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Genetic Polymorphisms of Urea Transporter B in Iraqi Patients with Sickle Cell Anemia Treated with Hydroxyurea

A Thesis

Submitted to the Council of College of Pharmacy/ University of Kerbala as Partial Fulfillment of the Requirements for the Master Degree of Science in Pharmacology and Toxicology

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بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

وَقُلْ رَبِّ زِدْنِي عِلْمًا

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Dedication

All the prayers to almighty ALLAH who guided us to this blessing which without Allah's blessing we will never reach this without Allah's guidelines. O Allah please give your blessing and prayers to greatest Massinger Mohammad and his family whom helped us to be your worshipers O Allah.

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List of Abbreviations

ACS	Acute Chest Syndrome
ADME	Absorption, Distribution, Metabolization, and Excretion
AI	Arab-Indian
ANOVA	Analysis of Variances
API	Average Pain Intensity
AR	Autosomal Recessive
ARC	Absolute Reticulocyte Count
ATP	Adenosine Triphosphate

BCAM	Basal Cell Adhesion Molecule
BH4	Tetrahydrobiopterin
BLAST	Basic Local Alignment Search Tool
BMI	Body Mass Index
BMR	Basal Metabolic Rate
Bp	Base Pair
Camp	Cyclic Adenosine Monophosphate
CBC	Complete Blood Count
CDC	Centers for Disease Control
CNS	Central Nervous System
COMT	Catechol-O-Methyltransferase
CPI	Composite Pain Index
CRISPR/Cas9	Clustered Regularly Interspaced Short Palindromic Repeats or CRISPR Associated Protein 9
DRD3	Dopamine Receptor D_3
DVT	Deep Vein Thrombosis
EDTA	Ethylenediaminetetraacetic Acid
eNOS	Endothelial Nitric Oxide Synthetase
g/dl	Gram per Deciliter
GTP	Guanosine-5'-triphosphate
Hb F	Fetal Hemoglobin
Hb S	Hemoglobin Sickle
HLA-G	Human Leukocyte Antigen G
HMCs	Hematopoietic Multipotent Cells
HPCs	Hematopoietic Progenitor Cells
HU	Hydroxyurea
IgG	Immunoglobulin G

IL	Interleukin
IVIG	Intravenous Immunoglobulin
NO	Nitric Oxide
NO3-	Nitrate
OATP	Organic Anion Transporting Polypeptide
PCR	Polymerase Chain Reaction
PE	Pulmonary Embolism
Pg/ml	Picogram per Milliliter
Pmol/µl	Picomole per Microliter
RBCs	Red Blood Cells
RNA	Ribonucleic Acid
Rs	Reference SNP
SCA	Sickle Cell Anemia
SCD	Sickle Cell Disease
SD	Standard Deviation
SLC	Solute Carrier Transporter
SNPs	Single Nucleotide Polymorphisms
SPSS	Statistical Package for Social Sciences
TALENs	Transcription Activator-like Effector Nucleases
TBE	Tris-borate EDTA Buffer
TNF-α	Tumor Necrosis Factor-α
UGT1A1	Uridine Diphosphate Glucuronosyltransferase
US	United States
UT	Urea Transporter
UTA	Urea Transporter A
UTB	Urea Transporter B
VCAM-1	Vascular Cell Adhesion Molecule-1
VOC	Vaso-Occlusion Crisis

WBC	White Blood Cell
WHO	World Health Organization
ZFN	Zinc Finger Nucleosomes
α	Alpha
β	Beta
γ	Gamma
δ	Delta
˚C	Centigrade
µl	Microliter
2,3-DPG	2,3-bisphosphoglycerate

Abstract

Introduction

Sickle cell disease (SCD) manifests in varying clinical severities influenced by genetic factors, including polymorphisms within the UTB gene.

Aim

To evaluate the association between the UTB rs12605147 (A>G) and rs2298720 (G>A) polymorphisms and response to hydroxyurea (HU) treatment in SCD patients, aiming to guide personalized medicine in SCD management.

Patients and Methods

A sample of 50 Iraqi SCD patients was genotyped for the UTB rs12605147 and rs2298720 polymorphisms using allele specific polymerase chain reaction, with comprehensive demographic and clinical data recorded. Pain severity was scored on a 10-point scale, pain crisis frequency was assessed over a 12-month period, and HbF levels were measured. HU response was evaluated in terms of reductions in pain crises and increases in HbF levels among patients with different genotypes.

Results

The UTB rs12605147 polymorphism significantly correlated with key clinical outcomes. Patients with the AA genotype reported a lower average pain score of 4.5 ± 0.8 compared to those with the GG genotype, who had an average pain score of 7.2 ± 1.1 ($p < 0.01$). Pain crisis frequency also varied by genotype, with AA genotype carriers experiencing an average of 2.3 ± 1.0 crises per year versus 5.8 ± 1.5 crises in GG carriers ($p < 0.05$). HbF levels were significantly higher in AA genotype patients (24.83 ± 4.33%) compared to GG genotype patients (20.80±0.00%) after HU treatment ($p < 0.001$).

These results indicate that the AA genotype may confer a more favorable clinical profile and better HU response in SCD patients. Conversely, all patients exhibited the wild-type genotype for the rs2298720 SNP, indicating no observed variability in this SNP among the study subjects, and thus no correlation could be drawn with clinical parameters for rs2298720.

Conclusion

The UTB rs12605147 polymorphism is associated with pain intensity, crisis frequency, and HbF levels in SCD patients, suggesting a potential role in determining HU efficacy and clinical severity. The absence of variation in rs2298720 suggests it may not contribute to clinical variability in SCD within this population. The findings support personalized medicine approaches for SCD, wherein UTB rs12605147 genotyping could inform treatment strategies aimed at optimizing patient-specific management and outcomes.

Chapter One

Introduction

1. Introduction

1.1 Sickle Cell Anemia

Sickle cell anemia (SCA) or sickle cell disease (SCD) consider as one of genetic disease caused by the hemoglobin S variation (HbS) that it inherits through both parents. In these condition the red blood cells (RBCs) under some conditions as hypoxia, it changed their shaped from biconcave to rigid sickle that aggregate on endothelial of blood vessel with other molecules like platelets and neutrophile caused obstruction of these vessels and results ischemia of organs and vasculopathy (dos Santos Neres et al., 2023).

Hemoglobinopathies represent a major public health problem, and globally they are classified as one of the most commonly inherited genetic disorders. World Health Organization (WHO) report showed that the worldwide prevalence of carriers with significant genetic variants for hemoglobinopathies (sickle cell disease and thalassemia) is at least 5.2%(Modell & Darlison, 2008). These diseases have significant morbidity and mortality with high health cost for individual families and for the public (Tosun et al., 2006). Sickle cell anemia is considered by healthcare organizations as an international health problem. It is believed that the sickle hemoglobin was generated due to the selective pressure of Plasmodium falciparum malaria. Individuals with sickle cell trait (single abnormal gene) are protected against endemic Plasmodium falciparum malaria infection which is an advantage of the sickle gene, however, carriers of homozygous mutation (Hb SS) are not protected against malaria transmission.(Steinberg et al., 2009)

There are different five distinct haplotypes of SCA which have been identified: the Benin, Senegal, Cameroon, Bantu haplotypes in Africa, and

the Arab-Indian (AI) haplotype in Asia .The severity of manifestations of SCA ranging from highest manifest represented in Bantu haplotype to lowest AI, depend on availability of fetal hemoglobin in each haplotype (Hardouin et al., 2023).

1.2 Global Epidemiology of Sickle Cell Anemia

Sickle cell trait prevalence is higher between individuals with ancestral roots in Africa, tropical and subtropical regions. Specifically in the United States, the prevalence is higher among African Americans compared to Caucasians, with rates of 9% and 0.2% respectively. On a global scale, it is estimated that there are at least 300 million individuals affected by the sickle cell disease and the trait, particularly in areas where malaria is endemic. In certain regions of Africa, the prevalence of sickle cell trait can reach as high as 25%, while some parts of Saudi Arabia have reported rates as high as 60% (Thein, 2011). It is important to note that in Western countries, there has been an increasing prevalence of sickle cell trait due to migration from Africa and the Middle East to European and American nations (Dede Meshay, Haseeb Khamees and Abdalzehra Wadaha, 2022).Sickle cell anemia affects millions of people worldwide, with over 305,000 annual births related to the condition. The global burden of SCA is increasing due to improved survival in regions with a high prevalence of SCA and population migration to countries with higher income levels (Yaseen et al., 2020).

1.3. Epidemiology of Sickle Cell Anemia in Iraq

Patients with sickle cell anemia in Iraq are predominantly found in two specific geographical regions, posing a significant health concern. The highest prevalence is observed among the Arabs residing in the extreme south, with 6.48% of the population in Basra being carriers of the sickle

cell trait (Hassan et al., 2003). Arab Indian haplotype is particularly prevalent among SCA patients from Basra province (Yaseen, Al-Mamoori and Hassan, 2020).

Kurdish population in the north of Iraq is second prevalent area. In Dohuk, around 1.2% of individuals are carriers for the sickle cell trait (N. A. Al-Allawi & Al-Dousky, 2010), (Yaseen et al., 2020). The most frequent haplotype among SCA patients in the north of Iraq is the Benin haplotype, which present in 69.5% of the patients, then AI 12.5% and Bantu 7.8% of the patients(N. A. S. Al-Allawi et al., 2012).

1.4. Molecular Basis and Pathophysiology of Sickle Cell Anemia

Sickle cell anemia is an inherited hemoglobinopathy disease caused by mutation in the sixth amino acid of β globin gene which occur in single nucleotide when replacement A to T in codon this leads to production of valine amino acid instead of glutamic acid in the sixth position of β chain in the hemoglobin. Consequently, a modified hemoglobin is formed referred to HbS (Tebbi, 2022).

The hemoglobin is oxygen carrier from the lungs and to several tissues and carbon dioxide from various parts of the body to the lungs. Approximately 70% of iron in the body presents in RBCs in form of hemoglobin which is an iron-protein complex that provides blood cell its red color. Also, it is consisting of two parts, first one is heme which is iron containing protein, and second part is globin chains which are proteins as shown in Figure 1-1. There are different types of hemoglobin:

-Hb A which is found in adults makes about 95-98%, it contains 2 alpha chain (2α) and two beta chain (2β).

- Hb A2 found in adults makes about 2-3%, it contains (2α) and two delta (2δ).

- Hemoglobin F (Hb F) found in adults makes about 1-2%. it has (2α) and two gamma (2γ).

- Hemoglobin S (Hb S): when mutation occurs in normal HbA at 6th codon of β globin gene Hb S will produce (El-Hazmi et al., 2011).

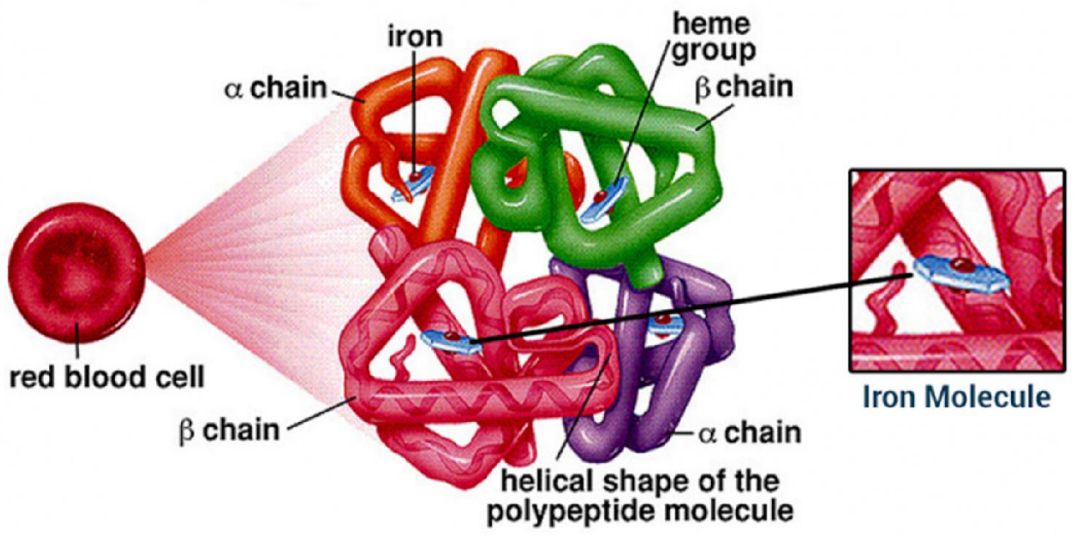


Figure 1-1: molecuar structue of hemoglobin (Jiang et al., 2022).

1.4.1 Hemoglobin S (Hb S) and Hb S Polymerization

Hemoglobin S is soluble in oxygenated environment, but once it delivers the oxygen to the tissues, the Hb S in deoxygenated form which subject to change in their conformational, which gives rise to generate long fibrous accumulates (polymers) attributed to hydrophobic interactions among the valines in the beside Hb S molecules. These polymers in RBCs

deform its shape from biconcave disc-spherical normally to distinct sickle shape, generate RBCs rigidity, sickled which aggregates on endothelial blood vessel causing obstruction (vaso-occlusion) as shown in Figure (1-2). The polymerization of hemoglobin is basic mechanism to SCA pathophysiology (Steinberg and Sebastiani, 2012).Polymerization of RBCs in deoxygenated conditions is affected by many factors involving level of fetal hemoglobin which reduction of incidence the polymerization. Additional to temperature, acidity and dehydration because the kinetic of polymerization process depended on dehydration of erythrocytes which leads to increasing the concentration of hemoglobin inside these cells (Piccin et al., 2019; Tebbi, 2022).

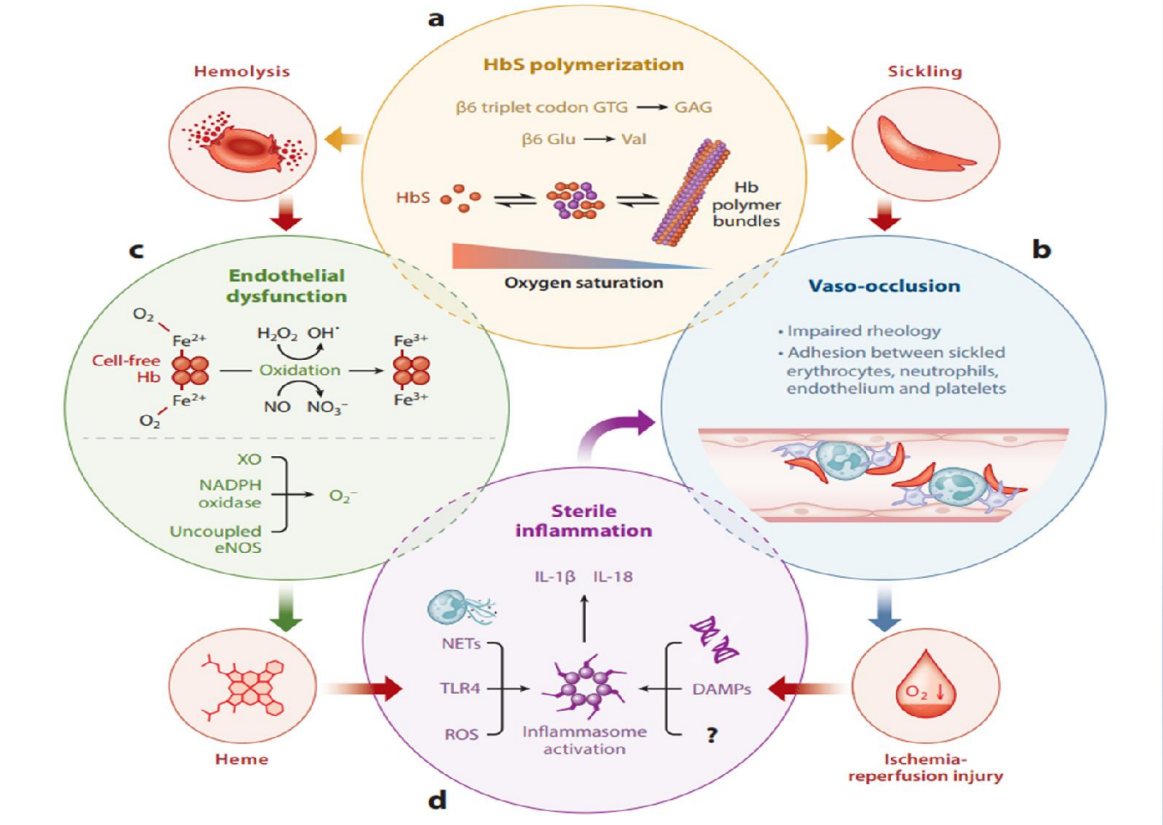


Figure 1-2 shows the pathophysiology of sickle cell anemia (Sundd et al., 2019).

1.4.2. Vaso- Occlusion and Molecules Adhesion

Vaso-occlusion refer to not only sickled red blood cells adhering to the vascular endothelium in small blood capillaries but also adherent leukocytes, platelets and neutrophils additional to adhesion molecules. These aggregation processes lead to obstruction of capillaries causing tissue infarction and vasculopathy. Additionally impaired blood rheology and promote stasis of blood flow (Darbari, Sheehan and Ballas, 2020).Many adhesion molecules have been associated with sickle cell disease, such as vascular cell adhesion molecule-1(VCAM-1) and basal cell adhesion molecule protein (BCAM) which expressing themselves on sickle cell compared to normal erythrocytes as shown in Figure (1-3) (Hebbel, 1997).

P-selectin and E-selectin are an additional adhesion molecule that appears significantly on the surface of platelets and endothelial cells in sickle cell anemia. Increased P-selectin expression on endothelial cells encourages leukocyte adhesion, and activated platelets attach to neutrophils in a P-selectin-dependent way to produce aggregates. These processes collectively contribute to the formation of vaso-occlusion crisis (VOC) i.e. painful crisis episodes (Wick and Eckman, 1996; Tebbi, 2022).

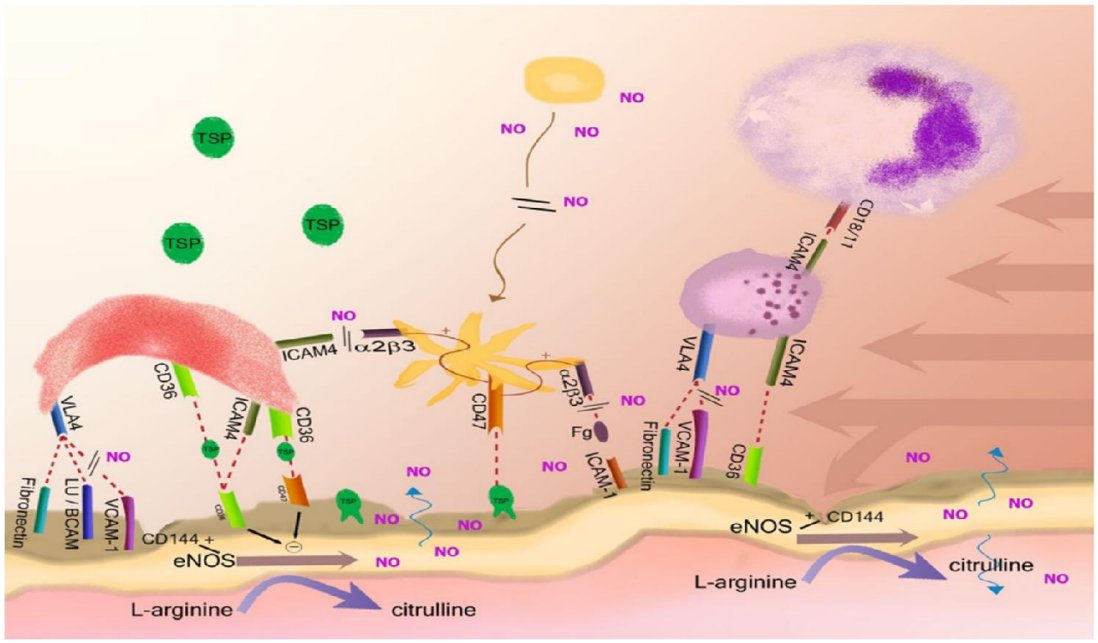


Figure 1-3: An illustration of how blood cells adhere to endothelium in SCA through adhesion molecules. Nitric oxide (NO), endothelial nitric oxide synthetase (eNOS), (ICAM-4) intracellular adhesion molecule (Piccin et al., 2019).

Figure (1-3) is an illustration of how blood cells adhere to endothelium in SCA through adhesion molecules. The production of nitric oxide (NO) is done by arginine-dependent endothelial nitric oxide synthetase (eNOS) on the endothelium of the vascular system. NO maintains platelet resting condition and inhibits blood cells- endothelial adhesion. Adhesion of sickle blood cells, from left to right: Sickle erythrocyte binds to endothelium via different adhesion molecules, it also attaches to activated platelet by ICAM-4 on the membrane of sickle erythrocyte. Reticulocytes also attaches endothelium through multi adhesive molecules. The large neutrophil attach to reticulocyte is also done by different adhesion molecules (Piccin et al., 2019).

1.4.3. Inflammation

The initial attachment of sickle RBCs to the endothelium of the blood vessels is largely mediated by adhesion molecules such as complement components and selectins, as well as circulating substances such as

chemokines, histamine, thrombin, cytokines and heme. Tumor necrosis factor-α (TNF-α), interleukin-6 IL-6, IL-17, and IL-8 are examples of inflammatory mediators have been demonstrated to be markedly elevated in SCA patients (Keikhaei et al., 2013), (Darbari et al., 2020; Steinberg, 2008a).

1.4.4. Hemolysis, Nitric Oxide and Endothelial Dysfunction

When RBCs deformability is damaged by sickling, the membrane of RBCs is disrupted polymers of free-Hb releasing directly into circulation, additional to ferric hemoglobin. Free plasma hemoglobin (Hb) may lower nitric oxide (NO) levels in the blood. This is more commonly referred to as the "free-Hb scavenging effect," in which Hb converts NO into nitrate (NO^{3-}). Comparably, the release of arginase from damaged red blood cells lowers plasma arginine levels, which is a crucial precursor to NO. This lowers NO levels even more and damages the processes of vasodilation that are sustained by NO. Moreover, platelet aggregation and vasoconstriction are facilitated by NO deficiency. Ferric hemoglobin causes pro-inflammatory damage that exacerbates endothelial dysfunction and encourages vaso-occlusion. All of these hemolysis-induced processes combined account for some of the vaso-occlusion. Vasoconstriction is exacerbated by hypoxia and decreased NO bioavailability (Piccin et al., 2019; Steinberg, 2008b).

1.5 The Clinical Hallmarks of Sickle Cell Anemia

The two main clinical features of sickle cell anemia are hemolysis and vascular occlusive symptoms. Vaso-occlusion causes multiple major organ system issues, including lifelong disability and even death, as well as recurrent painful episodes (formerly known as sickle cell crisis). Gallstones

with persistent anemia usually come by the hemolysis of red blood cells (Costa and Fertrin, 2016).

1.6 Clinical Manifestations and Complications

SCA is described by manifestations ranging from acute pain to very early onset stroke, ulcers of leg and the risk of earliest deaths from failure of multi-organ. Due to the influence of HbF cause clinical manifestations do not appear until the second or third quarter of the first year after birth, when adult hemoglobin has mostly replaced its.

Vaso-Occlusive Crisis (Pain crisis): The definition of a painful vaso-occlusive crisis (VOC) is a sudden attack of acute, persistent pain. The pain may be localized to a specific area or may be coupled with other complications like acute chest syndrome (ACS). The extremities, joints and lower back are common locations for pain (Brandow and Liem, 2022). Acute pain episodes, also known as sickle cell pain crises or VOC are the main cause of hospitalization in about 95% of people with sickle cell anemia and are also the majority of presenting morbidity (Ballas & Lusardi, 2005a). Moreover, ACS and VOC frequency are the most common indicators of mortality in SCA patients (Platt et al., 1994). The incidence of pain may have a higher effect on quality of life than cumulative organ damage because recurrent episodes have a considerable impact on quality of life (Van Tuijn et al., 2010).

Vaso-occlusive crises frequently have a one-to-two-day prodromal phase before the pain peaks on day three and lasts until day six or day seven, at which point it resolves. Adult hospital stays typically last between nine and eleven days. Generally, the frequency of VOC resulting in hospitalizations is increased with increasing age and lower fetal hemoglobin (Darbari et al., 2020).

Acute chest syndrome, in SCA patients, ACS increases the likelihood of respiratory failure, the onset of chronic lung disease and finally death. It is considered as the second most frequent reason for SCA patients to be admitted to the hospital (Tebbi, 2022).

Additional to others of clinical manifestations like anemia and infection beside to other complications. Many chronic complications affect older children, adolescents, and young adults: priapism, leg ulcers, stroke, avascular necrosis (of the humeral or femoral head), pulmonary hypertension, chronic pain, acute kidney injury, retinopathy, chronic kidney disease, hepatic sequestration and thromboembolic events as deep vein thrombosis (DVT) and pulmonary embolism (PE). cholecystitis and cholelithiasis (gallstones) are caused by the excessive formation and precipitation of bilirubin as a result of hemolysis. SCA also raises the risk of complications during pregnancy (Inusa et al., 2019; Tebbi, 2022).These complications come at early life, but appear more with advancing age. Many factors like fever, cold weather, infections and stress contribute the complications. Almost of the therapy directed intended towards preventions or reducing sickling, and reduction in clinical complications of SCA (El-Hazmi et al., 2011; Sundd et al., 2019).

1.7 Pattern of Inheritance of Hemoglobin Disorders

An autosomal recessive (AR) condition is caused by defective hemoglobin, in which the offspring inherits the defective genes from their carrier parents. The probability of getting homozygous HbSS (sickle cell anemia, or SCA) is 25% if both parents are heterozygotes for HbS as illustrate in Figure (1-4), shown the all the probabilities which may be occur:

•There is a 50% risk that the child may inherit sickle cell trait. (one is sickling cell gene; one is normal gene).

• There is a 25% risk that the child may not inherit sickle cell trait. (two genes normal).

•There is a 25% risk that the child may inherit sickle cell anemia (two genes of sickle cell).

The majority of heterozygotes are asymptomatic carriers (traits) (El-Hazmi et al., 2011).

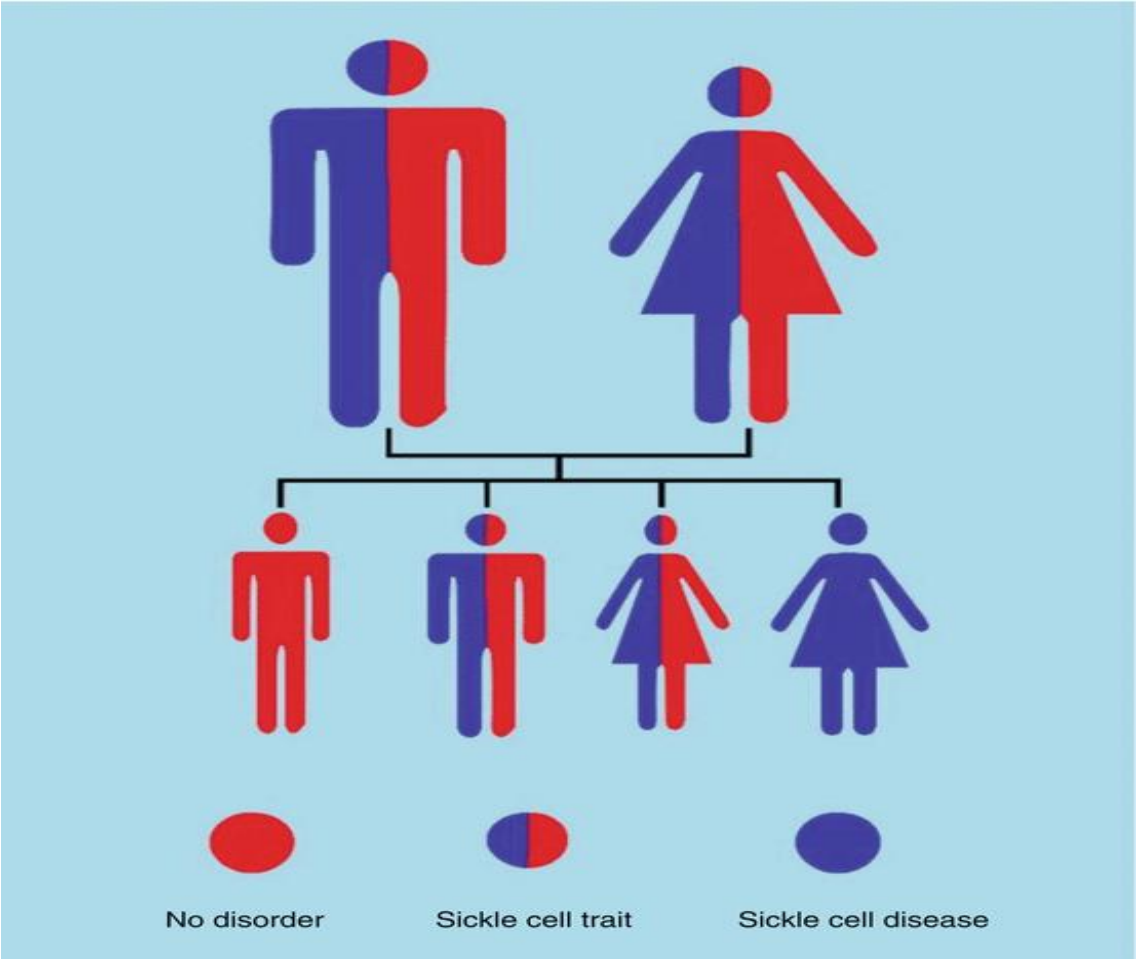


Figure 1-4: Diagrammatic illustration of inheritance in the case where sickle cell trait is present in both parents *(Genetics and Pathophysiology of Sickle Cell Anemia _ Oncohema Key, n.d.).*

1.8 Fetal Hemoglobin (HbF)

Fetal hemoglobin is the predominant hemoglobin found in fetuses during their embryonic development. It is synthesized by erythroid precursor cells starting around the 10th to 12th week of pregnancy and continues to be produced during the first six months of postnatal life. HbF consists of two alpha and two gamma subunits. It plays a crucial role in facilitating the transfer of oxygen from the maternal bloodstream to the fetal circulation during pregnancy and it exhibits a high affinity for oxygen but a reduced affinity for 2,3-bisphosphoglycerate (2,3-DPG) when compared to HbA, 2,3-DPG plays a crucial role in proper oxygen unloading .The properties of HbF find a valuable medical application in the management of SCA (Kaufman et al., 2023).

In individuals with SCA, the presence of HbF in their hemoglobin composition typically ranges from 2% to 20%, which varies depending on various patient-specific factors, sufficient HbF in each erythrocyte can prevent deoxyHbS polymerization and abrogate the tissue injury and hemolytic anemia. HbF inhibits HbS polymerization because it is not incorporated into the polymer phase. The concentrations of HbF vary among patients as well as the distribution of HbF-containing cells and the amount of HbF per F-cell, F-cells are subgroup of RBCs that containing HbF. The main target of HbF-inducing therapy is to reach HbF levels in every sickle erythrocyte enough to inhibit or highly retard HbS polymerization. Although any increase in HbF is clinically beneficial, at least 20% HbF in all erythrocytes should cure most features of the disease. Remarkably, the pharmaceutical drug Hydroxyurea (HU) has been shown to increase the fraction of HbF in patients with SCA and effectively reduces the need for blood transfusions (Kaufman et al., 2023),(Maier-

Redelsperger et al. 1994 ; Brittenham et al. 1985),(Ferreira Costa, n.d.),(Steinberg, 2008b).

1.9 Treatment Strategies of Sickle Cell Anemia

The recent advances therapy of SCA include the following :

1.9.1 Targeting Hemoglobin S Polymerization

- Hydroxyurea (HU) acts via induction synthesis fetal hemoglobin (HbF).

- Voxelotor reduces the tendency to sickle by binding selectively to the N-terminus of the alpha subunit of HbS, stabilizing the oxygenated hemoglobin state (Inusa et al., 2019a).

1.9.2 Targeting Vaso occlusion:

- Crizanlizumab is a monoclonal antibody that targets P-selectin; it works by preventing activated platelets, neutrophils, and erythrocytes from adhering.

- L-Glutamine reduces endothelial adhesion(Salinas Cisneros and Thein, 2020).

1.9.3 Targeting Inflammation

-The anti-inflammatory effects of Statins and intravenous immunoglobulin (IVIG) on monocyte and neutrophils adhesion have been investigated. C-reactive protein, soluble ICAM1, soluble E-selectin, and vascular endothelial development were all lower in statin-using patients. It was discovered that Simvastatin decreased white blood cell adhesion (Salinas Cisneros & Thein, 2020).

1.9.4 Modifying the Patient's Genotype

-Allogeneic stem cell transplant

-Autologous transplant

1. Gene therapy is strategies of Lentiviral: Inducing of fetal hemoglobin
2. Gene editing uses transcription activator-like effector nucleases (TALENs), CRISPR/Cas9, and zinc finger nucleosomes (ZFN) techniques (Inusa et al., 2019a; Salinas Cisneros & Thein, 2020).

1.10 Hydroxyurea (HU)

1.10.1 General View

Hydroxyurea or Hydroxycarbamide is chemotherapy that inhibits DNA synthesis through inhibition ribonucleotide reductase enzyme which convert ribonucleoside di-phosphate to deoxyribonucleotide tri-phosphate which consider the cornerstone in DNA repair and synthesis. It received approval in 1999 by US of Food and Drug Administration for use in the treatment of sickle cell anima due to its ability to reduction the sickling of red blood cells, making it an effective treatment option for this condition (Yahouédéhou et al., 2018).

In SCA, the main effect of HU occurs through increasing HbF production and decreasing Hb S synthesis. Beside other effects which include reduction the number of platelets, neutrophils and reticulocytes in circulation, reduction the expression of adhesion molecules on surface of RBCs, neutrophils and endothelium of vascular. Additionally, it increases the size of RBCs and enhances cellular deformability that leads to

improvement in blood flow and reduction of vaso-occlusion. Moreover, it works as donor of NO (Inusa et al., 2019; Piccin et al., 2019).

1.10.2 The Mechanism of Action of Hydroxyurea

1.10.2.1 Increased HbF Production

Hydroxyurea causes a change in gene expression at the beta-globin locus so that expression from the gamma-globin locus is increased compared to expression from the beta-globin locus. This is the opposite of the normal fetal switch, resulting in increased formation of fetal hemoglobin (HbF: 2α2γ) and decreased formation of adult hemoglobin (HbA: 2α2β). Since the sickle mutation does not impact the gamma globin chain, the overall consequence in SCA patients is a reduction in the relative concentration of hemoglobin S (HbS; 2α2βS). HbF production increases as a result of HU to at least twice the starting value and frequently much more. There is an increase in HbF concentration per cell, HbF-containing cell percentage, and HbF overall percentage. As a consequence, drop of intracellular concentration of HbS result in less frequently hemoglobin polymerizes and precipitates. These results decrease the sickling, also increases red blood cells vitality and their hydration improves as well as reduced of hemolysis and cell adhesion to the vascular endothelium. Therefore it improves microcirculation blood flow and reduces the risk of occlusive events. This is believed to be the primary mechanism by which HU reduces the risk of vascular occlusion. Hospitalization, acute chest syndrome, fever and vasoconstrictor pain were dramatically reduced in children with SCA (HbF > 20%) in a prospective observational trial involving 230 patients receiving HU treatment (Goldberg et al., 1990; Rodgers et al., 1990).

Although the exact mechanism(s) by which hydroxyurea promotes the production of HbF is unknown, it may involve impacts on gene transcription, cell signaling, and epigenetic alteration (Pule et al., 2015). Through genes that control fetal hemoglobin transcription and translation, such as BCL11A, a crucial regulator of baseline HbF levels. Elevated nitric oxide (NO) levels and cyclic nucleotides like guanylyl cyclase may increase HbF transcription (Cokic et al., 2003).

1.10.2.2 Other Mechanisms

Although lowering sickle blood levels through high HbF levels is the main beneficial effect of hydroxyurea in SCA, other mechanisms may also play a role:

●Nitric oxide -HU may raise levels of nitric oxide (NO) through two different mechanisms. Hemolysis releases free hemoglobin, which acts as a natural NO scavenger. Because HU lowers hemolysis, it may also lower free hemoglobin, which raises NO levels. In RBCs and endothelial cells, Hydroxyurea induces the creation of NO intracellularly by a variety of pathways, including direct NO generation through interactions with heme proteins, moreover, HU metabolism releases nitric oxide directly which might contribute also to local vasodilation. Increases in NO may improve blood flow in specific circulatory beds, such as the pulmonary vasculature. NO is a strong vasodilator, and its depletion in some vascular beds may contribute to vaso-occlusion (Gladwin et al., 2002).

●Red blood cells rheology – Independent of Hb F levels, the decreased reticulocyte count and accompanying shift to more mature red blood cells may also can enhance RBC volume, adhesion, density, and passage via the microcirculation. This could be partly explained by a decrease in the percentage of young, low-density RBCs and reticulocytes, which are more

likely to stick to the vascular endothelium. Additionally, Hydroxyurea's effects on cellular signaling pathways may lessen adhesion (Bridges et al., 1996), (Orringer et al., 1991).

●White blood cells – Reductions in neutrophil adhesion to the vascular endothelium and/or white blood cell (WBC) numbers may also be factors in decreased vaso-occlusion (Ware, 2010).

1.10.3 Pharmacokinetics

Absorption: Readily absorbed ≥80%. Distribution: extensively is distributed into the tissues including into the brain. V_d: approximately total body water and concentrates in erythrocytes and leukocytes. Metabolism: Up to 60% via hepatic metabolism and intestinal bacteria's urease. Half-life elimination: 3 to 4 hours. Excretion: in urine (50%-80% as unchanged drug, additional to 30% as urea) (Lexi-Comp, 2010).

1.10.4 Drug Interactions

1.10.4.1 Increased Effect/Toxicity

-HU may increase the effects/levels of Didanosine; Natlizumab, Vaccines (live).

-The effects/levels of HU may be increased by Tacrolimus; Pimecrolimus, Topical Trastuzumab (Lexi-Comp, 2010).

1.10.4.2 Decreased Effect/Toxicity

-HU may decrease the effects/levels of Vaccines (live), Vaccines (inactivated).

- The effects/levels of HU may be decreased by Echinacea (Lexi-Comp, 2010).

1.10.5 Uses

-The management of chronic myeloid leukemia.

-Neck and head cancer management.

-In the management of SCA, reducing the number of painful crises and reducing the requirement for blood transfusions in patients who frequently experience moderate to severe painful crises are the main goals (Costa & Fertrin, 2016; Lexi-Comp, 2010).

1.10.6 Indications in Sickle Cell Anemia Patients

In adults, the indications for HU may include:

1. More than three painful episodes per year or persistent, crippling pain that is not managed according to standard protocol.
2. Stroke history, heightened risk of stroke, or other serious vaso-occlusive events.
3. Severe symptoms of anemia.
4. Acute chest syndrome history (Inusa et al., 2019a).

1.10.7 Dosing and Monitoring

The adult dose ranges between 10mg/kg/day to maximum 35mg/kg/day. The primary, patient-important therapeutic outcome of Hydroxyurea therapy is the reduction of vaso-occlusive pain episodes and other vaso-occlusive consequences. The main hazard that limits dosage is bone marrow suppression. Patients on HU undergo regularly monitoring

for the develop of anemia, neutropenia or thrombocytopenia (Inusa et al., 2019a). CBC, reticulocyte count test, platelet count and hemoglobin all of them monitoring, if toxicity occurs, hold HU until the recovers of bone marrow. Toxic range: platelet count < 80,000/μL, neutrophil <2000 cells//μL, hemoglobin<4.5g/dl.(Lexi-Comp, 2010), reticulocyte <80,000//μL (Costa & Fertrin, 2016; McGann et al., 2019).

1.10.8 Predictors of Response

Because HbF response to HU is variable and deeply complex, there is no distinct characteristic that can be utilized to predict the degree of clinical benefit or whether a patient receiving Hydroxyurea would expose any benefit at all. Despite there are many studies shown efficacy of HU in patients with SCA as it significantly reduced the incidence of acute painful events, the frequency of hospitalization, the requirement for blood transfusion and acute chest syndrome. However, there is still variation in the HU response between patients with SCA, which may be brought on by genetic, physiological, environmental, socioeconomic or metabolic factors. HU metabolism and transporter pathways suggested as important markers for HU response, which will facilitate pharmacogenomic studies and help in identification of candidate genes for predicting HU response(Yahouédéhou et al., 2018), (Steinberg, 2008b).

1.11 Hydroxyurea as a Substrate for Drug Transporter

1.11.1 Solute Carrier

The solute carrier (SLC) gene superfamily consists of membrane transport proteins that are absolutely crucial function in the pharmacokinetics and pharmacodynamics of numerous drugs. These proteins affect the absorption, metabolism, distribution, and elimination of drugs (Kitamura et al., 2008; Oostendorp et al., 2009). While SLC transporters are found throughout the body, their expression in the kidneys and liver is particularly influential in drug pharmacology (Grover & Benet, 2009).

1.11.2 Urea Transporters

Urea transporters (UT) belong to the SLC superfamily; it transfers endogenous urea through cell membranes, and also plays crucial role in recycling of urea, concentration of urine and function of kidney(Shayakul and Hediger, 2004),(Walker et al., 2011). Hydroxyurea considers as substrate for some SLC transporters as UTA and UTB and its movement across them is bidirectional i.e. in both the apical-basal and basal-apical directions occur via facilitated diffusion and significantly inhibits by competitive substrates such as dimethylurea.

1.12 Pharmacogenomics of Hydroxyurea

1.12.1 The Role of Genetic Polymorphisms of UTB rs2298720 G>A And UTB rs12605147 A>G on Hydroxyurea Response

The UTB gene, also known as SLC14A1, is situated on chromosome 18 (18q12.3) and spans approximately 13 kb. It encodes a urea transporter primarily involved in urea transport across cell membranes, playing significant physiological roles in erythroid and renal tissues. This transporter gene is located on the sense strand and contains both intronic and exonic regions, with rs2298720 and rs12605147 polymorphisms found within intronic regions, potentially affecting gene regulation rather than protein coding directly (Walker, Franke, et al., 2011)..

The presence of polymorphisms in UTB, particularly the intron variants rs2298720 (G>A) and rs12605147 (A>G), may significantly influence hydroxyurea (HU) pharmacokinetics and pharmacodynamics, given the transporter's role in HU distribution and elimination. Specifically, HU's biodistribution is influenced by UTB's expression in multiple tissues, including high levels in erythroid cells, peripheral blood, bone marrow, and renal tissues. The widespread expression of UTB, particularly in these tissues, suggests its crucial role in HU distribution across physiological compartments, including the central nervous system and breast milk. As HU is primarily cleared by the kidneys, the presence of renal UTB transporters may also enhance drug elimination (Wagner et al., 2002).

Research indicates that UTB genetic polymorphisms, notably rs2298720, could alter HU's pharmacodynamic outcomes in sickle cell anemia (SCA) treatment by modulating the therapeutic increase in fetal hemoglobin (HbF), which is a primary goal of HU therapy. Variants in UTB have been associated with diverse clinical outcomes, including HbF levels, myelosuppression risk, and overall HU efficacy. These genetic insights suggest UTB polymorphisms as potential biomarkers for optimizing HU therapy in SCD, helping to predict patient response and minimize adverse effects, ultimately supporting personalized treatment strategies (Tozatto-Maio et al., 2020).

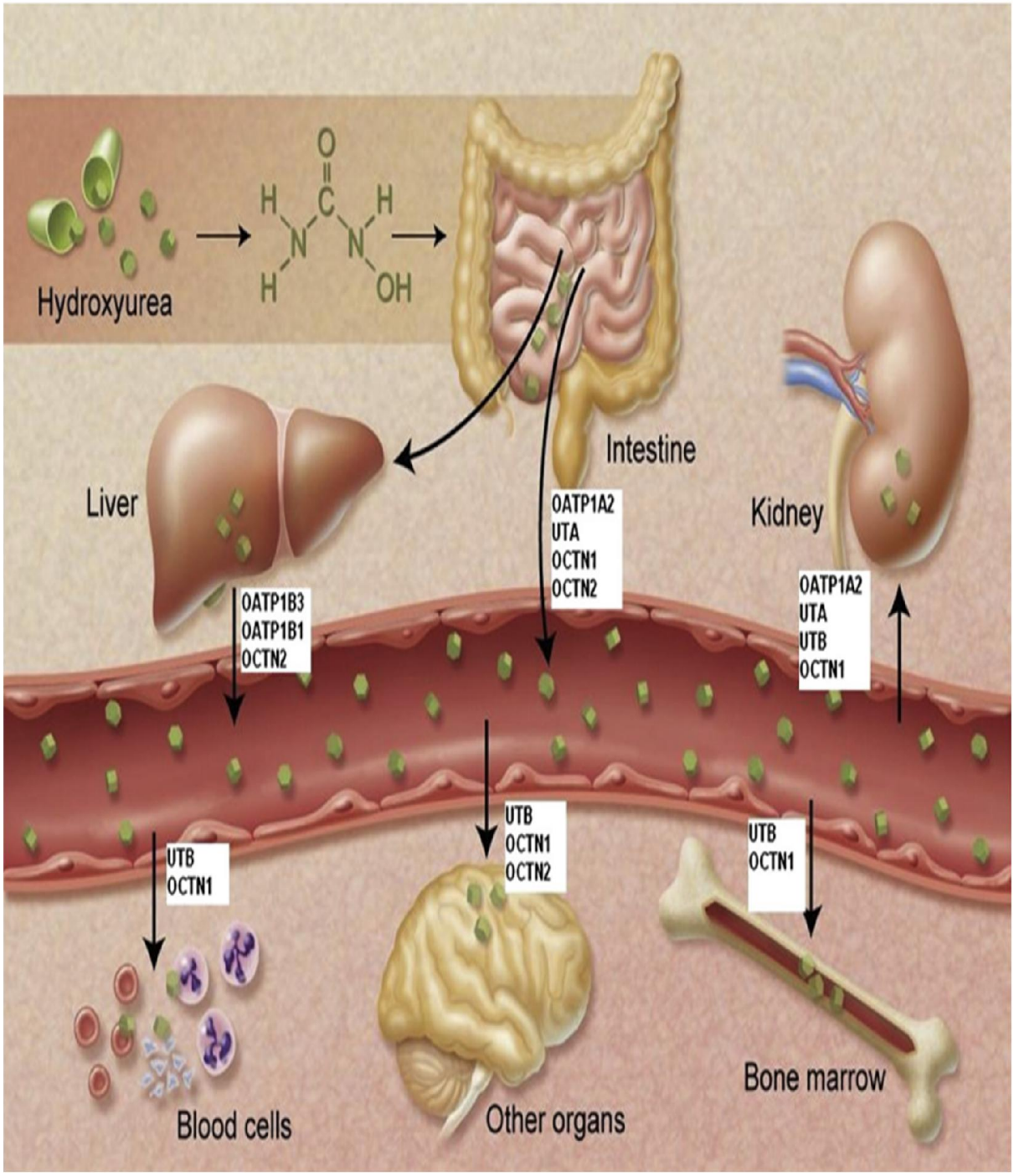


Figure1-5: Hydroxyurea in vivo movement and UTB distribution among different compartments and organs (Walker et al., 2011).

1.13 Objectives

1- To investigate the distribution genotypes of UTB transporter in the patients with sickle cell anemia on Hydroxyurea therapy.

2- To investigate the effects of UTB-genetic polymorphisms on the efficacy and safety of Hydroxyurea in sickle cell anemia patients.

3- To study the association between genetic polymorphism and clinical outcomes.

Chapter Two

Patients, Materials & Methods

2. Materials, patients and methods

The following presents the materials, patients, and methods were used in the current study.

2.1 Materials

2.1.1 Instruments

The instruments used in this investigation are given in the table (2-1) along with their manufacturers and places of origin.

Table (2-1): Instruments used in this study with their manufactures and origins .

Instrument	Manufacture	Origin
CBC machine	SYSMEX XN-350	Japan
Centrifuge	SIGMA	Germany
Distillator	GFL	Germany
Electrophoresis apparatus	TECHNE ME	England
Freezer (-20 ˚C)	TOST	Philippine
Hood	LabTech	Korea
Hot plate Stirrer	LabTech	Korea
iPhone Camera	Apple	USA
Micropipettes	SLAMMED	Japan
Nanodrop	Bio Drop	England
PCR machine (Thermocycler)	Verity	USA
Sensitive balance	DENVER	Germany
UV-transilluminator	Syngene	England
Vortex mixer	Human Twist	Germany
Water bath	LabTech	Korea

2.1.2 Chemicals and Kits

Table (2-2) contains a list of the specific compounds and kits utilized in this investigation.

Table (2-2): Kits and chemicals used in this study with their Manufactures and origins .

Kits and Chemicals	Manufacture	Origin
100 bp DNA ladder	Bioneer	Korea
10X TBE (Tris-Borate-EDTA) Buffer	iNtRON	Korea
Absolute ethanol	BDH	Germany
AccuPower® PCR PreMix	Bioneer	Korea
Agarose D1 low EEO	CONDA	Spain
BIO-RAD D-10 Electrophoresis Kit	BIO-RAD D-10	France
CBC kit	Sysmex XN-350	Japan
Ethidium bromide	iNtRON	Korea
Geneaid for blood genomic DNA extraction kit	Geneaid	Taiwan
Nuclease free water	Promega	USA
Primers	Macrogen	Korea
Reticulocyte stain	Bio research	Germany

2.2 Patients

2.2.1 Study Population

This cross-sectional observational study was conducted from August 2023 to November 2024 at the Inherited Hematological Center disorders in Karbala at Karbala Teaching Hospital for Children, Al-Najaf at Al-

Zahraa Teaching Hospital, and Basra at Al-Basrah Children's Hospital. The Scientific and Ethical Committee of Pharmacy College in the University of Kerbala accepted protocol of the study, and each subject signed an informed consent form after being fully informed about the nature of studes and objectives. Fifty patients, both male and female, ranging in age from 14 to 65 years, participated in the trial. They took 10–20 mg/kg of hydroxyurea orally every day.

2.2.1.1 Inclusion Criteria

The inclusion criteria included the fallowing: all patients with Hb SS and good medication compliance, and all males and females had been receiving HU as monotherapy for at least six months prior to the commencement of the trial.

2.2.1.2 Exclusion Criteria

The patients who met the exclusion criteria were those who had thalassemia and HbSS, began HU therapy concurrently, and had received a blood transfusion within the last six months, or were on non-compliance therapy. Additionally, patients taking medications like L-glutamine that alter pharmacokinetics or pharmacodynamics.

2.2.2 Clinical Data Collection

To ensure that all potentially interfering medications were recorded in the database, each patient was asked if she/he had consumed any drugs that might impact the activity of the transporter or interfere with HU metabolism, at the time the blood sample was taken. The following information was gathered from the consenting male and female patients' medical records as well as from the patients themselves: age, weight, the date that HU therapy was started, medication compliance,

and the most recent blood transfusion needed. In addition, the frequency and severity of pain crises, the length of each hospital stay, fever, ICU admission, acute chest syndrome, and the need for blood transfusions while receiving HU therapy

2.2.3 Sample Collection and Analysis

Following the approval of the College of Pharmacy Scientific and Ethical Committee at the University of Kerbala, blood samples were drawn from qualified patients who had provided signed informed consent. Every patient who participated in this study had 5 milliliters of venous blood taken from them. A two milliliter vial containing blood was used for the genetic analysis. A further 3 ml was added to an EDTA tube for hematological and biochemical examination, which included the Hb-electrophoresis test, reticulocyte count test, and CBC test.

2.3 Methods

2.3.1 Molecular Analysis

2.3.1.1 DNA Extraction

Genomic DNA was extracted from blood sample as stated by the protocol Genomic DNA Mini Kit for blood genomic DNA extraction kit. The following method is suitable for DNA isolation from blood :

1. Transferred 200 µl of blood to a 1.5 ml microcentrifuge tube.
2. Added 20 µl of Proteinase K (make sure ddH2 O was added) then mixed by vortex for 10 seconds.
3. Incubated at 60ºC for at least 10 minutes. During incubation inverted the tube every 3 minutes

4. Step 2 Lysis. Added 200 µl of GB Buffer to the sample and mixed by vortex for 10 seconds.
5. Incubated at 70ºC for at least 10 minutes to ensure the sample lysate is clear. During incubation, inverted the tube every 3 minutes.
6. At this time, pre-heated the required Elution Buffer (200 µl per sample) to 70ºC (for step 5 DNA Elution).
7. DNA Binding, added 200 µl of absolute ethanol to the sample lysate and mixed IMMEDIATELY by shaking vigorously. If precipitate appears, break it up as much as possible with a pipette.
8. Placed a GD Column in a 2 ml Collection Tube. Transferred mixture (including any insoluble precipitate) to the GD Column then centrifuged at 14-16,000 x g for 2 minutes.
9. Discarded the 2 ml Collection Tube containing the flow-through then placed the GD Column in a new 2 ml Collection Tube.
10. Wash, added 400 µl of W1 Buffer to the GD Column.
11. Centrifuged at 14-16,000 x g for 30 seconds then discard the flow-through.
12. Placed the GD Column back in the 2 ml Collection Tube. Added 600 µl of Wash Buffer (make sure ethanol was added) to the GD Column.
13. Centrifuged at 14-16,000 x g for 30 seconds then discarded the flow-through.
14. Placed the GD Column back in the 2 ml Collection Tube. Centrifuged again for 3 minutes at 14-16,000 x g to dry the column matrix.
15. Elution Standard elution volume is 100 µl. If less sample is to be used, reduced the elution volume (30-50 µl) to increased DNA concentration. If higher DNA yield is required, repeated the DNA

elution step to increased DNA recovery and the total elution volume to approximately 200 µl.

16. DNA eluted in water should be stored at -20ºC to avoid degradation

2.3.1.2. Determination of Purity and Concentration of DNA

The Nano Drop, a Nano-spectrophotometer, was used to assess the concentration and purity of DNA. The A260/A280 ratio was used to calculate the DNA purity. A single microliter of DNA was applied to the nanodrop's micro detector. The instrument's concentration and purity were recorded.

2.3.2. Primers Design

The primers were created using Primer-BLAST software, which dictated how they should be made. The position of UTBrs2298720 G>A and UTB rs12605147A>G at chromosome 18, and the gene consequence is SLC14A1 intron variant as presented in appendix. Tables (2–3) and (2-4) display the primer sequences that were used for the UTB gene amplification analysis for SNP identification.

Table (2-3): Primers sequences of SNPs (rs12605147 A>G) with their product sizes.

Rs12605147 A>G	Sequence (5'->3')	Product length
Forward primer	TGTGCTGTTGGCTGTTCT	985bp
Reverse allele A	GCCTGGTTACAGTAGAAGAt	
Reverse allele G	GCCTGGTTACAGTAGAAGAc	

Table (2-4): Primers sequences of SNPs (rs2298720 G>A) with their product sizes.

rs2298720 G>A	Sequence (5'->3')	Product length
Forward primer	GCCCAAAGCTGGCTAATCTG	237bp
Reverse allele G	TTAAGCTGGTTGGCAAGTTc	
Reverse allele A	TTAAGCTGGTTGGCAAGTTt	

As directed by the manufacturer, lyophilized primers were dissolved in a specific amount of nuclease-free water to yield a concentration of 100 pmol/µl, which is equivalent to a stock solution. To acquire 10 pmol/µl as the final concentration for the working solution, 10 µl of stock solution was diluted with 90 µl of nuclease-free water. Before being used, the stock and working solutions were stored at -20 ℃.

2.3.3 Polymerase Chain Reaction (PCR)

Primer-BLAST program was used in this study to design primer for the following SNPs UTB rs12605147 and UTB rs2298720 of the UTB gene. Allele Specific-PCR method was employed for this purpose.

2.3.3.1 Optimization of the PCR Conditions

After conducting several trials, the PCR was optimized to determine the ideal primer concentration and annealing temperature. The optimal circumstances that yielded the greatest outcomes for the detection of UTB rs12605147 and UTB rs2298720 are displayed in tables (2-5), (2-6), (2-7) and (2-8) correspondingly.

2.3.3.2 Running the PCR

The PCR premix formula, as indicated in tables (2–5) and (2–6), was used to create the PCR mixture.

(2-5): PCR reaction mixture to amplify a DNA fragment encompasses rs12605147 A>G SNP

Component	Volume (µl)
Forward primer	0.5
Reverse primer	0.5
DNA sample	3
Accupower® PCR PreMix	5
Nuclease free water	16
(total volume)	25

Table (2-6): PCR reaction mixture to amplify a DNA fragment encompasses rs2298720 G>A SNP

Component	Volume (µl)
Forward primer	2
Reverse primer	2
DNA sample	3
Accupower® PCR PreMix	5
Nuclease free water	13
(total volume)	25

In the current study, the thermal program for detecting UTB rs12605147 is demonstrated in table (2-7).

Table (2-7): PCR program for detecting rs12605147 A>G SNP.

Steps	Temperature(ºC)	Minute: second	Cycles
Initial denaturation	95	03:00	1
Denaturation	95	00:30	32
Annealing	59	00:40	
Extension	72	00:40	
Final extension	72	05:00	1

The thermal program for detecting UTB rs2298720 is demonstrated in Table (2-8).

Table (2-8): PCR program for detecting rs2298720 G>A SNP.

Steps	Temperature(ºC)	Minute: second	Cycles
Initial denaturation	95	03:00	1
Denaturation	95	00:30	32
Annealing	65	00:40	
Extension	72	00:40	
Final extension	72	05:00	1

2.3.3.3 Agarose Gel Electrophoresis

1. To create the agarose gel, 1.5g of agarose powder were dissolved in 100ml of 1x TBE buffer (PH 8).
2. Bring the mixture to a boil on a heated plate.
3. After allowing the mixture to cool, 4 µl of ethidium bromide was added.

4. To create wells for the loading of PCR products, the comb was fastened at the end of the tray.
5. After carefully pouring the agarose into the tray, it was left to harden for 30 minutes at room temperature.
6. The comb was carefully taken out of the tray.
7. A TBE buffer was added to the tray, which was then fixed in an electrophoresis chamber.
8. The PCR products were put straight into the wells.
9. The voltage of the electrophoresis device was adjusted to produce an electrical field of 5 volts for every cm that separated the cathode and anode.
10. Band detection was done using a UV trans-illuminator set to 320–336 nm at the conclusion of the run, or around 60 minutes.
11. A digital camera was used to take pictures of the gel.(Fischbach & Dunning III, 2013)

2.3.4 Hematology Parameters

2.3.4.1 Complete Blood Count:

An automated hematology analyzer typically performs the complete blood count (CBC), counting cells and gathering data on their size and structure. Hematology Analyzers are used in accordance with manufacturer instructions to measure hemoglobin concentration and generate red blood cell indices from measurements of red blood cell (RBC), white blood cell (WBC), hemoglobin, and platelet counts.

One blood cell at a time may fit through the tiny hole used to transmit blood between two electrodes. The impedance fluctuates while a cell passes through. Impedance changes correlate with cell volume, allowing for the calculation of both cell count and volume.

For every participant, the following blood tests were performed: Red cell distribution width (RDW), mean platelet volume (MPV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet distribution width (PDW), and white blood cell differential.

2.3.4.2 Reticulocyte Count Test Procedure

Immature red blood cells (RBCs) known as reticulocytes have significant amounts of mitochondrial and ribosomal RNA. Peripheral blood typically contains a modest number of reticulocytes that remain there for 24 to 48 hours while they mature.

2.3.4.2.A. Sample Required

The EDTA blood is the best sample.

2.3.4.2.B. Principle

The ability of ribosomal RNA to react with an isotonic solution of a supravital stain, such as brilliant cresyl blue or new methylene blue, is the basis for the reticulocyte count. Stains that bleed into living things are known as supravital stains. Hence, reticulocytes should be fixed in order to detect ribosomal RNA. After adding stain, blood is incubated. The reticulum, or dark blue network, is the result of the RNA precipitating in the cell. A blood smear is created and looked at under a microscope. Since a direct count is not feasible, the percentage of RBC is obtained by comparing the relative count to the total number of RBCs.

2.3.4.2.C. Requirements Specimen

EDTA whole blood/capillary blood

2.3.4.2.D. Reagent

- New Methylene Blue

New methylene blue = 1.0 gm Sodium citrate = 0.6 gm Sodium chloride = 0.7 gm Distilled water = 100 ml

2.3.4.2.E. Procedure

1. Fill a test tube with 2-3 drops of dye solution.
2. Mix with 2-4 drops of thoroughly blended blood sample.
3. Close the tube and let it sit at 37°C for ten to fifteen minutes.
4. After incubation, thoroughly combine and create a thin, blood-stained smear.
5. After the films are dry, look at them without fixing or counterstaining.
6. Count one thousand RBCs and record how many reticulocytes are present. In reticulocytes, there will be a dark-blue reticulum or network.In.(Fischbach & Dunning III, 2013)

2.3.3.2.F. Calculation

1. Reticulocyte Percentage

This is the percentage of reticulocytes per 1000 RBCs.

2. Absolute Reticulocyte Count

The absolute reticulocyte count (ARC) is the precise amount of reticulocytes in one milliliter of whole blood. (Fischbach & Dunning III, 2013)

$$\text{Absolute Reticulocyte Count} = \frac{\text{Reticulocyte (\%)} \times \text{RBC Count } (10^{12}/\text{L})}{100}$$

2.3.4.2.G. Normal Values:

Adult = 0.5 to 1.5 % of total RBCs counted.

2.3.4.3 Hemoglobin Electrophoresis Test

The hemoglobin electrophoresis test is a diagnostic laboratory technique that aids in the diagnosis and characterization of hemoglobin disorders by separating and identifying various forms of hemoglobin in the blood. A modern method called capillary electrophoresis divides hemoglobin variants according to size and charge using a narrow capillary tube filled with a buffer solution. It provides excellent sensitivity and resolution.

2.3.4.3.A. Test Procedure of Hb Electrophoresis

-Prepare Electrophoresis Gel or Medium: A gel or alternative separation medium is set up in a dedicated electrophoresis chamber in advance of electrophoresis. Polyacrylamide, agarose, cellulose acetate, or other substances could be used to create this medium.

1. **Load Sample:** after filled the designated wells with the prepared medium, and wait around 15-20min to cool down, the extracted hemoglobin sample was gently loaded.
2. **-Apply Electrical Current:** An electrical current is run through the gel via the electrodes in the electrophoresis- chamber. Hemoglobin molecules can flow in a sieve-like pattern through the gel based on their charge and size.
3. **-Separation:** hemoglobin migrates through the gel at various speed in response to the current. The migration speed allows the different types of hemoglobin to be spatially separated along the gel.

2.3.4.3.B. Staining and Visualization

-**Staining:** an appropriate dye or reagent was mixed with the gel following electrophoresis, which, when combined with hemoglobin, makes the bands or spots visible. Many dyes may be used, depending- on the particular gel medium and staining- method.

-Distaining: this step used to remove excess dye to be ready for visualization. In the visualization step, the bands or spots represent the different hemoglobin variants- that are present in the sample.

2.3.4.3.C. Analysis

Interpretation: The types and relative concentrations of hemoglobin present are measured by visually inspecting and analyzing the stained and destained gel. On the gel, each hemoglobin variant is represented by a unique band or spot.(Fischbach & Dunning III, 2013; Hultin, 2012)

2.3.5 Measuring Intensity of Pain

In this study used the Visual Analog Scale (VAS) to gauge how severe pain crises were for patients receiving HU therapy. The VAS rates the intensity of pain on a scale of 0 to 10, with "no pain" denoting no pain at all to "worst pain." The innovative work of Hayes and Patterson served as the model for this scale. It used the VAS into the assessment methods to measure and quantify the pain intensity of the patents in a methodical manner. This study achieved numerical measures of pain intensity by using a consistent methodology. This method offered insightful information and a greater comprehension of the participants' experiences.

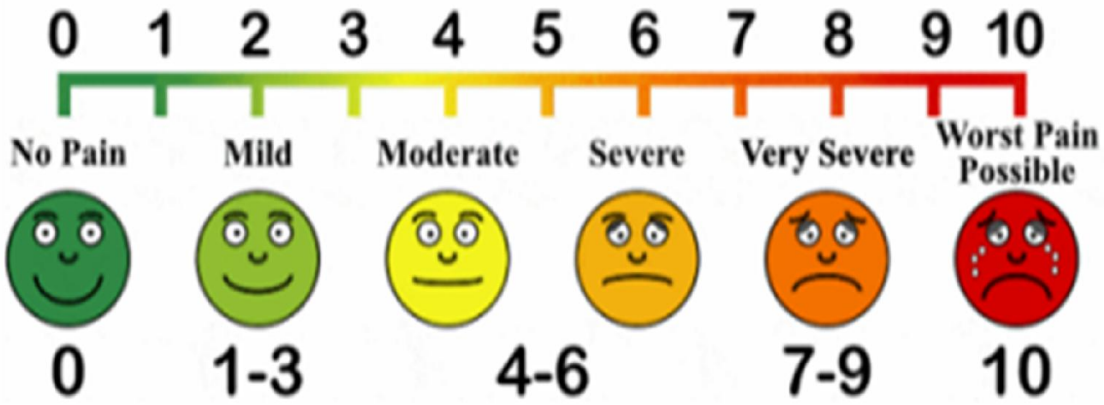


Figure (2-1): represents Visual analog scale VAS that used in this study to evaluates pain scores (Hong, 2019).

2.3.6 Measuring Frequency Of Painful Crisis

The patients undergoing HU treatment had been asked questions about the number of the painful crisis that they experienced through each year. The frequency of painful crises is determined as numbers (none, once, twice, thrice, and more than three times.

2.3.7 Statistical Analysis

The Statistical Package for the Social Sciences (SPSS version 26) was used to analyze the data gathered for this study, which were entered from patient sheets. In the relevant tables and graphs, the data were shown as frequencies and percentages or as mean and standard deviation. Where appropriate, the independent ANOVA (post hoc) analysis were employed. The observed values and the expected values were compared using the chi-square statistic. To ascertain whether there was a statistically significant difference between the observed and expected values, this test statistic was utilized. Additionally, to ascertain the relationship between the parameters

under investigation, a statistical Pearson correlation between the numerical was employed. Statistical association was considered significant when p value equal or less than 0.05 or 0.01 (P value $\leq$ 0.05 , 0.01).

Chapter Three

Results

3. Results

3.1 Patient's Demographic Information

The table (3-1) displays the demographic information for the patients who completed in this study. The total number of participants 50 patients involved both sex, male was 19 (38%) and female was 31(62%). The age range of sickle cell anemia patients was (14-65) years with mean±SD for male was (17.16± 4.902) and for female was (21.97±10.62). The mean±SD for body weight for male was(47.92±11.16) and for female was (51.87±12.57). According to guidelines of the treatment of SCA patients with HU, three types of doses are observed among participants, 22(44%) patients who administered 10mg/kg/day, 20(40%) patients are administered 15mg/kg/day and 8(16%) patients are administered 20mg/kg/day. In the 34(68%) patients who treated with HU there is no need for hospitalization annually, while the others 16(32%) require the hospitalization with interval times ranging between one to more than three days for admission that followed the discharge. Additionally, 34(68%)of patients were fever free while the remaining of percentage (32%) had been shown fever and their temperature was range from 38.1°C to above 39°C. Noted 40(80%) of participants were not required for blood transfusion. Regarding to pain intensity, category 2 (moderate) had more frequency among the other categories, it was 18(36%)patients while "sever" category was less frequency 6(12%)patients. The "No pain" category was the highest one 20(40%) in frequency of pain crisis, while "three times /year" was the lowest one 5(10%)patients.

Table1(3-1): Description of demographic information and disease features of the individuals under study (n=50)

Sex %	Male, N=19			38%
	Female, N=31			62%
Age (Years) Mean±SD	Male, N=19			17.16±4.902
	Female, N=31			21.97±10.62
BWt. (Kg) Mean±SD	Male, N=19			47.92±11.16
	Female, N=31			51.87±12.57
HU dose (mg/Kg/day), (N)%	10			(22), 44%
	15			(20), 40%
	20			(8), 16%
Frequency of hospitalization (N)%	0			(34), 68%
	1			(9), 18%
	2			(5), 10%
	3			(1), 2%
	4			(1), 2%
Duration of hospitalization (days)	0			(34), 68%
	2-4			(7), 14%
	>4			(9), 18%
Frequency of Fever	Non			(34), 68%
	Once			(6), 12%
	Twice			(5), 10%
	Thrice			(2), 4%
	More than three times			(3), 6%
Temp (N), %	36.5-37 °C			(34), 68%
	37.1-38 °C			(2), 4%
	38.1-39 °C			(13), 26%
	≥39 °C			(1), 2%
Frequency of blood transfusion (N), %	Non			(40), 80%
	Once			(6), 12%
	Twice			(2), 4%
	Three or more			(2), 4%
Intensity of pain (N), %	categories	0	No pain	(7), 14%
		1	Mild	(11), 22%
		2	moderate	(18), 36%
		3	Sever	(6), 12%
		4	Worst pain	(8), 16%
Frequency of pain (N), %	categories	0	Non	(20), 40%
		1	Once/year	(10), 20%
		2	Twice/year	(6), 12%
		3	Thrice/year	(5), 10%
		4	>3 times/year	(9), 18%

*(N)%, N refers to the patient's number and % refers to the percentage, (means± SD) refers to the means and Standard Deviation of the means.

3.2. Hematology Parameters

3.2.1. Recent Hb F% Levels

The recent Hb F% level in SCA patients is divided to two groups according to Hb F% response, involving responders who Hb F% value equal and more than 20, and non-responders who Hb F% less than 20, as shown in Table (3-2).

The total number of responders was 20 patients of both genders, as presentative 6(12%) males' patients with mean ± SD 28.07± 5.332, and 14(28%) females' patients with mean ± SD 22.92± 2.125, with statistically significant P value <0.0055. while the non-responder was 30 patients, 13(26%) males with mean ± SD 12.92± 4. 362, and 17(34%) females with mean ± SD12.36± 4.726, statistically not significant P value 0.7436, the Figure (3-1) shown distribution of both response groups between male and female.

Table(3-2): Recent HBF% in sickle cell patients grouped by gender and HbF% values

Recent HbF%	Patients gender		95%confidence interval	P value
	Male Mean ±SD	Female Mean ±SD		
Non-responder HbF<20, N=30	12.92± 4. 362	12.36± 4.726	-4.008 to 2.895	0.7436
	N=13 (26%)	N=17 (34%)		0.0007
Responder HbF≥20, N=20	28.07± 5.332	22.92± 2.125	0.4811 to 9.809	**<0.0055**
	N=6 (12%)	N=14 (28%)		<0.0001
*The mean values are presented along with 95% confidence intervals and corresponding P values, (means± SD) refers to the difference of means and standard deviation of the means, N refers to the patient's number.				

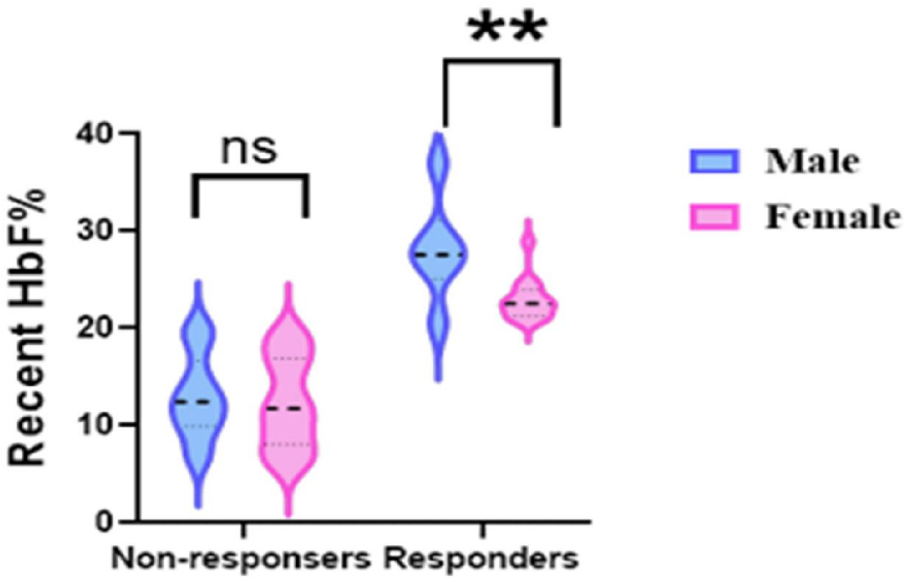


Figure (3-1): Distribution of recent HBF% in responders and nonresponders SCA patients. Data was showed by mean±SD.

3.2.2 Effectiveness of Hydroxyurea Treatment: Recent HbF% Response in Sickle Cell Patients

Table (3-3) and figure (3-2) present recent hemoglobin F (HbF%) data in sickle cell patients categorized by their response to hydroxyurea (HU) treatment. Patients are divided into non-responders (HbF<20, N=30) and responders (HbF≥20, N=20). The mean recent HbF% for non-responders is 12.60 (±4.502), while responders exhibit a higher mean of 24.47 (±4.053). The statistical analysis shows a highly significant difference in HbF% between the two groups, as indicated by a P value of less than 0.0001 for non-responders. This suggests that sickle cell patients responding to HU treatment (HbF≥20) have significantly higher recent HbF% levels compared to non-responders.

Table(3-3): Response of sickle cell patients undergoing HU treatment based on recent HbF% values

Recent HbF%	Mean ±SD	P value
Non-responder/HbF<20 N=30	12.60±4.502	<0.0001
Responder/HbF≥20 N=20	24.47±4.053	
*The mean values are presented along with 95% confidence intervals and corresponding P values, (means± SD) refers to the difference of means and standard deviation of the means, N refers to the patient's number.		

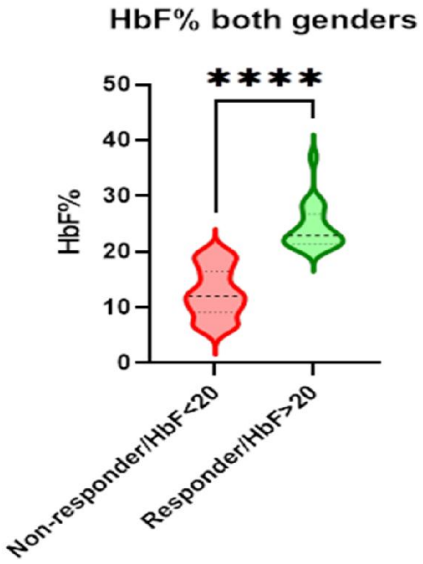


Figure (3-2): Distribution of recent HBF% in responders and nonresponders SCA patients based on HbF% values. The data was presented as mean±SD, ** refers to p<0.0001**

3.2.3. Markers of Hemolysis and Myelosuppression in Sickle Cell Anemia Patients Treated with Hydroxyurea.

The markers of hydroxyurea toxicity (myelosuppression) should be monitoring including platelet count, neutrophil count, reticulocyte count and Hb in SCA patients treated with HU as shown in Table (3-4) which demonstrate comparative analysis of hematological parameters: means, standard deviation and percentages in reference and toxic range. As observed, all patient values of markers were within the accepted range for all hematological parameters, and statistically non-significant P values. Except for 5 patients, 3 males and 2 females, their values of neutrophil count were within toxic range with mean ± SD 1.633±0.378 and 1.490±0.0141 respectively, and statistically non-significant P value 0.6466.

Table (3-4): A comparative analysis of hematological parameters

Hematological Parameters	**Male mean±SD**	**Female mean±SD**	**P value**
Absolute reticulocyte count 20-80X10^3 cells/ µl			
Accepted range ≥ 80X10^3 cells/ µl	234.4±78.42	214.3±71	0.3483
	(N=19), 36%	(N=31), 62%	<0.0001
Toxic range < 80X10^3 cells/ µl	-	-	
Neutrophile 2-8X10^3 cells/ µl			
Accepted range ≥ 2000 cell/ µl	4.968±1.506	4.248±1.544	0.1383
	(N=16),32%	(N=29),58%	<0.0001
Toxic range < 2000 cell/ µl	1.633±0.378	1.490±0.0141	0.6466
	(N=3),6%	(N=2),4%	<0.203
Platelets count 150-400X10^9/L			
Accepted range ≥80X10^3 cell/µl	407.3±209.5	381.1±182.5	
	(N=19),36%	(N=31),62%	<0.0001
Toxic range <80X10^3 cell/µl	-	-	
Hb g/dl Male 13-17 g/dl Female 12-15 g/dl			
Accepted range >5.3 g/dl	9.442±1.399	9.419±1.359	0.954
	(N=19),36%	(N=31),62%	<0.0001
Toxic range < 4.5 g/dl	-	-	

*Data was presented as means, P value adjusted <0.05 considered significant, (means± SD) refers to the difference of means and standard deviation, N refers to the patient's number

3.3. Molecular Analysis

3.3.1. Genotyping of UTB rs2298720 G>A SNP

The genetic polymorphism for the genotype UTB rs2298720 SNP showed a distinct band with a molecular size of 237 bp. The size of amplicon was ascertained by comparing it to a 100–1000 bp DNA ladder, and the UTB rs2298720 genotype was categorized into three genotypes:

1. GG (Wild) consider a major genotype
2. GA (Mutant heterozygote)
3. AA (Mutant homozygote)

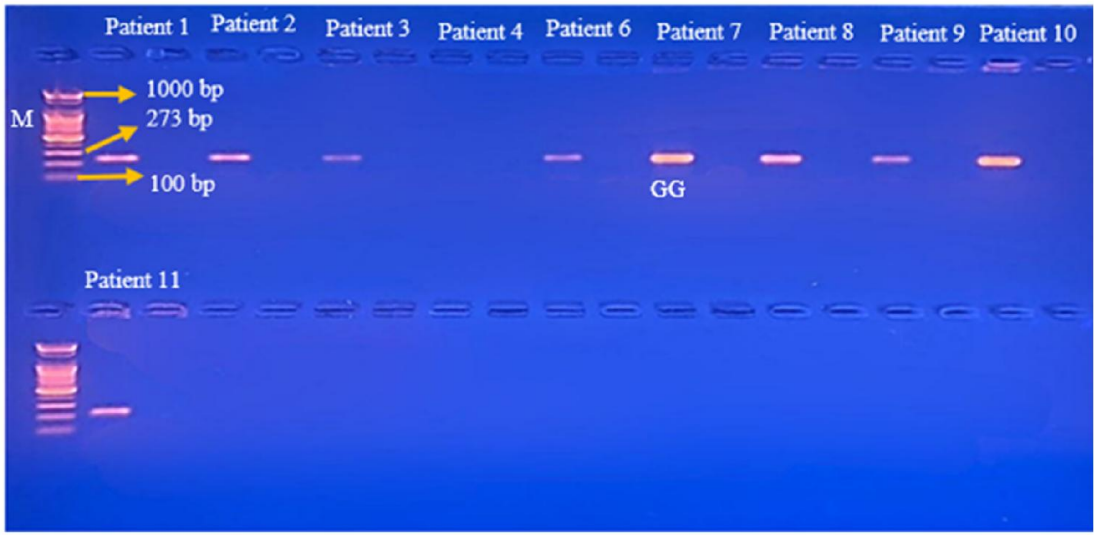


Figure (3-3): Agarose gel electrophoresis to detect UTB re2298720; G>A SNP using allele-specific PCR technique. M: DNA ladder, all the lanes represent the wild type (GG).

All results of PCR for this gene were wild genotype (GG)for all the participants in the current study as demonstrated in Figure (3-3).

3.3.2 The genetic distribution of UTB rs2298720 G>A SNP

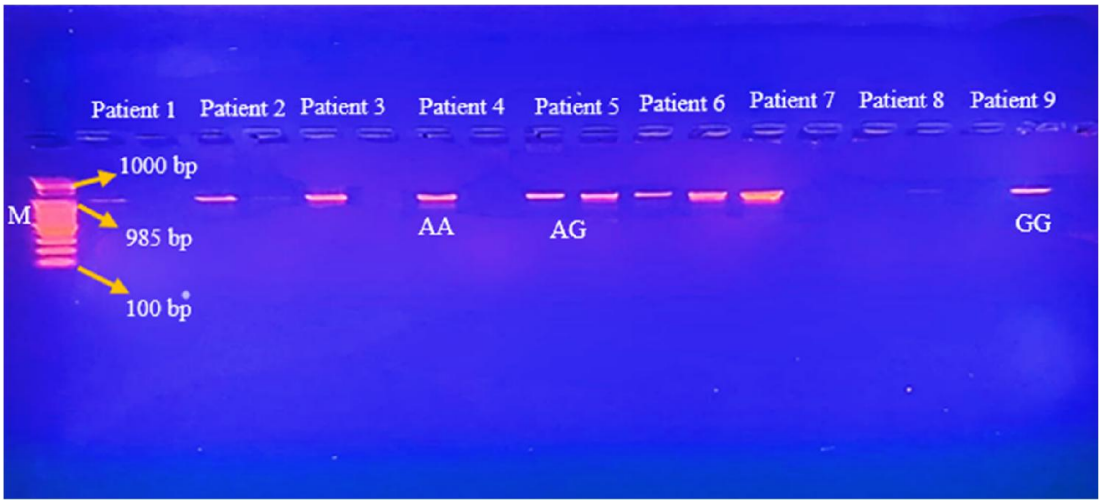
All participants in the present study who were wild genotype (GG) while the other mutant genotypes either the heterozygote (GA)and homozygote(AA) were absent. Therefore, there is no comparison between the observed and expected according to Hardy–Weinberg equilibrium.

3.3.3. Genotyping of UTB rs12605147 A>G SNP

The genetic polymorphism rs12605147 A>G SNP genotype UTB showed a distinct band with a molecular size of 985 bp. The amplicons size was detected by comparing it to a 100-1000 bp DNA ladder. Three genotypes were identified based on the UTB rs12605147 A>G allele genotyping:

1. Major genotype group for wild (AA).
2. Mutant heterozygote (AG).
3. The mutant homozygote genotype group (GG).

The results of PCR for this gene were multiple varying between wild and mutant , as shown in Figure (3-4)



Figure(3-4): Agarose gel electrophoresis to detect UTB rs12605147 A>G SNP using allele-specific PCR technique. M: DNA ladder, the lanes represent the wild type (AA), mutant heterozygote(AG) and mutant homozygote(GG).

3.3.4. The Genetic Distribution of UTB Rs12605147 A>G SNP in Patients with Sickle Cell Anemia

The distribution of UTB rs12605147 genotype diverse among SCA patients as 39 (78%) of patients had a wild (AA), 9(18%) of patients had a mutant heterozygote(AG), and 2(4%)of patients had a mutant homozygote as displayed in table (3-5).

Table (3-5): The distribution of SNP rs12605147 in sickle cell patients

Gene SNP	Genotype	Group N=50	Frequency %	Allele		Significant P < 0.05
				A	G	
rs12605147 A>G	AA (Wild)	39	78	0.87	0.13	**<0.0001*** **
	AG (Mutant heterozygote)	9	18			
	GG (Mutant homozygote)	2	4			
*Data was shown as percentage and number of patients, *P<0.05, **P<0.001, ***P<0.0001						

Under the Hardy-Weinberg equilibrium, the distribution and percentage of participants with UTB rs12605147 differ between number of observed and expected as in : AA (0.87, 0.75) was statistically highly significant p**<0.0048****, GG(0.13, 0.016) was statistically highly significant p<0.0001*** ; and AG/GA (0.226) also was statistically highly significant p<0.0001*** , as shown in Table(3-6) and Figure (3-5).

Table(3-6): Hardy-Weinberg equilibrium to individuals with the rs12605147 genotype of UTB

Genotype*100% Observed, N=50		**Hardy–Weinberg equilibrium*100% Expected, N=50**			**Chi square test**	**Significant P < 0.05**
A	G	A	G	AG/GA	AA/observed vs AA/expected	**<0.0048****
0.87	0.13	0.7569	0.0169	0.2262	AG/observed vs AG/expected	**<0.0001*****
					GG/observed vs GG/expected	**<0.0001*****
*Data was shown as percentage and number of patients, *P<0.05, **P<0.001, ***P<0.0001						

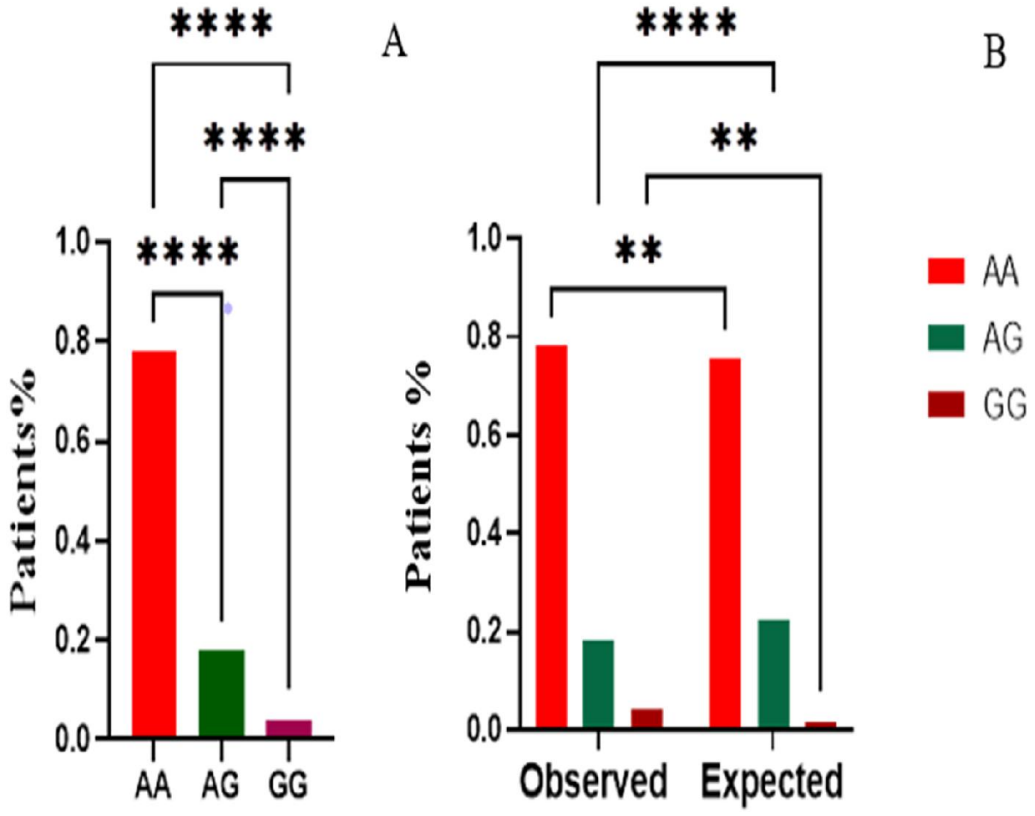


Figure (3-5): The genetic distribution of UTB rs12605147 A>G SNP in SCD patients. A) histogram depicts distribution of UTB rs12605147 alleles. GG (mutant homozygote), AA (wild), and AG (mutant heterozygote), with percentages of 78%, 18%, and 4%, respectively. The SNP genotypes are shown on the x-axis, and the percentage of patients is displayed on the y-axis. Significant differences are found in the Fischer exact tests between AA and AG (< 0.0001*), AA and GG (< 0.0001***), and AG and GG (< 0.0001***). A visual representation of the rs12605147 SNP distribution is provided by the notation *P<0.05, **P<0.001, and ***P<0.0001, which also highlights genotype variations in the patient cohort.**

3.4 Association Between UTB Rs12605147 A>G SNP and the Demographic Features in Patients with Sickle Cell Anemia Treated by Hydroxyurea.

The variety and distribution of UTB rs12605147 A>G alleles based on demographic characteristics in SCA patients who treated with HU as shown in Table(3-7). As observed, the predominant of genotyping distribution was the wild genotype (AA) for all demographic parameters and their subdivided categories. Followed by mutant heterozygote(AG),then the mutant homozygote was the least one. In gender category, the wild genotype(AA) was 32% for male and 46% for female, mutant heterozygote (AG) was 6% and 12% for male and female respectively. In the age category, it also was found that wild genotype was predominant in each subdivided of age, as shown in first category of 14-23 years was the distribution of genotype 62%,14% and 2% for AA,AG and GG respectively. This was exactly the same case on dose category, that for patient's dose 10 mg/kg/day was 32%,10%and 2% for AA, AG and GG genotype respectively. It also was found that result was seen in others parameters like frequency of hospitalization, frequency of fever, pain intensity and frequency of painful crisis.

Table (3-7): Difference among demographic characteristic in UTB rs12605147 genotyping A>G

Parameters				AA (N), %	AG (N), %	GG (N), %
sex%	Male, N=19			(16), 32	(3), 6	-
	Female, N=31			(23), 46	(6), 12	(2), 4
Age (Years)	14-23			(31), 62	(7), 14	(1), 2
	24-33			(6), 12	(1), 2	-
	34-43			(1), 2	-	-
	44-53			(1), 2	(1), 2	(1), 2
HU dose (mg/Kg/day), (N), %	10, N=22			(16), 32	(5), 10	(1), 2
	15, N=20			(16), 32	(3), 6	(1), 2
	20, N=8			(7), 14	(1), 2	-
Frequency of hospitalization (N), %	0, (non)			(27), 54	(5), 10	(2), 4
	1, (once/year)			(6), 12	(3), 6	-
	2, (twice/year)			(4), 8	(1), 2	-
	3, (thrice/year)			(1), 2	-	-
	4, (> three times/year)			(1), 2	-	-
Duration of hospitalization/days (N), %	0			(27), 54	(5),10	(2), 4
	2-4			(6), 12	(1), 2	-
	>4			(6), 12	(3), 6	-
Frequency of Fever	Non			(30),60	(4), 8	-
	Once			(3), 6	(2), 4	(1), 2
	Twice			(4), 8	(1), 2	-
	Thrice			(1), 2	-	(1), 2
	More than three times			(1), 2	(2), 4	-
Temp (N), %	36.5-37 °C			(30), 60	(4), 8	-
	37.1-38 °C			(1), 2	(1), 2	-
	38.1-39 °C			(7), 14	(3), 6	(2), 4
	≥39 °C			(1), 2	-	(1), 2
Frequency of blood transfusion (N), %	Non			(33), 66	(5),10	(2), 4
	Once			(5), 10	(1), 2	-
	Twice			(1), 2	(1), 2	-
	Three or more			-	(2), 4	-
Intensity of pain (N), %	categories	0	No pain	(6), 12	(1), 2	-
		1	Mild	(9),18	(2), 4	-
		2	moderate	(15), 30	(1), 2	(2), 4
		3	Sever	(6), 12	-	-
		4	Worst pain	(3), 6	(5),10	-
Frequency of pain (N), %	categories.	0	Non	(17),34	(2), 4	(1), 2
		1	Once/year	(8), 16	(2), 4	-
		2	Twice/year	(5), 10	(1), 2	-
		3	Thrice/year	(3), 6	(2), 4	-
		4	>3times/year	(6), 12	(2), 4	(1), 2

*(N) %, N refers to the patient's number and % refers to the percentage.

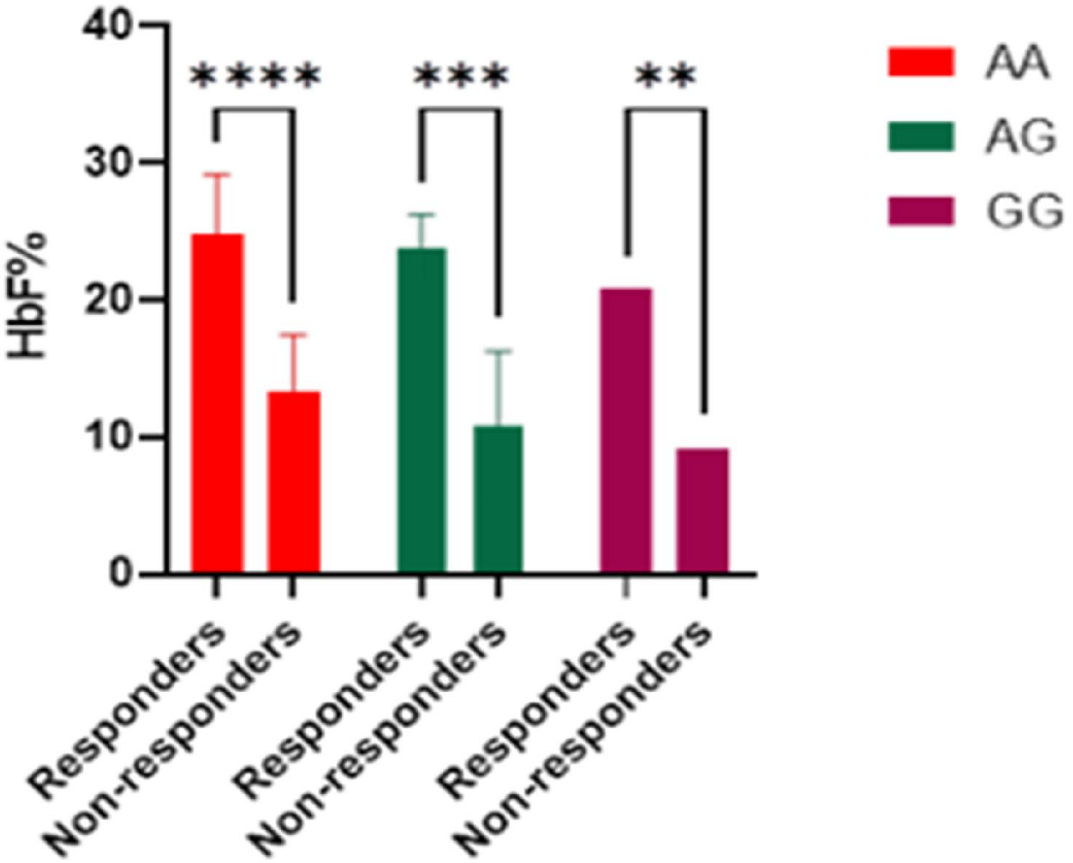
3.5 Association between UTB Rs12605147 A>G SNP and Recent Hbf%.

3.5.1 Impact of UTB rs12605147 SNP on Recent HbF% in Patients Percentage Undergoing HU Treatment

Regarding the distribution of UTB rs12605147 genotypes alleles according to responses of recent HbF%, reviews the results in table (3-8) demonstrated that the wild allele (AA) had considerably higher levels than the mutant heterozygote and homozygote in both groups responders and non-responders with percentage (32%)and (46%) respectively, statistically high significant p value <0.0001 in both groups. In addition, the percentage level of the mutant heterozygote(AG) in non-responders was double comparing with responder group, (AG 12% vs 6%), statistically high significant p value <0.0001 in both groups, and the same percentage for homozygote(GG) alleles(GG 2% vs 2%) as shown in Table (3-7), also statistical highly significant p values in both groups as mentioned in the Figure (3-5).

Table (3-8): Impact of UTB rs12605147 SNP on HU response according to recent HbF% .

Patients' response	**UTB rs12605147 alleles**			**Chi square test**	**Significant P < 0.05**
	AA n=39	**AG n=9**	**GG n=2**		
Non-responders % HbF<20, N=30	0.46	0.12	0.02	AA vs AG	**<0.0001**
				AA vs GG	**<0.0001**
				AG vs GG	**<0.0001**
Responders% HbF≥20, N=20	0.32	0.06	0.02	AA vs. AG	**<0.0001**
				AA vs. GG	**<0.0001**
				AG vs. GG	**0.0010**
*Data was shown as percentage and number of patients, *P<0.05, **P<0.001, ***P<0.0001 The percentages in the table refer to the patients response or non-response to the HU treatment.					



Figure(3-6): Comparison between responders and non responders according to HBF%. The genotype alleles (AA, AG, GG) of rs12605147 and their effect on recent HbF% in patients treated with Hydroxyurea (HU) are compared in this histogram. With respect to each allele, non-responders (HbF < 20%, N = 30) and responders (HbF ≥ 20%, N = 20) exhibit different mean HbF% values. There are notable pairwise comparisons between non-responders and responders (AA vs AG, AA vs GG, AG vs GG; all <0.0001, AG vs GG; 0.0010 for responders). The x-axis displays the genotype alleles, while the y-axis displays the mean HbF% values. .

3.5.2 Correlation of Rs12605147 SNP with Recent HbF% Means in Patients Undergoing HU Treatment

Table(3-9) illustrates the correlation of UTB rs12605147 A>G genotype alleles among responders and non-responders of the means recent HbF% in SCA patients undergoing HU treatment. Clearly observed, according to Šídák's multiple comparisons test there was highly significant with p value <0.0001 between responders vs. non-responders with wild (AA) genotype, the mean±SD value 24.83±4.33 and 13.23±4.236 for responder and non-responders respectively. It was also found statistical high significant with p value 0.0002 between responders vs. non-responders in mutant heterozygote (AG) genotype, the mean±SD value 23.73±2.53 and 10.77±5.519 for responder and non-responders respectively. Additionally, a statistically significant difference (p value 0.0042) was observed between responders vs. non-responders in mutant homozygote (GG) genotype; the for responders and non-responders were 20.80±0.000 and 9.200±0.000, respectively.

Table (3-9): Impact of rs12605147 SNP on recent HbF% means in patients undergoing HU treatment

Patients' response	**UTB rs12605147, Mean±SD**			**Šídák's multiple comparisons test**	**Significant P < 0.05**
	AA n=39	**AG n=9**	**GG n=2**	Non-responders vs Responders	
Non-responders	13.23±4.236	10.77±5.519	9.200±0.000	AA	**<0.0001**
Responders	24.83±4.33	23.73±2.53	20.80±0.000	AG	**0.0002**
				GG	**0.0042**

*Data was presented as means, P value adjusted <0.05 considered significant, (means± SD) refers to the difference of means and standard deviation.

3.6. Impact of UTB rs12605147 SNP on Hematological Parameters in SCA Patients Undergoing HU Treatment.

In this experiment evaluated the impact of gene polymorphism variations on patient toxicity responses to HU treatment which represented as myelosuppression, with a specific focus on measuring hematological parameters markers like absolute reticulocyte count, neutrophil count, platelet count and Hb levels as an indicators of treatment toxicity. This study assessed the relationship between UTB alleles and how they correlate with changes in hematological parameters levels in response to HU therapy in SCA patients as summarized in the Table(3-10) and Figure (3-6). For UTB alleles (AA, AG, GG), the data indicates that all patients were within the accepted range and all of them out the toxic rang regarding to all measuring the marker except neutrophile.

Regarding to absolute reticulocyte count, the AA allele exhibited mean±SD 226.2±74.62, no significant change in absolute reticulocyte count levels in response to HU, while AG allele showed mean±SD180.2±44.82, with significant p value 0.0293for AG vs GG according to Tukey's multiple comparisons test. Similarly, for GG, the mean±SD 335.7±30.17 had highly significant change in absolute reticulocyte count p value 0.0042.

For neutrophile according accepted range, the AA allele presented mean±SD 4.436±1.479 , no significant change in neutrophile count levels in response to HU, while AG allele showed mean±SD4.584±1.367, with highly significant p value 0.0091 for AG vs GG depending on Tukey's multiple comparisons test. Similarly, for GG, the mean±SD 7.267±2.329 had highly significant change in neutrophile count p value 0.0042, show figure (3-7). While in toxic rang, as observed the mean±SD 1.500±0.300

and 1.690±0.2970 for AA and AG alleles respectively, with statistically no significant (p value0.5364).

For platelet count and Hb level, all patients were within accepted range, additional to Tukey's multiple comparisons test showed no significant correlation between UTB alleles and toxic response of platelet count and Hb for HU therapy.

Table(3-10): Impact of UTB rs12605147 alleles on hematological parameters in SCA patients undergoing HU treatment

Parameters	**AA n=39**	**AG n=9**	**GG n=2**	**Tukey's multiple comparisons test**	**Significant summary**	
Absolute reticulocyte count 20-80X10^3 cells/ µl	**mean±SD**	**mean±SD**	**mean±SD**	**Tukey's multiple comparisons test**	**P < 0.05**	**95.00% CI**
Accepted range ≥ 80X10^3 cells/ µl	226.2±74.62	180.2±44.82	335.7±30.17	AA vs. AG	0.1814	-107.8 to 15.89
				AA vs. GG	**0.0293**	9.273 to 209.7
				AG vs. GG	**0.0042**	43.93 to 266.9
Toxic range < 80X10^3 cells/ µl	-	-	-	-	-	-
Neutrophile 2-8X10^3 cells/ µl						
Accepted range ≥ 2000 cell/ul	4.436±1.479	4.584±1.367	7.267±2.329	AA vs. AG	0.9696	-1.371 to 1.667
				AA vs. GG	**0.0091**	0.6210 to 5.040
				AG vs. GG	**0.0362**	0.1451 to 5.220
Toxic range < 2000 cell/ µl	1.500±0.300	1.690±0.2970	-	AA vs. AG	0.5364	-0.6786 to 1.059
				-		
				-		
Platelets count 150-400X10^9/L						
Accepted range ≥80X10^3 cell/µl	387.0±190.7	377.7±179.1	531.0±342.2	AA vs. AG	0.9907	-182.3 to 163.7
				AA vs. GG	0.5632	-195.1 to 483.2
				AG vs. GG	0.5713	-212.3 to 519.0
Toxic range <80X10^3 cell/µl	-	-	-	-	-	-
Hb g/dl Male 13-17 g/dl Female 12-15 g/dl						
Accepted range >5.3 g/dl	9.495±1.364	9.367±1.306	8.400±1.980	AA vs. AG	0.9654	-1.355 to 1.098
				AA vs. GG	0.5178	-3.500 to 1.310
				AG vs. GG	0.6416	-3.560 to 1.626
Toxic range < 4.5 g/dl	-	-	-	-	-	-
*The mean values are presented along with 95% confidence intervals and corresponding P values,(means± SD) refers to the difference of means and standard deviation.						

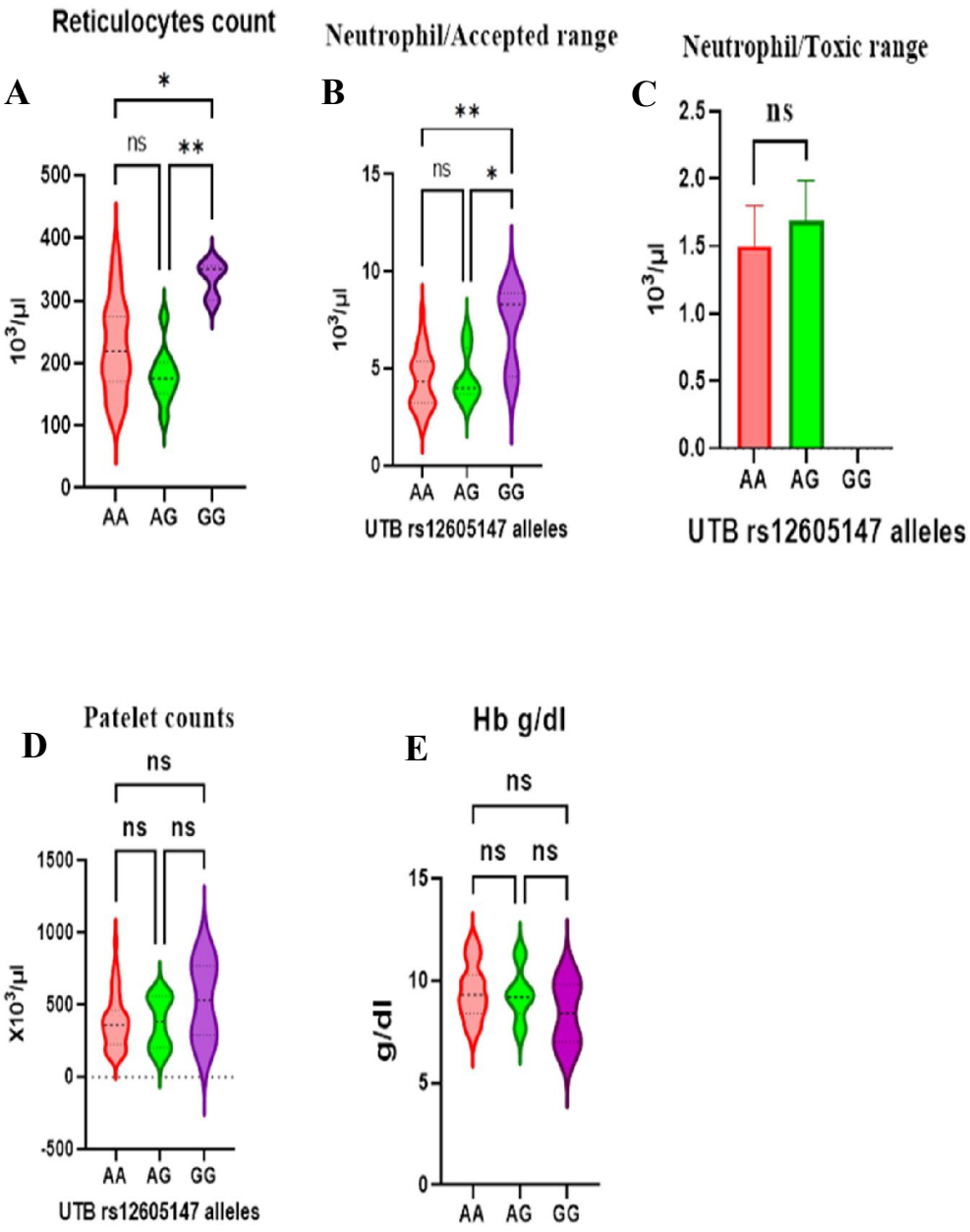


Figure (3-7): Impact of UTB rs12605147 SNP on hematological parameters. The genotype alleles (AA, AG, GG) of rs12605147 and their effect on hematological parametersvin patients treated with Hydroxyurea (HU) are compared in the histograms. A) reticulocytes count. B)Neutrophil accepted range. C) Neutrophile toxic range. D) platelets count. E) Hb g/dl. Data was shown as mean±SD. ns=non-significant, *P<0.01, **P<0.001.

3.7 Impact of UTB rs12605147 SNP on Intensity of Pain in Patients Undergoing HU Treatment

Table (3-11) illustrates the impact of UTB rs12605147 genotype alleles on intensity of pain in patients undergoing HU treatment, the observed predominant and high percentage was wild (AA) allele in each category of pain intensity score which ranging from "no pain" to "worst pain" and appear with highest expression in "moderate" category was 30%. Followed by heterozygote mutant (AG) allele which observed expression in highest percentage in " worst pain " category, it was 10% of comparing of the total percentage 18% which distribute among other categories. The homozygote mutant (GG) allele was the last one and presented 4% of total genotypes and expression at only one category "moderate". According to Nested analyses and Wilcoxon test was used to determine the significance of the differences across genotype groups. The results included the 95% Confidence Interval (CI) of the difference, significance ($P < 0.05$), and the actual mean. There was a significant correlation between effect of genotype variations and pain intensity score , as AA allele was highly significant P value (two tailed) 0.0186, and AG allele with P value (two tailed) 0.0979 as shown in table(3-12) and Figure (3-8).

Table (3-11): Impact of rs12605147 SNP on intensity of pain in patients undergoing HU treatment

Pain intensity score		UTB rs12605147, (N) %		
		AA, n=39	**AG, n=9**	**GG, n=2**
0	No pain	(6), 12%	(1), 2%	-
1	Mild	(9), 18%	(2), 4%	-
2	Moderate	(15), 30%	(1), 2%	(2), 4%
3	Sever	(6), 12%	-	-
4	Worst pain	(3), 6%	(5), 10%	-
Total		**(39), 78%**	**(9), 18%**	**(2), 4%**
*Data was shown as percentage and number of patients				

Table(3-12): Impact of rs12605147 SNP on intensity of pain in patients undergoing HU treatment analyzed by Nested analyses, Wilcoxon test

Statistical report	**AA**	**AG**	**GG**
Theoretical mean	0.000	0.000	0.000
Actual mean	7.800	2.250	2.000
Number of values	**5**	**4**	**1**
P value (two tailed)	**0.0186**	**0.0979**	**-**
95% Confidence Interval	-0.7621 to 5.262	2.151 to 13.45	
*The mean values are presented along with 95% confidence intervals and corresponding P values			

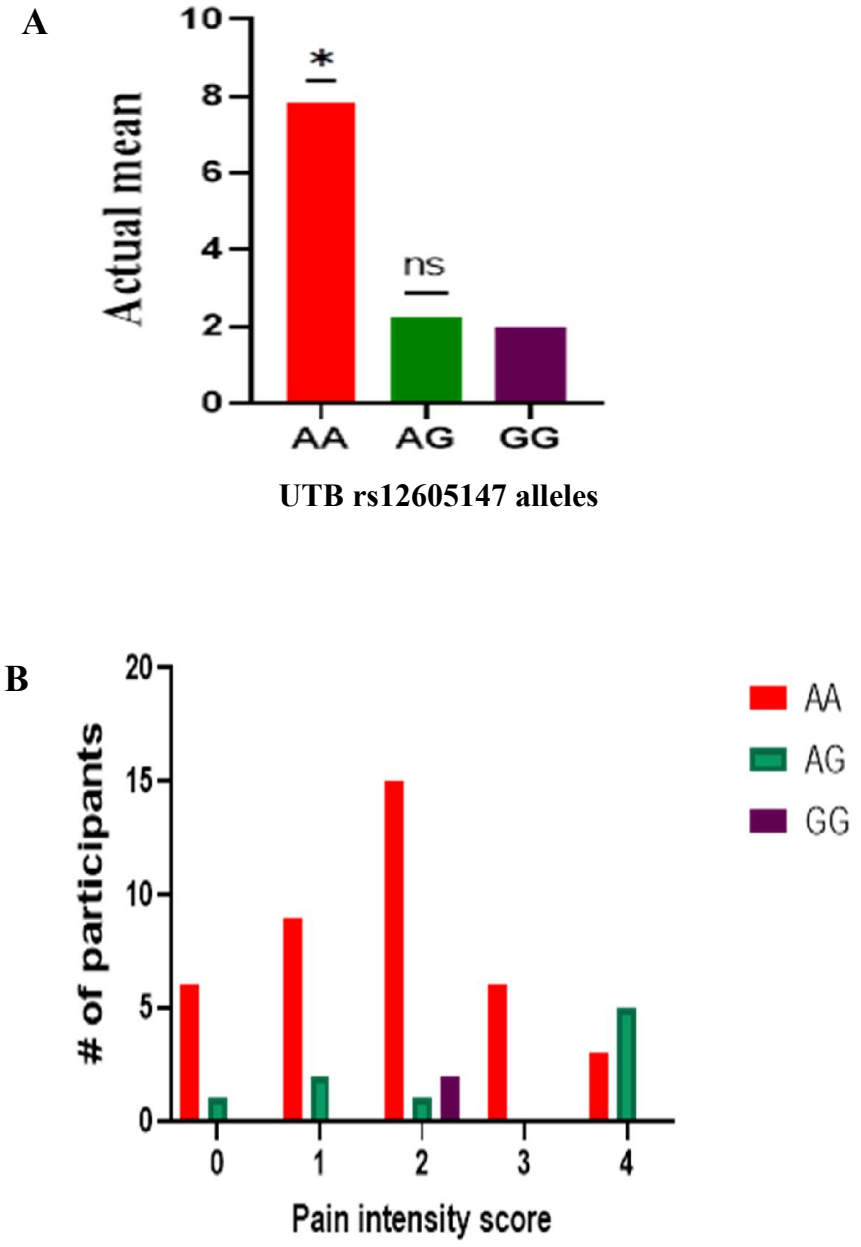


Figure (3-7): Impact of rs12605147 genotype alleles on intensity of pain in patients undergoing HU treatment. A) Nested analyses and Wilcoxon tests were used in the analysis to compare the mean pain intensity for each genotype between theory and reality. B) The variability was shown in bar charts with 95% Confidence Intervals, and statistical significance is indicated by two-tailed p-values.

3.8 Impact of UTB rs12605147 SNP on Frequency of Pain Crisis in Patients Undergoing HU Treatment

Table(3-13) illustrates the impact of genotype alleles among different categories of frequency of painful crisis, the wild allele (AA) was predominant totally and high percentage in each category, with the highest percentage in" no pain" category was 34%, followed by heterozygote mutant (AG) allele was expression with nearest values among the categories, while the homozygote mutant (GG) allele was the lowest one expression in only two categories: "no pain " was 2% , and " more than three times per year" also was 2%. Nested analyses and Wilcoxon test were used to determine the significance of the differences across genotype groups. The results included the 95% Confidence Interval (CI) of the difference, significance ($P < 0.05$), and the actual mean. There was a significant correlation between effect of genotype variations and frequency of pain crisis, where the result showed that AA alleles had statistically significant effect p (two tailed) value 0.0329, while the AG allele had highly significant effect p value 0.0008 as illustrated in Figure (3-9) and Table(3-14).

Table (3-13): Impact of rs12605147 SNP on frequency of pain crisis in patients undergoing HU treatment

Frequency of pain crisis categories		UTB rs12605147, (N), %		
		AA, n=39	**AG, n=9**	**GG, n=2**
0	No pain crisis	(17), 34%	(2), 4%	(1), 2%
1	Once/year	(8), 16%	(2), 4%	-
2	Twice/year	(5), 10%	(1), 2%	-
3	Thrice/year	(3), 6%	(2), 4%	-
4	> 3times/year	(6), 12%	(2), 4%	(1), 2%
	Total	**(39), 78%**	**(9), 18%**	**(2), 4%**
*Data was shown as percentage and number of patients.				

Table (3-14): Analyses of impact of UTB rs12605147 SNP on frequency of pain crisis in patients undergoing HU Treatment

One sample t test statistical report	**AA, n=39**	**AG, n=9**	**GG, n=2**
Theoretical mean	0.00	0.00	0.00
Actual mean	0.1560	0.03600	0.02000
Number of values	5	5	2
P value (two tailed)	**0.0329**	**0.0008**	
SD of discrepancy	0.1090	0.008944	
SEM of discrepancy	0.04874	0.004000	
95% confidence interval	0.02066 to 0.2913	0.02489 to 0.0471	
*The mean values are presented along with 95% confidence intervals and corresponding P values			

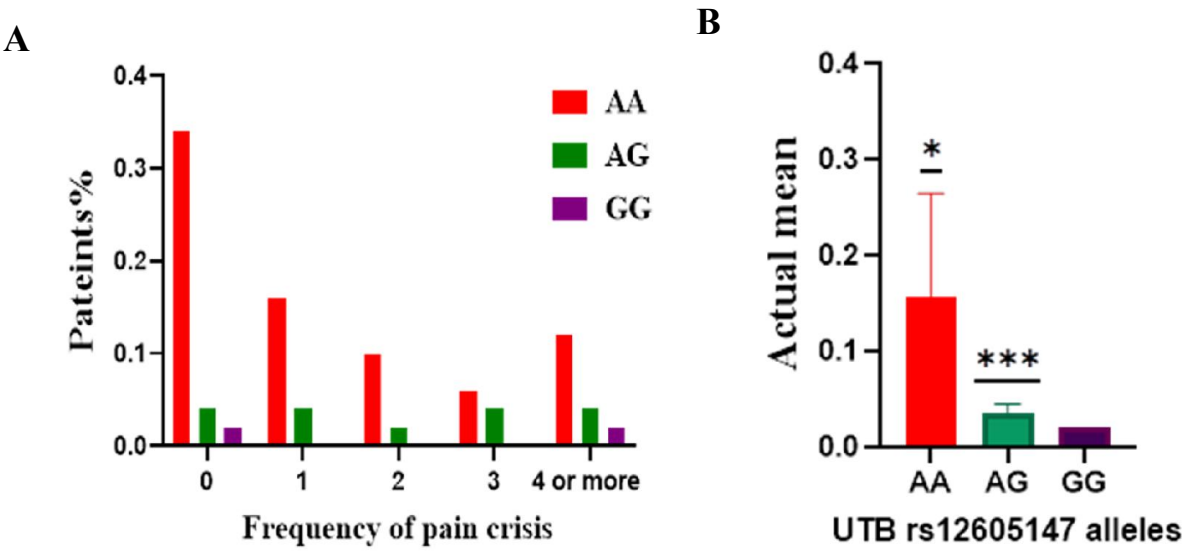


Figure (3-9): Impact of UTB rs12605147 SNP on frequency of pain crisis. A-B) histogram depict how UTB rs12605147 alleles (AA, AG, GG) influence pain crisis frequency in Hydroxyurea (HU)-treated patients. The One-sample t-test compares theoretical and actual mean frequencies, revealing potential genotype-associated variations

3.9 Correlation between Pain Intensity and Recent HbF% in Patients with Sickle Cell Anemia with or without UTB rs12605147 Mutation

The current study, assessed the relationship between UTB alleles and how they correlate with pain intensity and changes in HbF% levels in response to HU therapy in SCA patients as summarized in the Table(3-15). This table details into the complex relationship between gene variation and intensity of pain scores and mean of recent HbF% level among SCA patients. It investigates the correlation while categorizing intensity pain scores by severity, ranging from 'No pain' to 'worst of pain'. The analysis encompasses patients with mutant and no mutant gene variations, providing a holistic perspective on how genetic factors may influence intensity of pain. With the aim of shedding light on the potential impact of these gene variations, we present R Square values, p-values, and confidence intervals. These findings not only enhance our understanding of HbF% role in SCA but also hold promise for tailoring personalized treatment strategies, particularly involving HU therapy. This study examined the correlation between UTB gene variation and pain intensity scores and recent HbF% in SCA patients, a critical aspect considering the association of pain intensity with both the development HbF% and responses to HU treatment as depicted in table(3-17). For patients with mutant and no mutant gene variations, the analysis reveals a strong correlation (R Square =0.9518), and according to Pearson value there was negative correlation and highly significant p value 0.0046 between pain intensity scores and mean of recent HbF% level. Interestingly, the proportion of pain intensity notably decreased with increase recent HbF% , as showed in the table. The confidence intervals provide a range for this correlation's strength.

Subdividing pain intensity scores from "No pain" to "worse of pain " alongside corresponding mean HbF% levels, allows for a comprehensive assessment of the gene variation's impact across different pain intensity. Additionally, for patients with mutant gene variations, a negative correlation and not significant were observed (p-value =0.1777), while patients with no mutant gene variations(wild AA allele) showed a negative correlation and statistically significant (p-value =0.0175).

Table (3-15): Correlation between pain intensity and recent HbF% in patients with SCD with or without rs12605147 mutation

Parameters			**Mutant and no mutant**	**R Square**	**P-value**	**Pearson value**	**95% Confidence Intervals**
Pain intensity score	**Scores**	**# of patients**	**Mean of Recent HbF%**				
No signs	0	7	21.8714				
Mild	1	11	18.1818	0.9518	**0.0046**	-0.9756	-0.9985 to -0.6705
Moderate	2	18	17.9777				
Severe	3	6	14.5666				
Worst pain	4	8	12.987				
Total		50					
Parameters			**Mutant**	**R Square**	**P-value**	**Pearson value**	**95% Confidence Intervals**
Pain intensity score	**Scores**	**# of patients**	**Mean of Recent HbF%**				
No signs	0	1	22.8				
Mild	1	2	19.55		0.1777	-0.8223	-0.9961 to 0.6618
Moderate	2	3	12.166				
Severe	3	-	-				
Worst pain	4	5	13.48				
Total		11		0.6762			
Parameters			**No mutant (Wild)**	**R Square**	**P-value**	**Pearson value**	**95% Confidence Intervals**
Pain intensity score	**Scores**	**# of patients**	**Mean of Recent HbF%**				
No signs	0	6	21.716666				
Mild	1	9	17.8777	0.8837	**0.0175**	-0.9401	-0.9961 to -0.3387
Moderate	2	15	19.14				
Severe	3	6	14.5666				
Worst pain	4	3	12.1666				
Total		39					
The mean values are presented along with 95% confidence intervals and corresponding P values							

3.10 Correlation between Frequency of Pain Crisis and Recent HbF% in Patients with Sickle Cell Anemia with or without UTB rs12605147 Mutation

This experiment, assessed the relationship between frequency of pain crisis and UTB alleles and how they correlate with changes in recent HbF% levels in response to HU therapy in SCA patients as summarized in Table(3-16).

This table delves into the complex relationship between gene variation and frequency of painful crisis scores and mean of recent HbF% among SCA patients. It investigates the correlation while categorizing frequency scores ranging from 'No pain' to 'more than three times per year.' The analysis encompasses patients with mutant and no mutant gene variations, providing a holistic perspective on how genetic factors may influence frequency of pain crisis. With the aim of shedding light on the potential impact of these gene variations, we present R Square values, p-values, and confidence intervals. These findings do not only enhance our understanding of HbF% role in SCA but also hold promise for tailoring personalized treatment strategies, particularly involving HU therapy. This study examined the correlation between UTB gene variation and frequency of painful crisis scores and recent HbF% in SCA patients, a critical aspect considering the association of frequency of painful crisis with both the development HbF% and responses to HU treatment as depicted in table(3-18). For patients with mutant and no mutant gene variations, the analysis reveals a strong correlation (R Square =0.9541), depending on Pearson value a negative correlation and highly significant p value 0.0042 observed between the frequency of painful crisis and recent HbF%. Interestingly, the proportion of instances frequency of painful crisis

notably decreased with increase recent HbF%. The confidence intervals provide a range for this correlation's strength.

Subdividing frequency of painful crisis scores from "No pain crisis" to "more than three times per year," alongside corresponding mean HbF% levels, allows for a comprehensive assessment of the gene variation's impact across different frequency of painful crisis. Additionally, for patients with mutant gene variations, a negative correlation and non-significant were observed as in (p-value = 0.2755), while patients with no mutant gene variations showed a negative correlation and statistically significant (p-value =0.0110).

Table (3-16): Correlation between frequency of pain crisis and recent HbF% in patients with SCD with or without UTB rs12605147 mutation

Parameters	**Mutant and no mutant**		**R Square**	**P-value**	**Pearson value**	**95% Confidence Intervals**
Frequency of pain crisis	**# of patients**	**Mean of Recent HbF%**	0.9541	**0.0042**	**-0.9768**	-2.955 to -0.3961
No pain crisis	**20**	**20.06**				
Once /year	10	17.83				
Twice/year	6	15.516				
Thrice /year	5	14.42				
>3times/year	9	13.62				
Total	50					
Parameters	**Mutant**		**R Square**	**P-value**	**Pearson value**	**95% Confidence Intervals**
Frequency of pain crisis	**# of patients**	**Mean of Recent HbF%**	0.3711	0.2755	-0.6092	-0.9701 to 0.5904
No pain crisis	3	23.4				
Once /year	2	13.75				
Twice/year	1	6.5				
Thrice /year	2	16.05				
>3times/year	3	9.83				
Total	11					
Parameters	**No Mutant(Wild)**		**R Square**	**P-value**	**Pearson value**	**95% Confidence Intervals**
Frequency of pain crisis	**# of patients**	**Mean of Recent HbF%**	0.9141	**0.0110**	-0.9561	-0.9972 to -0.4716
No pain crisis	17	19.47				
Once /year	8	18.937				
Twice/year	5	17.32				
Thrice /year	3	13.333				
>3times/year	6	12.96				
Total	39					
The mean values are presented along with 95% confidence intervals and corresponding P values						

Chapter Four

Discussion

4. Discussion

This study employed to understand the effect of UTB rs12605147 and rs2298720 gene polymorphisms within the context of SCD patients undergoing HU treatment, especially among the Iraqi population. UTB gene is responsible for encoding urea transporters in erythrocytes and bone marrow, which has been recognized for its role in influencing HU pharmacokinetics and mediating recent HbF induction. The impact of UTB gene SNPs, rs12605147 and rs2298720 on treatment response in SCD patients remain unmasked globally, particularly within the unique genetic landscape of Iraqi individuals. By studying genetic variations unique to Iraqis with SCD, the goal is to develop personalized treatments that are more effective. This groundbreaking study aims to improve understanding of SCD's causes and develop more targeted and successful treatment approaches for Iraqi patients.

The demographic area of this study involved 50 patients with SCA, including 38% males and 62% females, with age range of 14 to 65 years. The males had a mean weight of (47.92±11.16) compared with the females mean weight (51.87±12.57, $P<0.0001$). Hydroxyurea treatment was varied, with 44% receiving 10mg/kg/day, 40% receiving 15mg/kg/day, and 16% receiving 20mg/kg/day. Notably, 68% of patients treated with HU did not require annual hospitalization, while 32% needed hospitalization with admission-discharge intervals ranging from one to more than three days. The 32% of patients suffered from fever, with a temperature range of 38.1°C to above 39°C. Blood transfusion was not required for 80% of participants. Pain intensity varied, with 14 % experiencing "No Pain," 36% in the "Moderate" category, and 12% in the "Severe" category, making this data crucial for understanding the demographic and clinical aspects of sickle cell anemia and HU treatment outcomes. This study explored the

demographic pictures including age, BW, HU treatment course, frequency of pain, intensity of pain, frequency and duration of hospitalization, which implement the role of genetics in influencing variability among different groups, this research gains greater credibility and applicability. Taking into account a diversity of demographic data dose not only enhances the generalizability of the findings, but also allows for a more thorough analysis by stratifying results and controlling for potential confounding factors (G. et al., 2017; Sarri et al., 2018).

Table (3-2) provides a comprehensive overview of recent HbF% levels in patients with sickle cell disease, defined by gender and HbF% values. The data is grouped into two distinct categories: non-responders (HbF < 20) and responders (HbF ≥ 20). In the non-responder group, the average HbF% for male patients (N=13) is 12.92±4.362, while it is 12.36±4.726 for female patients (N=17), yielding a P value of 0.7436, which is not statistically significant. Conversely, in the responder group, male patients (N=6) demonstrate a significantly elevated mean HbF% of 28.07±5.332, as opposed to females (N=14) who have a mean HbF% of 22.92±2.125, with a P value less than 0.0055. The 95% confidence interval for the male responder group spans from 0.4811 to 9.809. Interestingly, the distribution of non-responders and responders varies between genders, with 26% of males and 34% of females classified as non-responders, and 12% of males and 28% of females classified as responders. This data implies that there are gender-specific differences in HbF% response to treatment, especially in the responder group where males show a significantly higher HbF% compared to females. The observed P values highlight the statistical significance of these differences, with a notably low P value (<0.0001) in the responders, emphasizing the impact of gender on HbF% response to HU in SCA.

The findings of this study and the data published by previous studies both focused on patients with SCD, but they explored the topic from different angles. Previously, scientists explored at a broad range of demographic characteristics and treatment outcomes. However, this study examined specifically the levels of recent HBF% in the patients that were categorized by gender and response to treatment. In respect to the previous context, this new data adds another layer of understanding SCD pathophysiology. It suggests that there might be gender-related differences in HbF% response to treatment. This could potentially explain some of the variations in treatment outcomes observed in the previous study. For example, if males generally have a higher HbF% response to treatment, this could contribute to better overall outcomes in male patients. Hence, further research would be needed to fully understand the relationship between gender, HbF%, and treatment outcomes in sickle cell anemia patients (Acharya et al., 2023; Badawy et al., 2021; Shah et al., 2019; Treadwell et al., 2022). The observed difference in response to hydroxyurea (HU) between male and female SCD patients, with males showing a more significant increase in fetal hemoglobin (HbF%) levels, could stem from a combination of biological and pharmacological factors. Hormonal influences, for instance, might play a role; testosterone, which is typically higher in males, supports erythropoiesis and may enhance HU's effects on HbF production, whereas estrogen in females could have a moderating impact on this process (Pencina et al., 2023; Valancy et al., 2021). Additionally, sex-based differences in HU pharmacokinetics could influence drug efficacy; males and females may metabolize HU differently due to variations in drug-metabolizing enzymes or transporters, potentially resulting in higher effective drug levels in males or faster clearance in females (Ware et al., 2011). Genetic variability also warrants consideration, as sex-specific differences in HbF-related regulatory genes,

such as BCL11A, might affect HU responsiveness (Ginete et al., 2023; Sales et al., 2022). Furthermore, baseline disease characteristics, such as disease severity or comorbidities, might differ between male and female SCD patients, impacting overall HU response (McGann et al., 2016; Phillips et al., 2018; Thornburg et al., 2010). Understanding these factors could pave the way for personalized HU therapies, optimizing HbF induction across sexes and improving outcomes for SCD patients.

A comparative study of hematological parameters in SCA patients which grouped by gender was based on the accepted and toxic ranges. Both male and female patients had shown average values for each parameter, including absolute reticulocyte count, neutrophil counts, platelet counts, and hemoglobin concentrations. However, there were significant gender differences ($P < 0.0001$). Most males and females had values within the normal range for each parameter, with a certain percentage falling below or above the range. For example, 36% of males and 62% of females had an absolute reticulocyte count greater than or equal to 80x10^3 cells/µl. The majority of patients also had safe levels for each parameter, as evidenced by the absence of any toxic ranges. Overall, these findings demonstrate the overall health of the patients and suggest that they were well within acceptable levels for these parameters. Raymond and his colleague suggested that age and gender are significantly impact the prevalence of organ damage in SCD patients. However, current study added a new layer to this study by suggesting that gender might influence hematological parameters in SCD patients (Osarogiagbon et al., 2009). Scientists have recently discussed the challenges of managing SCD in women, in particular in a cost-effective manner. The findings of this current study about gender-related variations in hematological parameters could suggest effective management strategies for women with SCD (Afolabi et al.,

2022; Jaber et al., 2019). Intriguingly, in this study findings strongly agree with Ritta and her research team which they suggested that SCD manifestations and complications are strongly based sex-specific dissimilarities (Masese et al., 2021).

Gene variations or single nucleotide polymorphism might play a crucial role in the pathophysiology of SCD by influencing the way it presents in various individuals. For example, polymorphisms in genes encoding inflammatory proteins have been shown to enhance the incidence of SCD complications (Fertrin & Costa, 2010). However, it must be acknowledged that numerous studies have revealed that gene variations in sickle cell anemia is crucial as these variations can dramatically influence the clinical manifestations and severity of the disease. For instance, in the study of 500 SCD patients, the researchers suggested that TLR2 rs4696480 TA, TLR2 rs3804099 CC, and HLA-G rs9380142 AA genotypes were clearly associated with improve SCD outcomes while the HLA-G rs9380142 G allele might increase risk of cholelithiasis. More studies have suggested that certain gene polymorphisms like UGT1A1 gene and alpha thalassemia enhance bilirubin levels and cholelithiasis in sickle cell anemia (Inusa et al., 2019b; Knisely et al., 2023; Tozatto-Maio et al., 2019, 2020a, 2020b).

UTB is a gene encoded by SCL14A1. It is mostly expressed in erythrocytes and bone marrow. Researchers have suggested the crucial role of UTB in the HU pharmacokinetics. They found that overexpression of UTB gene in erythrocytes strongly associated with HU enhancement that mediate HbF production. Furthermore, studies have specifically implicated that UTB rs12605147 and rs2298720 SNPs in influencing in HU pharmacokinetics parameters in SCD patients (Marahatta et al., 2020; McDade et al., 2009; Olives et al., 1994; Walker, Steward, et al., 2011;

Walker & Ofori-Acquah, 2017; Yahouédéhou et al., 2018b). Notably, specific studies on the UTB gene polymorphism are limited, and there is no such study exist within Iraqi population. Therefore, the current study aims to further explore UTB rs12605147 and rs2298720 SNPs impact on the response to HU in the individuals with sickle cell disease and the possibility of UTB gene variations influence the SCD clinical manifestations and severity.

It has been found that a homogenous genotype distribution of the UTB rs2298720 SNP with all the individuals with SCD that possessing the GG (wild alleles). Clearly, the results did not find variations in the in the genotypes of the heterozygous (GA) or homozygous mutants (AA). Interestingly, these results consistence and supported by the Hardy-Weinberg equilibrium analysis, as the observed genotype frequencies closely mirrored the expected frequencies. In fact, the GG genotype was present in all individuals in the population, indicating a lack of genetic diversity at this particular locus in the group of patients under investigation. The homogeneity of the GG alleles provides thorough onto genetic of SCD of the studied patents. This potentially might help to develop of new therapeutic mechanisms. Interestingly, these findings can be used as a reference in comparative studies involving a verity of populations or different genotypes. Furthermore, the lack of GA or AA alleles in the studied population could lead to significant association studies. For example, previously scientists have identified that genetic variants associated with extreme levels of fetal hemoglobin in SCD patients in Tanzania. In the other hand, the homogeneity of GG genotype may be potential prediction modeling for SCD patients. Hence, it is worth to suggest that these findings apply only to the specific population that was studied. Genetic diversity can greatly vary between different groups, so

caution should be taken when extrapolating these results to other populations (Da Guarda et al., 2020; Kosiyo et al., 2021; Nkya et al., 2020).

Previously, scientists suggested that no SNPs were defined as predictive of hydroxyurea pharmacokinetic (PK) parameters, which limited understanding of HU absorption, distribution, metabolism and excretion. Nevertheless, several studies predicted an association between measures of exposure to HU and gene variations in the genes that encode the urea transporters including UTA and UTB, which may warrant further exploration (Galactéros et al., 2019; Sales et al., 2022; Ware et al., 2011; Wyszynski et al., 2004). This study examined UTB rs12605147 SNPs in a cohort of 50 patients with SCA. It has been found that the AA (wild type) genotype is the most prevalent at 78%, followed by the AG (mutant heterozygote) genotype at 18%, and the GG (mutant homozygote) genotype at 4%. The allele frequencies indicate a higher prevalence of the A allele (87%) compared to the G allele (13%). Fischer exact tests reveal significant differences in genotype distributions, with highly significant P-values (< 0.0001) for AA vs AG, AA vs GG, and AG vs GG comparisons, suggesting a strong association between the rs12605147 SNP and sickle cell anemia. Intriguingly, Hardy-Weinberg predicted that the rs12605147 genotype significantly deviated from the equilibrium for AG/GA, AA, and GG genotypes, with P-values below 0.05. These findings suggest a deviation from the expected genetic equilibrium at the rs12605147 locus in the studied sickle cell anemia patient population.

Exploring the correlation between gene variations and patient's demographic characterization can contribute to the field of personalize medicine. Treatment and managing care can be modified based on a person's genetic background to increase efficacy and reduce side effects and therapy resistance. Furthermore, studying the impact of gene variations

on the persons demographic characterizations can contribute to the drug development. If ascertain genotype is associated with a particular health outcome, it could potentially be targeted with specific drugs (Beichman et al., 2017; Yashin et al., 1999). Therefore, this study explored the demographic characteristics grouped by UTB rs12605147 genotypes (AA, AG, GG). The data reveals that among females, 46% have AA, 12% AG, and 4% GG. According to the age category14-23, 62% have AA and 14% AG, while in the 24-33 group, 12% have AA and 2% AG. The majority receiving 10 mg/kg/day have AA (32%), while those receiving 15 mg/kg/day are 32% AA, 6% AG, and 2% GG. Hospitalization frequency varies, with 54% of AA genotype individuals having none, while the remaining 24% required hospitalization. Fever is absent in 60% of AA individuals, while the 18% having fever. Temperature is normal (36.5-37°C) in 60% of AA individuals. Blood transfusions are rare in the AA group (66% none, 10% once). Pain intensity and frequency vary; 12% of AA individuals report no pain, while 30% report moderate pain. Among AG individuals, 2% report mild pain and 4% moderate pain. The Centers for Disease Control and Prevention (CDC) provides prevalence data suggesting that SCD impact around 100000 American, occurring in approximately 1 out of every 365 black African American births and 1 out of every 16300 Hispanic American births. Additionally, studies have delved into the relationship between gene polymorphism in inflammatory genes and SCD complications, taking into consideration geographical origin, SCD genotype, red blood cell transfusion rates, and gender. Furthermore, the impact of SCD type, age, and gender on the prevalence of end-organ damage and morbidity is being investigated by researchers. Lastly, the connection between genetic variations and the severity of sickle cell disease is also being explored, providing valuable insights into the matter (Osarogiagbon et al., 2009; Snyder et al., 2022; Tozatto-Maio et al.,

2019, 2020b, 2020a; Zhang et al., 2016). The current study, it has been unable to locate any studies that directly compare UTB rs12605147 genotypes based on demographic information. This could be due to the fact that this particular area of research is relatively new and novel, or that the relevant studies are not easily accessible online. Indeed, recent HbF concentration is a major marker for the response to treatment in SCD patients. HbF is a major genetic modifier of clinical expression in SCD. For instance, levels of HbF between (10-20) % have been suggested to improve survival and reduce vaso-occlusive complications (VOCs). Recently, hematologists found a significant positive correlation between HbF concentration and the percentage of hematopoietic multipotent cells (HMCs). Unlikely, a negative correlation was observed between HbF levels and hematopoietic progenitor cells HPCs in the patients with SCD undergoing HU treatment (Buchanan, 2014; Minniti et al., 2020; Steinberg et al., 1997, 2014; S. Tolu et al., 2019; S. S. Tolu et al., 2019). Therefore, this study examined the effect of rs12605147 gene variations on recent HbF% in patients with SCD undergoing HU treatment. The current study categorized the study subjects into two groups: non-responders with HbF concentration less than 20% and responders with HbF concentration more or equal to 20%. The distribution of the AA, AG, and GG alleles among non-responders showed significant variations ($p<0.0001$), as revealed by the Fischer exact test. In responders, the same trend is observed with significant differences found for AA vs AG and AA vs GG (both $p<0.0001$), highlighting the influence of these genotypes on treatment response. in addition, AG was significantly different comparing to GG allele ($p=0.0010$). These findings align with previous studies that have shown a clear variation in HU response among SCD patients due to gene polymorphisms (Allard et al., 2022; Minniti et al., 2020; S. Tolu et al., 2019). Recently, studies suggested a significant correlation between

rs12605147 genotypes and patient responsiveness to HU treatment that emphasize the necessity for personalized treatment regime in SCD based on genetic factors. Taking all together, it is important to appreciate the potential effect of genetic variations and genetic backgrounds in the individualized treatment regimens for SCD patients. Proposing such strategies could improve patient outcomes and patient's well-being. It is important to show that the responses to treatments can differ among individuals, which may lead to the needily to consult with a healthcare professional when formulating a treatment plan (Minniti et al., 2020; Yahouédéhou et al., 2018b).

Recently, researchers discovered that people who have the GG genotype have more neutrophils and reticulocytes compared to those with the AA and AG genotypes. However, it was surprising to see that platelet counts were also not found to be different between these groups. Moreover, although people with the AA genotype generally had a higher level of hemoglobin (Hb), the difference was not significant enough from a statistical point of view. The results point out a likely association between the UTB rs12605147 genotype and the therapeutic effect of hydroxyurea in patients suffering from SCD. In this case, it can be assumed that the response to HU therapy in SCD is determined by individual genetic predisposition. It is worth to mention that these findings were limited the generalizability due to the small sample sizes for AG and GG gene variations. Hence more extensive studies are needed to prove these correlations between genetic factors and SCD and to better understand their clinical significance. It is important to shed the light of these discoveries as they are a part of the ongoing genetic studies into SCD, which might lead to develop more personalized treatment strategies. Therefore, this research underscores the understanding of the genetic factors that involved

in order to enhance to the development of more successful and effective treatments approach (Allard et al., 2022; Aninagyei et al., 2018; Antwi-Boasiako et al., 2018).

Examining pain pattern in patients with SCD is crucial due to its potential complexity, persistence and lasting even neuropathy. it is crucial to score pain intensity to generalize their influence on overall patient wellbeing. Interestingly, evaluating pain patterns in SCD patients has helped a shift in the evaluation and treatment their pain, which led to enhance their quality of life. Surprisingly, gene polymorphisms can influence pain experience in the patients with SCD. For instance, researchers found a significant association between chronic pain and certain gene polymorphisms. More studies have suggested that gene variations are linked to both acute and chronic SCD related pain (Chandrakala, 2013; Glaros & Brandow, 2020; Ramsay et al., 2021). Recently, scientists have explored the role of COMT and DRD3 SNPs in pain related care utilization among individuals with SCD. Other genes such as rs1611115, rs5324, and rs129882, have been widely suggested to associated with chronic pain in SCD patients (Chandrakala, 2013; Darbari & Brandow, 2017; Farrell et al., 2019; Glaros & Brandow, 2020; Matthie et al., 2020; Padhee et al., 2021; Ramsay et al., 2021). Therefore, this study interested to study the effect of rs12605147 gene variations impact on the intensity of pain in the SCD patients undergoing HU treatment. Surprisingly, the majority of AA genotype individuals reported mild to moderate pain, while the GG genotype showed fewer instances of pain, with none reporting the highest pain intensity. The present study further analyzed the pain intensity across genotypes using the Wilcoxon test, revealing a statistically significant difference between AA and AG genotypes ($P = 0.0186$). The actual mean pain intensity is highest in the

AA genotype (7.800), followed by AG (2.250) and GG (2.000). However, the small sample size for GG (n=2) necessitates caution in generalizing these findings. The findings suggest a potential association between UTB rs12605147 genotypes and pain intensity in HU-treated patients, which suggesting the need for larger studies to confirm and elucidate the clinical implications of these observations. To our knowledge, it is limited or no prior researches have explored the impact of UTB rs12605147 SNPs on pain intensity in SCD patients in particular among Iraqi population, rendering this study uniquely positioned to contribute novel insights into the genetic factors influencing pain experiences in this population. Lack of previous studies sparks the significance of current work and highlights the need for comprehensive research to establish a foundation for understanding the role of UTB rs12605147 SNPs in pain intensity. It is worth to mention that the existing literature predominately focused on other gene variations in different contexts. Recently, scientists have suggested that ICAM1 rs1799969 SNPs influencing pain experiences among the patients with SCD, and with those carrying one or more minor alleles displaying substantially lower average pain levels than their counterparts without a minor allele. More studies discovered DRD3rs6280 gene variations that noticeably associated with chronic pain intensity CPI scores, life quality and opioid equivalent doses, suggesting its role in opioid therapy for pain management in humans (E. Jhun et al., 2012; E. H. Jhun et al., 2018; Knisely et al., 2023).

The impact of UTB gene on the frequency of pain crisis in patients with SCD has been explored previously (Al-Habboubi et al., 2012). In this study, it aimed to examine whether UTB gene variations contribute to the severity and the frequency of pain crisis in SCD patients or not, which may improve of individualized and effective treatments for SCD patients in the

future (Darbari et al., 2020b; Gehling et al., 2023). The beauty of this study revealed that SCD patients with AA genotypes experienced significantly higher pain crisis frequency compared to those with AG and GG genotypes. The 95% confidence intervals provide a range for the likely true mean frequency of pain crisis. Interestingly, the majority of AA genotype individuals reported 34% "no pain crisis " and 16% " once per year, while the GG genotype showed fewer instances of pain crisis. This study further analyzed the frequency of pain crisis across genotypes using the Wilcoxon test, revealing a statistically significant difference between AA and AG genotypes (P = 0.0329). The actual mean frequency of pain crisis is highest in the AA genotype (0.156), followed by AG (0.036) and GG (0.0200). However, the small sample size for GG (n=2) necessitates caution in generalizing these findings. The findings suggest a potential association between UTB rs12605147 genotypes and frequency of pain crisis in HU-treated patients, which indicates further research will be required to validate these findings and clarify their therapeutic significance. Chronic pain is a substantial portion, and estimated at 30%–40%, of adolescents and adults with SCD. This group's experience with chronic pain is far from uniform, as the analysis of current scientific knowledge has shown. Some studies have delved into the correlation between gene polymorphism and persistent SCD pain, utilizing a Composite Pain Index (CPI) procured from a trusted tablet-based tool known as PAINReportIt to measure pain (Brandow et al., 2020; Hu et al., 2016; Powell-Roach, Yao, Jhun, et al., 2019; Sadhu et al., 2018). Meanwhile, other researchers have identified a chronic pain phenotype by utilizing Average Pain Intensity (API) scores, also derived from PAINReportIt, and a standardized approach to quantify sensory testing of heat, cold, and pressure thresholds that has been deemed safe for SCD patients (Ezenwa et al., 2016; Powell-Roach, Yao, Rutherford, et al.,

2019).

Understanding the complexity of pain in patients with SCD is challenging because the intensity and frequency of pain vary even among patients with the same genetic background. There are extensive studies have focused on understanding the mechanism of pain physiology signaling cascade in SCD patients. For example, scientists have appreciated the important role of ADRB2 gene in the pain signaling neurotransmitters in particular epinephrine. There is a strong correlation between ADRB2 gene variations and severity of pain perception in SCD patients. In addition, researchers have discovered GCH1 gene role in regulating chronic and acute pain by producing essential enzyme GTP cyclohydrolase for producing tetrahydrobiopterin (BH4), which a cofactor in neurotransmitter biosynthesis that affect the severity of pain perception (Belfer et al., 2014; Bonato et al., 2021; Ezenwa et al., 2016; E. Jhun et al., 2016; E. H. Jhun et al., 2019; Kushnir et al., 2013; Powell-Roach, Yao, Rutherford, et al., 2019; Wonkam et al., 2018). Not surprisingly, the current data align with previous findings and suggest that UTB genotype rs12605147 may be associated with the frequency of pain crises in patients receiving HU. More extensive studies are needed to deepen the molecular signaling pathways to fully understand the association between UTB rs12605147 gene variants and the severity of pain in SCD patients.

Around 80-100 SCD patients experience pain crises annually, and half of them required urgent medical intervention due to acute pain episodes. Recently scientists suggested a strong correlation between the increased pain rates and premature mortality in SCA patients aged over 20 (Heitzer et al., 2020; Higgs & Wood, 2008). In this current study, explored the link between clinical features of patients with SCD, such as pain intensity and frequency of pain crisis, and the presence of a genetic mutation known as

UTB rs12605147. When combining data from mutant and non-mutant individuals, an interesting finding became manifest: a strong negative correlation was observed (Pearson value = -0.9756, p-value = 0.0046). This suggests that pain intensity tends to decrease as the recent HbF percentage increases. A weak correlation (Pearson value = -0.8223, p-value = 0.1777) showed when calculate the strength of the group consisting only of mutant individuals, while the group without mutations or with the dominant alleles showed a strong negative correlation (Pearson value = - 0.9401, p-value = 0.0175). These findings unmask the possible impact of the rs12605147 mutation on the relationship between HbF levels and pain intensity in SCD patients. Likewise, in the combined group, the current study found a strong negative correlation (Pearson value = -0.9768, p-value = 0.0042), indicating that a higher current HbF percentage was associated with a lower frequency of pain crises. The Mutant-only and No Mutant-only groups show distinct correlations (Pearson values of -0.6092 and -0.9561, respectively), underlining the potential influence of the rs12605147 mutation on the relationship between recent HbF% and the frequency of pain crises in SCD patients. Previously, scientist explored the different type of gene SNPs impact on the association between pain crisis and the concentration on HbF% in SCD patients undergoing treatment. For instance, previously scientists revealed that there was an inverse association between HbF levels and pain rates. What is more intriguing is the fact that the G allele in the HMIP-2A rs7776196 SNP was the only one that was concerned with the lower incidence rate of painful episodes (Ballas & Lusardi, 2005b; Childerhose et al., 2023; Takaoka et al., 2021). Nevertheless, this study collectively reveals significant negative correlations between recent HbF% and both pain intensity and frequency of pain crises in SCD patients undergoing HU treatment, with distinct patterns observed in individuals with and without the rs12605147

mutation. This emphasizing the potential influence of this genetic variant on these relationships and highlighting the need for further investigation to guide personalized treatment approaches.

Conclusions

&

Recommendations

Conclusions

Based on the results of the present research, the following conclusions are below:

1. The study revealed a homogenous distribution of the GG genotype for UTB rs2298720. While UTB rs12605147 was polymorphic and detected in various genotypes frequencies in Iraqi SCA patients, the wild genotype AA was the dominant pattern among patients compared to mutant genotypes AG and GG.

2. Regarding the impact of UTB gene rs12605147 polymorphism, notably the rs12605147 genotype showed significant associations with treatment response, recent HbF levels, pain intensity, and pain crisis frequency.

3. Concerning to the effect of UTB gene rs12605147 genetic polymorphism, the present study demonstrated a significant and negative correlation between intensity of pain and recent HbF% in wild genotype, this means that the patients with wild genotype have good response to HU therapy and better improvement of clinical parameters than mutant genotypes who required higher doses of HU to reach the target of therapy.

4. Regarding the impact of UTB gene rs12605147 polymorphism, the existing finding revealed that was a significant and negative correlation between frequency of pain crisis and recent HbF% in wild genotype, while the mutant genotype was non-significant correlation.

5. The present results showed non-significant correlation between UTB gene rs12605147 polymorphism and hematological parameters at toxic range.

Suggestions and Future Studies:

More studies can be done to:

1. Increase the sample size to explore the robust effect of UTB gene polymorphisms on the disease progression and treatment response
2. Validate and replicate the findings in particular regarding the association of UTB (rs12605147 and rs2298720) SNPs with therapy and severity of clinical symptoms.
3. Conduct clinical trails to explore the development of treatment strategies based on the identified genetic variations.
4. Explore and investigate sex specific therapeutic approaches for treatment response given the observed sex differences in HbF%. Developing treatment strategies based on gender specific may shed the light to more effective management.
5. Conduct longitudinal studies that involve monitoring the long term effects of UTB gene polymorphisms on disease progression and severity of symptoms.
6. Explore the effectiveness of different molecular biology techniques such as gene therapy and CRISPR-Cas9 in light of UTB gene polymorphisms among Iraqi patients with SCD.

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Appendix

Ministry Of Higher Educatio
and Science Research
University of Karbala
College of Pharmacy
Department of Postgraduate Student

جمهـــورية العــراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الصيدلة
شعبة الدراسات العليا

Research title: Genetic Polymorphisms of Urea transporter B In Iraqi Patients with Sickle Cell Anemia Treated with Hydroxyurea.

MSc research project By Fatima Manssor

Supervised By prof. Dr. Ahmed Alkhezaly

2022/2023

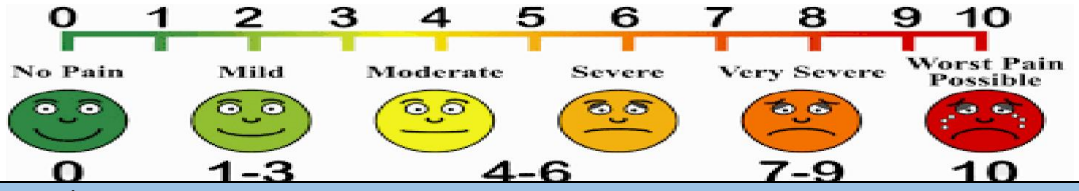
Patient Data

This form specific for sickle cell anemia (SCA) patients (HbSS) on hydroxyurea therapy.

Date:					
Patient no.:					
Name:					
Age:					
Date of birth:					
Gender: Male Female					
Marital status: Single Married					
Wight: kg					
Address: Phone no.:					
Information concern hydroxyurea therapy					
○ Dose of hydroxyurea: ○ Duration of treatment: ○ Date of starting therapy (if available):					
Clinical parameters					
Clinical parameters	None	One time/year	Two times/year	Three times/year	More than 3times/ year
1. Frequency of hospitalization					

2. Duration of hospitalization			Days/ time			Days/ time			Days/ time			Days/ time
3. Fever		37.5-38 ℃	38.1-39 ˚C	≥39.1℃	37.5-38 ˚C	38.1-39 ˚C	≥39.1˚C	37.5-38 ℃	38.1-39 ℃	≥39.1˚C	37.5-38 ˚C	38.1-39 ˚C	≥39.1℃
4. Acute chest syndrome												
5. Blood transfusion		No. pint			No. pint			No. pint			No. pint	
6. ICU admission												
7. Frequent painful crisis interferes with daily activities												

8. Intensity of pain by using pain scale: ……. degree of pain



Lap values

Element	Value	Normal range
1. Complete blood count (CBC)		
2. Platelet count		
3. Reticulocyte count		
4. Neutrophil count		
5. Hemoglobin		
6. HbF baseline level (Before start HU therapy)		
7. Recent HbF level (Patient on HU therapy)		

Genotyping analysis

SNPs	Genotypes	Frequency	Percentage
(rs2298720)			
(rs12605147)			

Consent

جمهـــورية العـــراق
وزارة التعليم العالي والبحث العلمي
جامعة كربلاء
كلية الصيدلة
شعبة الدراسات العليا

Ministry Of Higher Educatio
and Science Research
University of Karbala
College of Pharmacy
Department of Postgraduate Student

وثيقة الموافقة التحريرية

قد اطلعت على استمارة الموافقات الأخلاقية، او تمت قراءتها لي، وأدركت مضمونها ولقد أتيحت لي الفرصة لإجابتي على جميع الأسئلة التي تدور في ذهني، ولهذا انا بكامل الحرية والاختيار ولست مجبراً بالمشاركة في البحث ولقد فهمت ان الباحثة والطبيب المسؤول ومساعدين الباحثة سيكونون مستعدين للإجابة عن جميع اسئلتي المستقبلية.

كما أعلم أنني املك الحرية والاختيار في الانسحاب من البحث متى شئت ولو بعد الموافقة التحريرية ومصادقتها من دون ان يؤثر ذلك على العناية الطبية المقدمة لي.

اسم المشترك **توقيع المشترك** **التاريخ**

إذا كان المشترك امياً

لقد شهدت القراءة الدقيقة لنموذج الموافقة، وأتيحت الفرصة للمريض المشارك لطرح الأسئلة اوكد ان الفرد قد وافق بحرية.

أسم الشاهد **توقيع الشاهد** **التاريخ**

بصمة ابهام المشارك

بيان من الباحث

لقد قرأت بدقة ورقة المعلومات الخاصة بالمشارك، وأوكد ان المشاركين قد سئلوا عن الدراسة وتمت الإجابة على جميع الأسئلة بشكل صحيح والى أقصى حد قدرتي. وقد أعطيت هذه الموافقة التحريرية بحرية وطواعية.

اسم الباحثة **توقيع الباحثة** **التاريخ**

اسم الباحث:

الجزء الأول: ورقة المعلومات

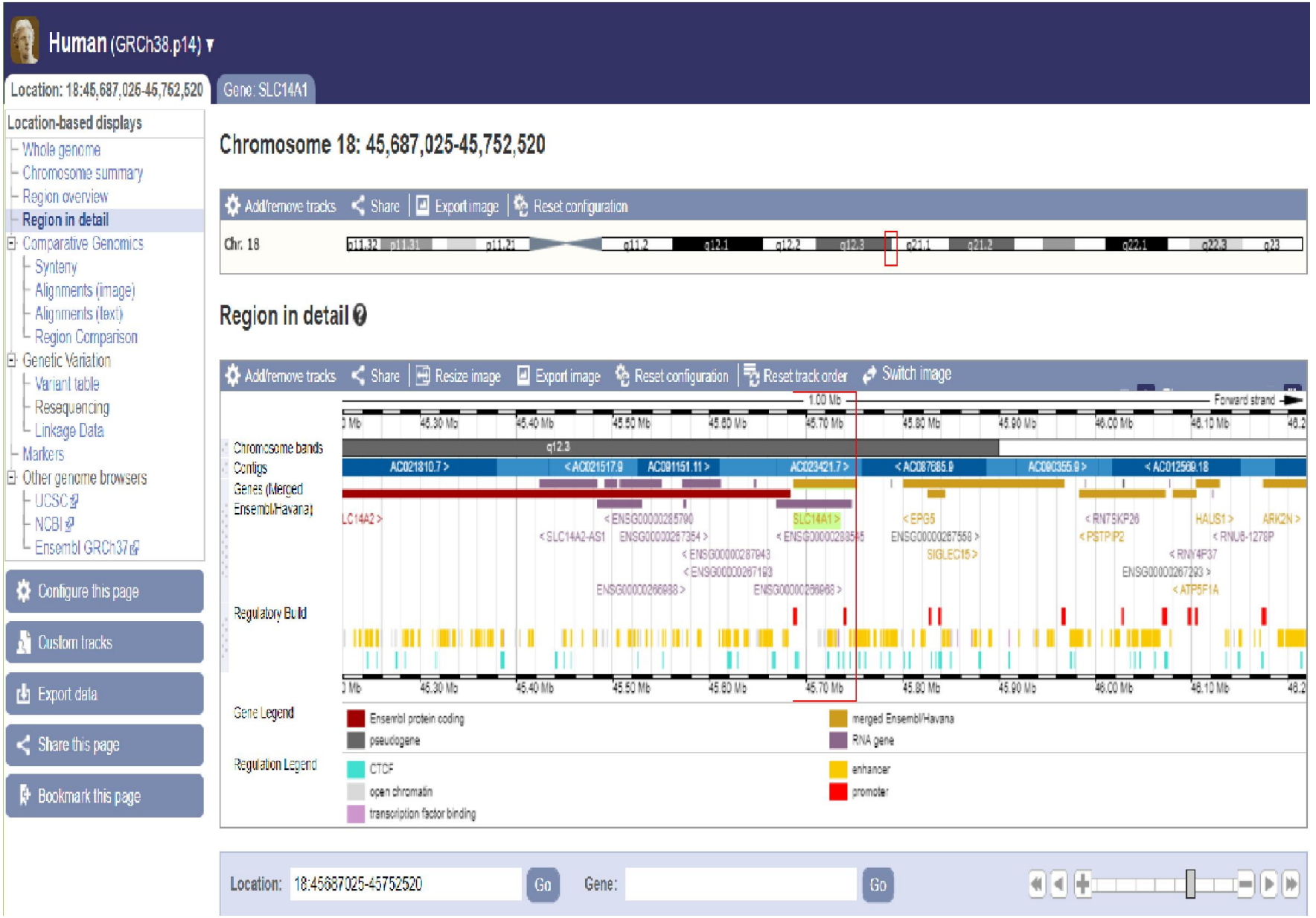
انا الباحثة/فاطمة بنت منصور ... أقوم بأجراء بحث حول مرضى فقر الدم المنجلي، وهو امر شائع جدا في هذا البلد سأقدم لك معلومات وأدعوك لتكون جزءاً من هذا البحث. قد تكون هناك بعض الكلمات التي لا تفهمها من فضلك أطلب مني التوقف عندما نتصفح المعلومات وسوف أقوم بتوضيحها لك بصورة مفصلة. أذا كانت لديك أي أسئلة، يمكنك ان تسألني أو تسأل طبيب الدراسة أو فريق العمل.

(مرض فقر الدم المنجلي هو أحد الامراض الوراثية شيوعا ومضاعفاته الخطيرة على المصابين به في جميع أنحاء العالم، العقار المفيد للغاية والرئيسي الذي يستخدم حالياً لعلاج المصابين بفقر الدم المنجلي هو عقار هيدروكسي يوريا والسبب في اجراء البحث اننا نحقق في الاستجابة السريرية للهيدروكسي يوريا وفقا للتركيب الجيني)

يتضمن هذا البحث سحب عينة من الدم من احدى اوردة اليدين اثناء زيارتك للمتابعة الدورية في المركز "مركز امراض الدم التابع لمستشفى الحسين ‘التعليمي "، مشاركتك في هذا البحث تطوعية تماما. سواء اخترت المشاركة أم لا، ستستمر جميع الخدمات التي ستتلقاها في المستشفى ولن يتغير شي.

نريد اخذ عينة من الدم من احدى اوردة اليدين، وفي نهاية البحث، وفي غضون سنة واحدة، سيتم تدمير أي من العينات المتبقية، البحث يجري لمدة شهر. خلال ذلك الوقت، سيكون من الضروري الذهاب للمركز المذكور أعلاه كل ثلاثة أشهر كمتابعه روتينية دورية سريرية دون أي تدخل في العلاج الموصوف أو تغيير في الجرعة وبدون سحب أي عينة دم من قبل الباحث فيما يخص البحث.

المعلومات التي نجمعها لهذا المشروع البحثي ستكون سرية تماماً، وأي معلومات عنك لا تتم مشاركتها مع أحد أو منحها لأي شخص باستثناء طبيبك وسوف يكون لها رقم بدلاً من اسمك، وسوف يعرف الباحثون فقط ما هو رقمك وسنقوم بحبس ذلك بقفل ومفتاح.


Human (GRCh38.p14)
Location: 18:45,687,025-45,752,520
Gene: SLC14A1
Location-based displays
Whole genome
Chromosome summary
Region overview
Region in detail
Comparative Genomics
Synteny
Alignments (image)
Alignments (text)
Region Comparison
Genetic Variation
Variant table
Resequencing
Linkage Data
Markers
Other genome browsers
UCSC
NCBI
Ensembl GRCh37
Configure this page
Custom tracks
Export data
Share this page
Bookmark this page
Chromosome 18: 45,687,025-45,752,520
Add/remove tracks
Share
Export image
Reset configuration
Chr. 18
Region in detail
Add/remove tracks
Share
Resize image
Export image
Reset configuration
Reset track order
Switch image
1.00 Mb
Forward strand
Chromosome bands
Contigs
Genes (Merged Ensembl/Havana)
Regulatory Build
Gene Legend
Ensembl protein coding
pseudogene
merged Ensembl/Havana
RNA gene
Regulation Legend
CTCF
open chromatin
transcription factor binding
enhancer
promoter
Location:
18:45687025-45752520
Go
Gene:
Go

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جمهـــورية العـــراق
وزارة التعليم العالي والبحث العلمي
جامـــعة كربلاء
كلـــية الصيـــدلـــة
شعبة الدراسات العليا

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امر اداري

استناداً الى اللجنة المشكلة بموجب الامر الاداري ذي العـــدد د.ع/6/ 1450 في 6 / 9 /2022 لمناقشة وأقرار البحوث المقدمة من قبل طلبة الدراسات العليا في فرع الادوية والسموم للعام الدراســـي 2022-2023

واستنادا الى الصلاحيات المخولة لنا تقرر اقرار بحوث طلبة الدراسات العليا /ماجستير/ الادوية والسموم والمدرجة تفاصيلهم في الجدول ادناه :

ت	أسم الطالب	عنوان البحث	المشرف
1	روان آزاد محمد	Effects of Genetics Polymorphism of ABCB1 Transporter on Prednisolone Thearpy in Iraqi Nephrotic Syndrome Patients	المشرف الأول :- أ.م.د. احمد صالح صاحب
2	فاطمة بنت منصور بن حسن	Genetic Polymorphisms of Urea transporter B In Iraqi Patients with Sickle Cell Anemia Treated With Hydroxyurea.	المشرف الاول :- أ.د. احمد صالح صاحب
3	ناديه صاحب عبد علي	impact of genetic polymorphism of Cytochrome P450 3A4 on clomiphene citrate clinical response in Iraqi woman with PCOs.	المشرف الأول :- أ.م.د. أثير ماجد رشيد
4	مريم حسين عبد علي	Impact of genetic polymorphism of Cytochrome P450 2D6 on clomiphene citrate clinical response in Iraqi PCOS	المشرف الاول :- :- أ.م.د. أثير ماجد رشيد المشرف الثاني:- حسن محمود موسى
5	نبأ زهير ميران	impact of the thyroid hormone receptor alpha gene 1 on therapeutic response of levothyroxine in iraqi hypothyroidism patients	المشرف الاول :- :- أ.م. زهراء عبد الكريم محمد علي

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الخلاصة

مقدمة: يظهر مرض فقر الدم المنجلي (SCD) بدرجات متفاوتة من الشدة السريرية تتأثر بالعوامل الوراثية، بما في ذلك تعدد الأنماط الجينية لناقل اليوريا داخل جين UTB

الهدف: تقييم هذه الدراسة تأثير تعدد الأنماط الجينية لـناقل اليوريا B rs2298720 و rs12605147 على الاستجابة لعلاج هيدروكسي يوريا(HU) ، بهدف توجيه الطب الشخصي في إدارة مرض فقر الدم المنجلي.

المنهجية: تم تحديد النمط الجيني لعينة من 50 مريضًا عراقيًا مصابًا بمرض فقر الدم المنجلي لتعدد الأنماط الجينية لناقل اليوريا B rs12605147 و rs2298720 باستخدام تفاعل الكوثرة المتسلسل المتخصص بالأليل ، مع تسجيل بيانات ديموغرافية وسريرية شاملة. تم تسجيل شدة الألم على مقياس من 10 نقاط، وتم تقييم تكرار أزمة الألم على مدى فترة 12 شهرًا، وتم قياس مستويات HbF . تم تقييم استجابة HU من حيث الانخفاض في أزمات الألم وزيادة مستويات HbF بين المرضى الذين لديهم أنماط وراثية مختلفة.

النتائج: ارتبط تعدد انماط UTB rs12605147 بشكل كبير بالنتائج السريرية الرئيسية. أفاد المرضى الذين لديهم النمط الجيني AA بمتوسط درجة ألم أقل بلغ 4.5 ± 0.8 مقارنة بأولئك الذين لديهم النمط الجينيGG ، والذين كان متوسط درجة الألم لديهم 7.2 ± 1.1($p < 0.01$) .

كما تباينت وتيرة أزمة الألم حسب النمط الجيني، حيث عانى حاملو النمط الجيني AA من متوسط 2.3 ± 1.0 أزمة في السنة مقابل 5.8 ± 1.5 أزمة في حاملي النمط الجيني GG ($p < 0.05$).

كانت مستويات HbF أعلى بشكل ملحوظ في مرضى النمط الجيني AA ($24.83 \pm 4.33\%$) مقارنة بمرضى النمط الجيني GG ($20.80 \pm 0.00\%$) ($p < 0.001$) بعد العلاج بدواء HU. تشير هذه النتائج إلى أن النمط الجيني AA قد يمنح ملفًا سريريًا أكثر ملاءمة واستجابة أفضل لـ HUفي مرضى فقر الدم المنجلي . وعلى العكس من ذلك بالنسبة لـ SNP rs229872، أظهر جميع المرضى النمط الجيني من النوع السائد ، مما يشير إلى عدم وجود تباين ملحوظ في هذا SNPبين المجموعة، وبالتالي لا يمكن رسم أي ارتباط بالمعايير السريرية لـ rs2298720 .

الاستنتاجات: يرتبط تعدد أنماط UTB rs12605147 باختلافات كبيرة في شدة الألم وتواتر الأزمات ومستويات HbF في مرضى فقر الدم المنجلي، مما يشير إلى دور محتمل في تحديد فعالية HU والشدة السريرية. يشير غياب التباين في rs2298720 إلى أنه قد لا يساهم في التباين السريري في مرض فقر الدم المنجلي ضمن هذه العينة. تدعم النتائج مناهج الطب الشخصي لمرض فقر الدم المنجلي، حيث يمكن أن يساعد تحديد النمط الجيني UTB rs12605147 في إعلام استراتيجيات العلاج التي تهدف إلى تحسين الإدارة والنتائج الخاصة بالمريض.

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة كربلاء

كلية الصيدلة

الانماط الجينية المتعددة الناقلة لليوريا B عند المرضى العراقيين المصابين بفقر الدم المنجلي المعالج بالهيدروكسي يوريا

رسالة

مقدمة الى كلية الصيدلة – جامعة كربلاء

كجزء من متطلبات الحصول على شهادة الماجستير في علم الادوية والسموم

من قبل

فاطمة بنت منصور بن حسن الطويل

بكالوريوس صيدلة (كلية الصيدلة\جامعة الملك سعود بالرياض 2008)

بإشراف

الأستاذ الدكتور
أحمد صالح صاحب

الأستاذ المساعد الدكتورة
شيماء اموري جبار

2024 ميلادي

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