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

Tafrahi, et al1 studied the MAP3K5 gene and found two single-nucleotide polymorphisms (SNPs) in the intron 1 region that were confirmed to have a correlation with
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 -globin haplotype, the CAR, was associated with a β-studied by Steinberg et al,\(^9\) who found that the absence of the ASS1, NOS1, and NOS2A genes in SCD compound heterozygous patients with SCD. Their study was specifically in β-thalassemia/SCD. 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 SARI 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.

### 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 locus in a murine model increased the level of embryonic murine
globin synthesis. It is suggested that this same principle could be applied to human models and HbF. Deng et al 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, 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, SINE3A. 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 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 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.20

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 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 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 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 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

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

<table>
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<tr>
<th>Genes</th>
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<tr>
<td>HU: XmnI, NOS1, NOS2A, ASS, ARG2, BCL11A, KLF10, PDE7B, TOX</td>
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**Opioids**

<table>
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<th>Genes</th>
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<td>HBB locus (2 SNPs), MAP3K5 (2 SNPs intron 1)</td>
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**Polymorphisms**

| XmnI, NOS1, NOS2A, ASS, ARG2, BCL11A, KLF10, PDE7B, TOX |

**Abbreviations:** HU, hydroxyurea; SNP, single-nucleotide polymorphism.
deficiency. In addition, low plasma arginine levels were shown to be predictive of hospitalization for children with SCD. 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. Understanding the eNOS polymorphisms can help direct arginine therapy.

Relationship with adhesion molecule upregulation

Vilas-Boas et al 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. VLA-4 is the very late activation antigen that is only expressed on sickled RBC membranes. 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.

References


