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REVIEW

Pharmacogenomics of sickle cell disease: steps toward personalized medicine

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Abstract: Sickle cell disease (SCD) is a monogenetic disease but has a wide range of phenotypic expressions. Some of these differences in phenotype can be explained by genetic polymorphisms in the human globin gene. These polymorphisms can result in different responses to typical treatment, sometimes leading to inadequate therapeutics. Research is revealing more polymorphisms, and therefore, new targets for intervention to improve outcomes in SCD. This area of pharmacogenomics is continuing to develop. We provide a brief review of the current literature on pharmacogenomics in SCD and possible targets for intervention.

Keywords: sickle cell disease, pharmacogenomics, hydroxyurea, opioids, HbF inducers, gene therapy

Introduction

Sickle cell disease (SCD) was the first described genetic disorder. It results from the substitution of a valine for a glutamine affecting the hemoglobin structure as a result of substitution of A to T on chromosome 11 in the β-globin gene. The resultant hemoglobin is more rigid and less soluble compared to its normal counterpart. The homozygous form results in vaso-occlusion in vascular beds, resulting in pain and chronic organ dysfunction.

Although SCD is a monogenetic disease, there is vast variation in the phenotypic expression. Data demonstrates that there are variable haplotypes in SCD that effect HbF expression. Additional genetic variations may affect the efficacy of both hydroxyurea (HU), a disease-modifying therapy, and opioid therapy.

HU

HU, also known as hydroxycarbamide, is a ribonucleotide reductase inhibitor that works via multiple mechanisms. It can help with HbF induction, decreases adhesion molecules, and decreases white blood cells.[1] HU increases HbF production through its effect on human β-globin genes (*HBB*). Although this medication is the only US Food and Drug Administration-approved treatment for SCD, there are nonresponders and patients with intolerance to HU.[2] There have been a reported 25% of SCD and β-thalassemia patients that are poor responders or nonresponders to HU and have variation in the production of HbF.[3] It is likely that genetic makeup plays a role, as evidenced by showing the hereditary component in HbF induction with HU treatment.[4] Tafrali, et al[5] studied the *MAP3K5* gene and found two single-nucleotide polymorphisms (SNPs) in the intron 1 region that were confirmed to have a correlation with

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increased HU response in heterozygous SCD/β-thalassemia patients. However, they also suggest that there may be an unknown associated genetic polymorphism that influences the *MAP3K5* gene as this gene has not been found to be clearly associated with erythropoiesis. Chalikiopoulou[6] also found an association between the *ASS1*, *NOS1*, and *NOS2A* gene polymorphisms and HU treatment efficacy. These genes are involved in enzyme processing for nitric oxide (NO) synthesis. NO is known to be associated with HbF induction.

The genetic modifier KLF10 may lead to haploinsufficiency and has been found in SCD patients.[7] One study analyzed the *KLF10* gene and found that it was one of the major genes expressed in vitro in human erythroid progenitor cells when treated with HU.[8] They proposed that KLF10 can be used as a pharmacogenomic marker for efficacy of HU treatment in β-hemoglobinopathy patients, including those with SCD. Their study was specifically in β-thalassemia/SCD compound heterozygous patients. Other markers were studied by Steinberg et al,[9] who found that the absence of a β-globin haplotype, the CAR, was associated with a higher HbF response with HU treatment. Alternatively, poor response and death from acute chest syndrome was seen in SCD patients who were homozygous in the BAN haplotype and heterozygous in the CAM haplotype.[10] Kumkhaek et al,[11] found five SNPs in the *SAR1A* gene that was also correlated with HbF expression after 2 years of HU treatment.

Gene markers found to have a response to HU in children include *ARG1*, *ARG2*, *BCL11A*, and two SNPs in the *HBB* locus.[12] Interestingly, *BCL11A* is not present on the β-globin gene cluster but still has an effect on HbF production.[13]

The *Xmn1* polymorphism has been associated with higher levels of HbF upon treatment with HU. However, this has been shown in patients with β-thalassemia.[14,15] Finding genetic variations that lead to a nonresponder phenotype is important as HU does have side effects. Some common side effects include cytopenias, hyperpigmentation, weight gain, and possible teratogenic effects.[16] If the patient is known to be a nonresponder, exposure to potential side effects may be avoided.

A full list of genes involved in response to HU is listed in Table 1.

Opioids

Opioids are currently the primary treatment modality for pain in sickle cell crisis. Many sickle cell patients also require chronic opioid therapy to optimize functional status so that they are not debilitated by their chronic pain. However, there is variability in response to opioids among SCD patients. Many times, a lack of response at typical opioid doses is perceived as an aberrant behavior by the medical team. However, known polymorphisms in the *COMT*, *OPRM1*, and *ABCB1* genes can lead to an altered perception of pain and/or a change in response to opioids.[17,18] Additionally, the pharmacokinetics of opioids can be altered by genetically mediated variability in the activity of CYP enzymes and by organ dysfunction of SCD. This combination of factors results in a complicated clinical presentation.

COMT is the enzyme that breaks down dopamine, norepinephrine, and adrenaline. All of these neurotransmitters play a part in the modulation of pain. Genetic polymorphisms can lead to a 3–4-fold difference in the activity of this enzyme, likely altering pain sensitivity.[19] *OPRM1* codes for the human mu-opioid receptor. Polymorphisms in *OPRM1* can lead to varied morphine metabolism and mu receptor expression. Although little research has been done regarding the predictive value of *COMT* or *OPRM1* polymorphisms in SCD, data in cancer patients suggests a significant contribution of the most common polymorphisms (*OPRM1* A118G, *COMT* Val158Met) to analgesic response to morphine. Patients with *COMT* and/or *OPRM1* polymorphisms required 23%–93% more opioid than subjects with wild-type genes to achieve adequate analgesia.[20]

Many opioids are primarily metabolized by CYP2D6 to a more potent opioid agonist than the parent drug, and these include drugs such as hydrocodone, oxycodone, codeine, and tramadol. Yee et al[21] analyzed CYP2D6 polymorphisms in African American children with SCD. Of the 75 children analyzed, 44% were intermediate CYP2D6 metabolizers and 5.3% were poor CYP2D6 metabolizers. Both of these phenotypes would decrease conversion of codeine, hydrocodone, and tramadol to their active metabolites, thereby decreasing analgesia.

A list of genes/polymorphisms involved in opiate metabolism is listed in Table 1.

HbF inducers

HbF is still present in normal adults, usually at levels <1%. This provides a therapeutic opportunity to upregulate HbF production. HbF expression has been found to be strongly associated with SNPs in three particular loci: Xmn1–HBG2, HBS1L–MYB, BCL11A.[22] However, further study on the polymorphism showed that a downstream SNP, rs10128556, is more strongly associated with HbF expression than the Xmn1 site itself in African Americans with sickle cell anemia.[23]

One study showed that disruption of the *BCL11A* loci in a murine model increased the level of embryonic murine

Table 1 Genes/polymorphisms involved in response to HU and leading to alterations in pain perception

HU			Opioids
Genes		Polymorphisms	Genes
Positive	Negative		
Xmn1	BAN haplotype	*HBB* locus (2 SNPs)	*CYP2D6*
NOS1	CAM haplotype	*MAP3K5* (2 SNPs intron 1)	*COMT*
NOS2A		*SAR1A* (5 SNPs)	*OPRM1*
ASS1		*HBS1L–MYB* SNPs	*ABCB1*
ARG1			*UGT2B7*
ARG2			*TLR4*
BCL11A			
KLF10			
PDE7B			
TOX			

Abbreviations: HU, hydroxyurea; SNP, single-nucleotide polymorphism.

globin compared to adult globin.[24] It is suggested that this same principle could be applied to human models and HbF. Deng et al[25] also showed that using zinc finger proteins to target *HbF* promoter genes lead to HbF reactivation while reducing adult globin synthesis.

The role of genes was also elucidated by Ma et al,[26] who highlighted the numerous SNPs in genes that can lead to increased HbF production, particularly the *FLT1* gene polymorphism rs2182008. Other *FLT1* gene polymorphisms were also found such as rs9319428 and rs8002446. Other genes found to be associated were *MAP3K5*, *PDE7B*, *ASS*, *TOX*, *ARG1*, *ARG2*, *NOS2A,* and *NOS1*.

Some studies have also suggested that KLF10 may act through a corepressor gene, *SIN3A*. It has also been hypothesized that SIN3A, in conjunction with *HDAC1* gene, binds to KLF1 and represses KLF1 activity. This represses β-globin synthesis and, consequently, leads to an increase in fetal globin synthesis.[27,28]

Sheehan et al[29] found a novel gene, *SALL2*, using whole exome sequencing, which was shown to lead to higher HbF expression level. The particular polymorphism is P840R SALL2.

Krivega et al[30] studied the epigenetic effects of the G9a H3K9 methyltransferase on HbF induction. HBB transcription is regulated by the locus control region. The LDB1 complex brings the locus control region into direct contact with the gene. A chemical compound, UNC0638, inhibits the G9a methyltransferase, which allowed for more interaction between the LDB1 complex and the gamma globin promoters. This led to increased HbF production. The UNC0638 compound has poor in vivo pharmacokinetic properties, and so has limited clinical applications. However, this discovery provides an opportunity to study similar types of compounds that can serve as improved HbF-inducing therapy.[30]

Additionally, a study found that expression of the *LIN28* gene was found to decrease the amount of sickling in cultured human sickle cell sample and increase HbF expression.[31]

NO inducers

Endothelial Nitric Oxide Synthase polymorphisms

NO deficiency has been shown to play an important role in the development of vaso-occlusive crises and endothelial dysfunction, of which a common complication is pulmonary hypertension.[32,33] NO is synthesized by nitric oxide synthase (NOS), and one isoform, endothelial NOS (eNOS), regulates the level of NO in the body.[34] It is suggested that eNOS polymorphisms can be a prognostic marker for severity of SCD.[35]

There are SNPs of the eNOS gene associated with variations in NO levels. Some of these include 786T>C (rs2070744) in promoter region, 894G>T in exon 7 (Glu-298Asp, rs1799983), and 4a/b, a 27-bp variable number of tandem repeats in intron 4 (chromosome 7q35–q36).[35] Nishank et al[36] found that there was a higher frequency of these particular polymorphisms in patients with severe SCD who had significantly lower levels of plasma nitrite compared to those in the mild SCD group. The homozygous 786T>C polymorphism decreases eNOS activity, leading to lower NO levels.[37] Sharan et al[38] found that the 786T>C polymorphism was associated with a higher likelihood of ACS in female African American patients with SCD. They proposed that this may be the first gender-specific modifier in SCD.

Patients with the eNOS4 alleles aa and ab genotypes were found to have a higher risk of vascular complications.[39] Yousry, et al[35] found the 4a/4b allele to be an independent risk factor for acute chest syndrome in their Egyptian population with SCD.

Conversely, Vargas et al[40] found no association between these three main eNOS polymorphisms and SCD severity. Alternatively, the wild-type 4a/4b genotype was found to be protective against vaso-occlusive crises and pulmonary hypertension.[35]

Relationship with L-arginine therapy

There are renal complications from SCD, and the kidney is sensitive to the hypoxia induced by vaso-occlusion.[41] Arginine is decreased in renal dysfunction given the loss of arginine synthesis from citrulline, which primarily occurs in the kidney. There can be a role for monitoring eNOS polymorphisms and arginine therapy. The 4a/4b allele had a higher frequency in SCD patients with nephropathy.[37,39,42] In adults with SCD, it has been found that there is an arginine

deficiency.[43] In addition, low plasma arginine levels were shown to be predictive of hospitalization for children with SCD.[43] Low arginine levels lead to increased arginase levels, which metabolizes arginine in another pathway, to ornithine and subsequently, polyamines and proline, which leads to smooth muscle proliferation airway remodeling.[44] Understanding the eNOS polymorphisms can help direct arginine therapy.

Relationship with adhesion molecule upregulation

Vilas-Boas et al[45] found that the SCD patients with the 786T>C polymorphism had higher levels of sVCAM-1 levels. NO normally inhibits the expression of adhesion molecules, like sVCAM-1, which maintains normal endothelial function and blood flow. The upregulation of adhesion molecules contributes to vascular inflammation.[46] VLA-4 is the very late activation antigen that is only expressed on sickled RBC membranes.[47] Its receptor is VCAM-1, another immunoglobulin like ICAM-1. It is proposed that a possible treatment modality is antibodies to these two factors, which could decrease adhesion molecule upregulation.

Conclusion

There are few therapies approved for SCD treatment. Those that have been the most helpful, HbF inducers, HU, and opiates, have not displayed consistent results among patients, suggesting genetic influences. Many studies in this report have shown that genetic polymorphisms can influence the response to these treatments. Further studies are warranted to evaluate the impact on SCD treatment. Eventually, this approach would lead to more personalized approach for therapies in the field of SCD.

Disclosure

The authors report no conflicts of interest in this work.

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