11p15, correlates with higher HbF levels in β-thalassemia and SCD patients, leading to decreased disease severity [17, 20, 23]. SNPs (e.g., rs11886868, rs46713993 and rs766432) in the intron 2 of the *BCL11A* gene located on chromosome 2p16 have been associated with high HbF levels in SCD patients and healthy individuals [17, 23]. SNPs (e.g., rs9399137 and rs4895441) in the *HMIP* region between genes *HBS1L* and *MYB*, located on chromosome 6q23, have also been associated with high HbF levels in SCD patients and healthy individuals [17, 23]. Wonkam and colleagues found associations between HbF levels and both *BCL11A* and *HMIP* SNPs in Cameroonian SCA patients [24].

Accordingly, it is likely that these QTL affect HbF levels in SCD patients on HU therapy [21, 23]. This hypothesis has been corroborated by several studies investigating associations between QTL and elevated levels of HbF in response to HU. In 2016, Friedrisch and colleagues analyzed Brazilian SCA patients and reported increased endogenous levels of HbF in association with both *BCL11A* and *HMIP*. Furthermore, *BCL11A* rs1427407, rs4671393 and rs11886868 were also associated with an increase of 2 to 4-fold in HbF level at maximum tolerated dose (MTD) [20]. Banan et al reported a strong correlation of TT genotype of *HBG2* and the C allele of *BCL11A* rs766432, with HU treatment response observed in Iranian β-thalassemia patients [25]. Moreover, the T allele of *HBG2* has also been associated with significant increase in hemoglobin (Hb) and HbF levels, in addition to HU therapy response in β-thalassemia intermedia patients, since the results showed that patients carrying the CT genotype presented, after HU therapy, an increase in Hb and HbF levels of 0.7 ± 1.26 mg/dl

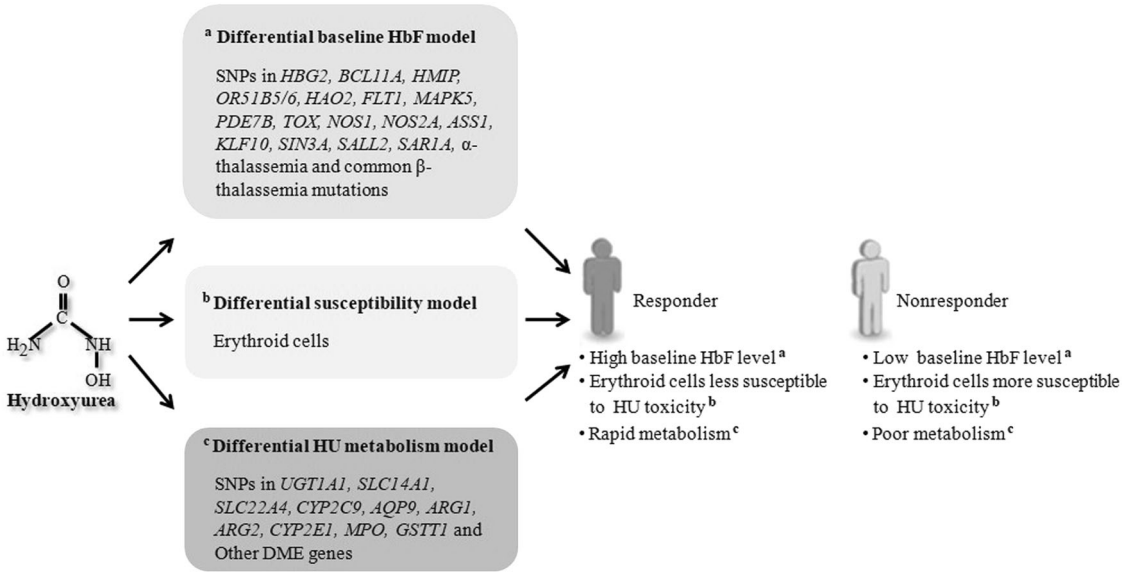

Fig. 2 Models attempting to elucidate differential hydroxyurea responses in SCD patients. Three models are presented, the differential susceptibility model, differential baseline HbF model and differential HU metabolism model. In the differential susceptibility model, it is supposed that erythroid cells can respond differently to HU in responders and non-responders. In the differential susceptibility model, the HbF production occurs only in patients with high cellular levels of HbF. In the differential HU metabolism model, we believe that, HU response will depend on capacity of each patient to metabolize HU. Hence, these three models can together determine the response of SCD patients on HU therapy. SCD patients can have a combination of models. *DME* drug metabolizing enzyme, *SNP* single nucleotide polymorphism

and 5.95 ± 14.8 mg/dl, respectively when compared with those carrying the CC genotype (0.26 ± 1.43 mg/dl and 0.8 ± 1.31 mg/dl, respectively) [26]. The TT variant genotype of *HBG2* has also been associated with higher HbF levels both pre and post-HU therapy in Kuwaiti Arab SCD and β-thalassemia patients on HU [21]. Recently, we found that SCA patients on HU therapy with the CC variant genotype of *BCL11A* rs766432 presented higher Hb concentration, red blood cell counts and hematocrit, in addition to lower direct bilirubin level and platelet count [23]. Furthermore, the multivariate linear regression analysis showed an independent and significant contribution of *HMIP* rs11759553 to modulate HbF levels.

However, other studies were unable to confirm this association, finding no significant correlations between the XmnIγ^G polymorphism and response to HU [27]. A study carried out in Iranian β-thalassemic patients also found no significant correlation between the XmnIγ^G polymorphism and HU response [28]. In a study conducted in SCD patients on HU, associations were found between the SNPs in the *BCL11A* gene (e.g., rs1427407, rs766432, rs4671393, rs7557939, and rs11886868) and baseline HbF% but none of them showed associations with MTD HbF levels [29]. A study involving Kuwaiti SCD and β-thalassemia patients on HU therapy reported that carriers of the C allele in *BCL11A* rs11886868, T allele in *HMIP* rs9376090, C allele in *HMIP* rs9399137 and A allele in *HMIP* rs9402686 had no significant differences in HbF levels either pre- or post-HU treatment when compared to carriers of respective wild-type alleles [21]. These findings seem to be consistent with the differential HU metabolism model, since no significant increases in HbF levels were observed in patients on HU therapy, regardless of whether patients were carriers of HbF QTL or not. Hence, it appears that in addition to HbF QTL, other genes are involved in the modulation of HbF production in SCD patients undergoing HU therapy.

Other genes associated with HbF production and HU response

It was reported that the olfactory receptors (*OR*) genes (*OR51B2*, *OR51B5* and *OR51B6*), located upstream of the *HBB* cluster locus control region may regulate the expression of the *HBG2* [30–32]. A GWAS performed by Green and colleagues in children with SCD on HU therapy found an association between *OR51B6* rs5024042 and baseline HbF [22]. On the contrary, in a study carried out in Cameroonian SCA patients, nonsignificant association was found between *OR51B5/6* rs5024042 and HbF level [24].

In a study carried out on SCA patients that received HU, it was discovered that SNPs within *HAO2*, *MAP3K5*, *PDE7B*, *TOX*, *NOS1*, *FLT1*, *ARG2* and *NOS2A* were significantly associated with the change of percent and/or absolute HbF. The most significant associations were found between SNP rs2182008 in *FLT1*, a vascular endothelial growth factor involved in cell proliferation and differentiation, and change of percent HbF as well as between SNP rs10483801 in *ARG2*, an enzyme involved in the

metabolism of drug, and the change of absolute HbF [14]. In another study involving SCD patients on HU therapy, SNPs *ARG1* rs17599586 and *ARG2* rs2295644 demonstrated significant associations with increased HbF levels [29]. A GWAS in hemoglobinopaties patients on HU treatment as well as erythroid progenitor cells from healthy and *KLF1*-haploinsufficient individuals treated with HU, found a significant association between intronic variants rs9483947 and rs9376230 of *MAP3K5*, a member of the p38 and the JNK MAPK pathway, and HU response [33].

The Krüppel-like family of transcription factors (KLF1, KLF2, KLF4, and KLF10) is a set of zinc finger DNA-binding proteins that can regulate the HbF production [34]. Research shows that KLF10 can repress the adult *HBB* gene interacting with SIN3A, leading to the repression of KLF1 activity, augmentation of γ-globin synthesis and hence HbF production [35]. Among the four SNPs identified within the *KLF10* gene in white population, it was discovered that *KLF10* rs3191333 can be considered as a pharmacogenomic biomarker [36, 37]. A pharmacogenomic study carried out earlier in Hellenic compound SCD/β-thalassemia patients as well as healthy and *KLF1*-haploinsufficient Maltese adults allowed the identification of *KLF10* rs3191333 (c.*141C > T) as an important pharmacogenomic biomarker, which facilitate the separation of responders and nonresponders to HU treatment [36]. Recently, in a cross-sectional study performed with Egyptian SCD and β-thalassemia (major and intermedia) patients on HU therapy, it was also found that *KLF10* gene plays a non-stand-alone role as HbF modifier and can be used as pharmacogenomic biomarker of HU treatment [38]. The participation of *SIN3A* gene in changes of *HBG2* expression was investigated in Hellenic β-hemoglobinopathies patients and it was found that the intronic *SIN3A* variants rs11072544 (C > T) and rs7166737 (T > C) were associated respectively with β-thalassemia disease severity and HU treatment response suggesting that they can be considered as pharmacogenomic biomarkers of HU response [35]. In a prospective study performed in SCA children treated with HU to MTD, Sheehan and colleagues using whole exome sequencing, investigated phenotype-genotype associations and they found out that a coding variant rs61743453 in *SALL2* (Spalt-like transcription factor) was associated with higher final HbF [39, 40].

The crucial role of the HU-induced small guanosine triphosphate-binding protein called secretion-associated and RAS-related (SAR) protein in *HBG2* induction has been demonstrated [41]. Report showed that some SNPs within the promoter region of *SAR1A*, a gene which acts in hemoglobin regulation, might contribute to the regulation of HbF expression and modulate the HU response in the patients [41, 42, 19]. A GWAS carried out in SCD patients identified SNPs in the promoter region of the *SAR* gene which were associated with the HU response [17].

In a recent study, it was suggested that SNPs (rs10901080 and rs10793902) within the *ASS1* gene, located on chromosomal region 9q34, can serve as pharmacogenomic biomarkers to predict HU treatment efficacy in compound SCD/β-thalassemia patients [43]. The G-T-T haplotype derived from the SNPs rs7860909, rs10901080 and rs10793902 was associated with higher HbF% and HU treatment efficacy. Furthermore, the in silico analysis performed by the authors revealed that these markers may induce NO biosynthesis, either altering splicing and/or miRNA binding, which consequently will activate the guanylate cyclase and increase γ-globin levels [43]. Multivariate analysis performed in SCA patients on HU therapy showed a strong influence of SNPs in *ASS* and *ARG1* on the change of HbF level, suggesting interactions between these genes and other genes for regulating the response to HU treatment [14].

Specific solute carrier membrane transporters (organic anion-transporting polypeptides—OATPs, cation/carnitine transporters—OCTNs, and urea transporters—UTs) regulate the transcellular traffic and accumulation of HU in the erythroid cells. The OATPs are more expressed in the intestine, liver and kidneys and associated with modulation of pharmacokinetics of HU, while UTB (encoded by *SLC14A1* gene) and OCTN1 (encoded by *SLC22A4* gene) are expressed principally in erythrocytes and bone marrow [44–46]. Walker and Ofori-Acquah carried out an experimental study which showed that the upregulation of UTB and OCTN1 in erythroid cells correlated with higher HU-mediated *HBG2* induction suggesting their important role in this process [47]. The result further showed that the OCTN1 expression mediated by HU was sustained and significantly correlates with *HBG2* induction, suggesting the pivotal role of OCTN1 in the efficacy of HU. Furthermore, the SNPs rs12605147 and rs2298720 in UTB demonstrated associations with pharmacokinetic parameters in SCD patients [29]. A GWAS performed in African-American SCA patients on HU therapy included various candidate genes (*ARG1*, *AQP9*, *ASS*, *PS1*, *SLC14A1*, *LOC57404*, *POR*, *CYP2C9*, and *CYP3A5*) with a potential role in HU metabolism and genes (*RRM1*, *RRM2*, *TK1*, *TYMS*, *MPO*, *SOD1*, *ABCB1*) mediating the effect of HU [48]. The results showed associations between SNPs in *CYP2C9* and *AQP9*, and HbF response to HU. In a recent study performed in Brazilian SCA patients on HU, we found independent associations between *MPO* rs2333227 and total cholesterol, *CYP2E1* rs3813867/rs2031920 and alpha 1-antitripsina, null *GSTT1* genotype and total bilirubin suggesting that these SNPs can be associated with alterations in lipid, inflammatory and hemolytic profiles, respectively, improving or compromising the treatment of the SCA patients under HU, depending on the SNP and parameter affected [49].

SNPs associated with alterations in clinical profile in response to HU therapy

There is evidence showing that beside factors such as reticulocytes count, frequency of vaso-oclusive events and α-thalassemia, genetic variants present in the promoter region of the uridine diphosphate (UDP)—glucuronosyl transferase 1A1 (UGT1A1), the main enzyme responsible for bilirubin conjugation, can be a risk factor for cholelithiasis [50–52]. In SCA patients on HU, an association was found between the $(TA)_7/(TA)_7$ repeats and higher bilirubin levels [52]. This result was confirmed by Adekile and colleagues that demonstrated that adults SCA patients under HU and carriers of $(TA)_7/(TA)_7$ repeats had higher levels of bilirubin [53]. Furthermore, a negative correlation was found between HbF% and bilirubin levels, suggesting contribution of this genotype in HU response [53]. Research from Italia and colleagues, showed that $(TA)_7/(TA)_7$ repeats were associated with elevated bilirubin levels among the different groups analyzed (SCA, β-thalassemia intermedia and HbE—β-thalassemia) and the use of HU therapy did not reduce the bilirubin levels under normal values [51]. Furthermore, the results showed that SCA and β-thalassemia intermedia patients on HU therapy with $(TA)_7/(TA)_7$ repeats and α-thalassemia had higher bilirubin levels in comparison with those without α-thalassemia [51]. However, further studies are needed to better understand this interaction between *UGT1A1* gene promoter polymorphism and HU response.

Report from Panigrahi and colleagues found that heterozygous $\alpha^{3.7}$ deletion carriers responded to HU therapy and were correlated with the XmnIγ^G polymorphism [54]. Hence, they suggested that besides the XmnIγ^G polymorphism, the $\alpha^{3.7}$ deletion can be associated with HU response in the patients. Genetic correlations between the XmnIγ^G polymorphism and common β-thalassemia mutations were investigated in different populations and the results were different according to the population studied. A research carried out in Iran showed that 61% of Iranian transfusion-dependent β-thalassemia patients on HU with molecular background favorable to HU (for example XmnIγ^G polymorphism), strongly associated with the IVS-II-I (G- > A) mutation in linkage with the haplotype I [+− − − − −] and internal β-globin framework 2, shifted from monthly blood transfusion dependency to a stable transfusion-free condition [55]. This result is supported by the works of Alebouyeh and colleagues that found a significant correlation between the XmnIγ^G polymorphism and IVSII-1 mutation [56]. They further demonstrated that the XmnIγ^G polymorphism and IVSII-1 mutation (homo- and/or heterozygosis) are important markers in most responding major β-thalassemic patients on HU.

One of the complications observed in patients treated with HU is cutaneous ulceration. In 2014, Crittenden and colleagues presented a case of a severe leg ulcer in an old woman with a history of myelodysplastic syndrome who has been on HU for 14 years, and homozygote for the C677T polymorphism of the methylene tetrahydrofolate reductase (*MTHFR*) gene, suggesting the implication of the *MTHFR* polymorphism or others thrombophilic genetic mutation in the development of cutaneous ulceration in patients on HU [57].

The above reports notwithstanding, little or no GWAS investigated association between genes encoding DME, especially those related to HU metabolism, and HU response (Table 1).

Enzymatic reactions of hydroxyurea

Metabolism of HU in liver and kidney

Currently, little is known about HU pharmacokinetics. It has been reported that approximately half of the drug is eliminated unchanged by the kidneys and the rest is metabolized in the liver [3]. Furthermore, it has been observed in mouse, that 30–50% of administered dose of HU-^{14}C was found in the urine as urea-^{14}C [58]. It has also demonstrated that mouse liver and kidney would convert HU to urea [58]. In 1970, Colvin and Bono suggested an enzymatic reduction of the hydroxylamine group and demonstrated the enzymatic reduction of HU to urea catalyzed by mouse liver tissue in the hepatic mitochondria [59]. Huang and colleagues demonstrated the efficient formation of NO from HU mediated by enzymes present in crude rat liver homogenate [10].

Potential enzymes involved in HU metabolism

In humans, the metabolic pathways of HU have not been established [3]. Fishbein and colleagues in 1965 reported that Davidson and Winter observed the breakdown of HU to hydroxylamine, ammonia, and carbon dioxide when treated with urease (Fig. 3) [60]. Another work suggests that urease greatly enhanced HbNO formation and that HU is partially metabolized to hydroxylamine, which quickly reacts with Hb to form methhemoglobin (metHb) and HbNO [61, 62]. It has been shown that 50% of HU dose administered intraperitoneally was metabolized in liver and kidney tissue, suggesting that NO formation from HU occurs in the liver rather than the blood [10]. In addition to acting as a substrate for urease, HU can act as an inhibitor of this enzyme [63].

Hepatic microsomes contain monooxygenases, which may be separated into two groups, the cytochrome (s) P-448 inducible by polycyclic aromatic hydrocarbons and the cytochrome (s) P-450, which predominate in the liver and accept as substrates hydrophilic and non-planar compounds [16]. Hence, HU is susceptible to being metabolized by a

Table 1 Summary of the association studies which have correlated SNPs located in genes within and outside the human globin gene cluster with HU therapy efficacy

Sample	HU therapy	Origin	Location	Gene and markers	Association with HU therapy response	References
SCD and β-thalassemia patients	No	NA	11p15	*HBG2*, XmnIγ^{G} rs7482144	High HbF level, decrease disease severity	[17, 20, 23]
SCD patients and healthy individuals	No	NA	2p16 6q23	*BCL11A* rs11886868, rs46713993, rs766432 *HMIP* rs9399137, rs4895441	High HbF level	[17]
SCA patients	No	Cameroon	2p16 6q23	*BCL11A* *HMIP*	Increased HbF level	[24]
SCD patients	Yes	NA	2p16	*BCL11A* rs1427407, rs766432, rs4671393, rs7557939, rs11886868	Baseline HbF% No association with MTD HbF levels	[29]
SCA patients	No	Brazil	2p16 6q23	*BCL11A* *HMIP*	Increased endogenous HbF level	[20]
	Yes		2p16	*BCL11A* rs1427407, rs4671393, rs11886868	Increase of HbF level	
β-thalassemia patients	Yes	Iran	11p15 2p16	*HBG2*, XmnIγ^{G} rs7482144 *BCL11A* rs766432	HU treatment response	[25]
β-thalassemia intermedia patients	Yes	Iran	11p15	*HBG2*, XmnIγ^{G} rs7482144	Significant increase in Hb concentration and HbF level; HU therapy response	[26]
SCA patients	Yes	Brazil	2p16	*BCL11A* rs766432	Significant increase in Hb concentration, RBC count and hematocrit, and significant decrease of DB levels and platelet count;	[23]
			6q23	*HMIP* rs11759553	High HbF level	
β-thalassemia patients	Yes	Iran	11p15	*HBG2*, XmnIγ^{G} rs7482144	No correlation with HU response	[28]
SCD and β-thalassemia patients	Yes	Kuwait	11p15	*HBG2*, XmnIγ^{G} rs7482144	Higher HbF levels pre- and post-HU therapy	
			2p16 6q23	*BCL11A* rs11886868 *HMIP* rs9376090, rs9399137, rs9402686	No significant difference in HbF levels pre- or post-HU treatment	[21]
Children SCD patients	Yes	NA	11p15.4	*OR51B6* rs5024042	Association with baseline HbF	[22]
SCA patients	No	Cameroon	11p15.4	*OR51B6* rs5024042	No association with HbF levels	[24]
SCD patients	Yes	NA	6q23.2 14q24.1	*ARG1* rs17599586 *ARG2* rs2295644	Increase HbF levels	[29]
Adults SCA patients	Yes	African-American	13q12.3 14q24.1 9q34.11 6q23.2	*FLT1* rs2182008 *ARG2* rs10483801 *ASS* rs7860909, rs10793902, rs10901080, rs543048 *ARG1* rs17599586, rs2781667, rs2246012	Change of percent HbF Change of absolute HbF Change of HbF level	[14]
Hemoglobinopathies patients; Erythroid progenitor cells from healthy and KLF1-haploinsufficient individuals	Yes	Western Greece	6q23	*MAPK5* rs9483947, rs9376230	HU response	[33]
SCD and β-thalassemia major and intermedia patients	Yes	Egypt	8q22.3	*KLF10*	HbF modifier; pharmacogenomic biomarker of HU therapy	[38]
SCD/β-thalassemia patients; Healthy and KLF1-haploinsufficient individual	No Yes	Greece Malta	8q22.3	*KLF10* rs3191333	Pharmacogenomic biomarker	[36]
β-hemoglobinopathies patients	No	Western–Greece	15q24.2	*SIN3A* rs11072544 and rs7166737	β-thalassemia disease severity and HU treatment response, respectively	[35]
SCA children treated with HU to MTD	Yes	NA	14q11.2	*SALL2* rs61743453	Higher final HbF	[39, 40]
Adults SCD patients	Yes	NA	10q22.1	*SAR1A* (SNPs in the promoter region)	Regulate HbF expression and modulate HU response	[17, 19]
SCD/ β-thalassemia patients	Yes	Western–Hellenic	9q34	*ASS1* rs10901080, rs10793902 *ASS1* rs7860909, rs10901080, rs10793902 –GTT haplotype	Predict HU treatment efficacy Higher HbF% and HU treatment efficacy	[43]
Human cell lines and oocytes overexpressing SLC transporters$_{\dagger}$	Yes	NA	12p12.1	*OATPs*	Modulation of HU pharmacokinetic	[44–46]

Table 1 (continued)

Sample	HU therapy	Origin	Location	Gene and markers	Association with HU therapy response	References
Erythroid cells[†]	Yes	NA	18q12.3 5q31.1	*SLC14A1* *SLC22A4*	Higher HU-mediated *HBG2* induction	[47]
SCD patients	Yes	NA	18q12.3	*SLC14A1* rs12605147 and rs2298720	Pharmacokinetic parameters	[29]
SCA patients	Yes	NA	10q24 15q22.1	*CYP2C9* *AQP9*	HbF response to HU	[48]
SCA patients	Yes	Brazil	17q22 10q26.3 22q11.23	*MPO* rs2333227 *CYP2E1* rs3813867/rs2031920 *GSTT1*	Alterations in lipid, inflammatory and hemolytic profiles, respectively	[49]
Pediatric SCA patients; Pediatric and adults SCA patients	Yes	NA	2q37.1	*UGT1A1* $(TA)_7/(TA)_7$ repeats	Higher bilirubin levels Influence HU response	[52] [53]
SCA, β-thalassemia intermedia and HbE/ β-thalassemia SCA, β-thalassemia intermedia	Yes	NA	2q37.1	*UGT1A1* $(TA)_7/(TA)_7$ repeats *UGT1A1* $(TA)_7/(TA)_7$ repeats and $\alpha^{3.7}$ deletion*	No reduction of bilirubin levels under normal values Higher bilirubin levels	[51]
Thalassemia intermedia patients	Yes	NA	16p13.3	$\alpha^{3.7}$ deletion	HU response	[54]
Transfusion-dependent β-thalassemia patients	Yes	Iran	11p15	*HBG2*, $XmnI\gamma^G$ rs7482144, IVS-II-I (G > A) in linkage with haplotype I […] and internal β-globin framework 2	Shift from monthly blood transfusion dependency to stable transfusion-free condition	[55]
Major β-thalassemia patients	Yes	NA	11p15	*HBG2*, $XmnI\gamma^G$ rs7482144 and IVS-II-I (homo- and/or heterozygosis)	HU response	[56]
Old woman**	Yes	NA	1p36.22	*MTHFR* C677T	Association with severe leg ulcer	[57]

SCA: sickle cell anemia; SCD: sickle cell disease; *HU* hydroxyurea, *MTD* maximum tolerated dose, *RBC* red blood cell, *DB* direct bilirubin, *Hb* hemoglobin, *HbF* fetal hemoglobin, *NA* not available *Co-heritance; **case report study; [†]experimental study

monooxygenase cytochrome (s) P-450 [9, 16]. In addition, Restituto and colleagues reported that 30 to 50% of HU dose administered is metabolized in the liver by the monooxygenated system [8].

A study with SCD patients on HU therapy showed evidence of in vivo NO formation from HU and identified peroxidases as potential oxidants for this conversion [63, 64]. Furthermore, Horseradish peroxidase and catalase catalyze the formation of NO and nitroxyl (HNO) from HU, which is then oxidized into NO [63]. Experiments carried out by Huang and co-workers using the 3 aminotriazole, a specific inhibitor of catalase, and heat-inactivated catalase demonstrated that, the catalase is necessary for the conversion of HU to nitrite/nitrate [63, 65]. The potential role of catalase in the in vivo metabolism of HU to NO was also revealed (Fig. 4) [10]. It was demonstrated in a study that the in vivo HU toxicity depends on catalase, which is a direct HU target since HU play a competitive inhibitor role of catalase-mediated hydrogen peroxide decomposition and HU decomposition *in vitro* can be accelerated by catalase [66].

Conclusion

In the present review, in addition to the previously proposed differential susceptibility model and differential baseline HbF model, we put forward for consideration a third model termed differential HU metabolism model to elucidate differential HU responses. These 3 models together can determine the response of SCD patients on HU therapy. In patients with hemoglobinopathies, various studies identified three important HbF QTL (*HBG2*, *BCL11A* and *HMIP*) and other genes involved in NO biosynthesis, genetic regulation

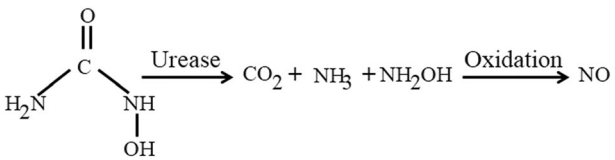

Fig. 3 Urease-mediated hydrolysis of hydroxyurea. Urease catalyzes the hydrolysis of hydroxyurea to hydroxylamine, carbon dioxide and ammonia following by nitric oxide production derived from reaction of oxidation. Figure adapted from [63]

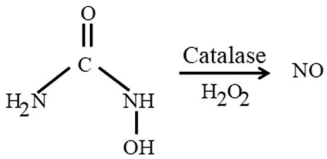

Fig. 4 Catalase-mediated hydrolysis of hydroxyurea. Hydroxyurea can be metabolized by catalase to produce NO in the presence of hydrogen peroxide

and drug metabolism (*ASS1, KLF10, HAO2, MAP3K5, PDE7B, TOX, NOS1, NOS2A*, *FLT1, ARG1, ARG2, UGT1A1*, *OR51B5/6*, *SIN3A*, *SALL2*, *SAR1A*, *UTB*, *OCTN1*, *CYP2C9*, *AQP9*, *MPO*, *CYP2E1*, and *GSTT1*). These genes considered as pharmacogenomic biomarkers can predict HU treatment efficacy. Despite the widespread use of HU and the diversity of its efficacy, the enzymes involved in its metabolic pathway are unknown. The few studies that investigated the enzymatic reactions of HU, had suggested the participation of enzymes such as catalase, urease, horseradish peroxidase and enzymes of CYP450 family. Therefore, further studies are necessary to elucidate the metabolic pathway of HU, which will allow identification and better selection of enzymes for pharmacogenomic studies in hemoglobinopathies patients under HU therapy.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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