Personalizing Pain Management in Sickle Cell Disease:
A Pilot Study in the Cytochrome P450 2D6 Gene Polymorphisms in Pediatric Sickle Cell Disease

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Abstract

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Introduction:

Opioid analgesic medications are used in patients with sickle cell disease (SCD) for the treatment of vaso-occlusive crisis (VOC). Oral opioids require enzymatic modification by the CYP2D6 enzyme to become active analgesic compounds. The CYP2D6 gene is highly polymorphic with varying degrees of activity from ultra rapid to no activity. The primary objective of this study is to determine the prevalence of cytochrome P450 CYP2D6 gene polymorphisms in a large sickle cell patient population.

Methods:

Patients were screened to determine eligibility at the time of routinely scheduled well-visit in Sickle Cell Clinic. Once consent and/or assent were obtained, the patient's medical history and medical record was reviewed to record the documented hemoglobinopathy. Whole blood samples were analyzed using CYP2D6 genotyping assay. The Luminex xTagTM assay system was used for PCR amplification of 12 small nucleotide polymorphisms (SNPs) and two gene rearrangements.

Results:

This was a prevalence study to determine the ratio of CYP2D6 polymorphisms among pediatric patients with SCD recruited from the Comprehensive Sickle Cell Program of Children's Healthcare of Atlanta at Egleston Hospital. The greatest percentage of patients genotyped were intermediate metabolizers (50%) compared to those who were poor metabolizers (19.74%), extensive metabolizers (18.42%) and ultra-rapid metabolizers (11.84%).

Conclusions:

The intermediate metabolizers were the dominant variant among the patients tested rather than the poor metabolizers as we had proposed at the beginning of the study. Although the poor metabolizers were not the majority, the percentage of 19.74 put them at the upper limit of the range given for the black adult population. Because intermediate metabolizers dominate the study population and are noted for a reduction of function of the CYP2D6 enzyme, this may contribute to the poor drug efficacy and hospitalizations in this pediatric population.

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I. Introduction

Opioid analgesic medications are used frequently in patients with sickle cell disease (SCD) for treatment of vaso-occlusive crisis (VOC). The commonly prescribed oral opioids codeine, hydrocodone, and oxycodone are inactive prodrugs that require enzymatic modification to become active analgesic compounds by the cytochrome 450 2D6 enzyme.

Cytochrome P450 enzyme CYP2D6 is involved in the metabolism of approximately 20-25% of commonly prescribed medications. (1) These include previously mentioned opiates as well as antidepressants, antipsychotics, beta-blockers, and anti-emetics. CYP2D6 is the enzyme that activates codeine, hydrocodone, and oxycodone via O-demethylation of these compounds. Two other enzymes within the CYP2 family (CYP2C19 and CYP2C9) are also able to activate the prodrugs via O-demethylation; however the role of these two enzymes is minor compared to CYP2D6. The cytochrome P450 enzyme CYP3A4 performs N-demethylation and is responsible for the inactivation of these prodrugs.

The CYP2D6 gene is highly polymorphic, with over one-hundred allelic variations discovered to date. These genetic polymorphisms can be classified according to their resultant CYP2D6 activity:

1. Extensive Metabolizers (EM) have normal (wild type) enzymatic activity
2. Intermediate Metabolizers (IM) have reduced enzymatic activity
3. Poor Metabolizers (PM) have very low or no enzymatic activity. This phenotype is the result of either homozygous or double heterozygous non-functional alleles

4. Ultrarapid Metabolizers (UM) have very rapid enzymatic activity. The active metabolites are produced at rapid rate, with potentially life-threatening intoxication as a result. The UM phenotype is a result of duplication of functional CYP2D6 alleles.

Vaso-occlusive pain crisis, one of the primary complications of Sickle Cell Disease, is responsible for a majority of medical visits for sickle cell patients. These patients are treated in outpatient setting with oral opioids such as codeine. Individual response to pain medications varies and this variation is linked to polymorphisms in the cytochrome P450 locus responsible metabolizing these medications. The goals of this study are to determine the prevalence of the CYP450 2D6 allele in a cohort of pediatric sickle cell patients and to obtain preliminary data for a pharmacokinetic study to determine the correlation between the CYP450 polymorphisms and oral opiate metabolism.

II. Background

Sickle Cell Disease and Vaso-Occlusive Pain:

Sickle Cell Disease (SCD) is an inherited monogenetic disorder caused by a mutation encoding abnormal adult hemoglobin. Normal red blood cells are round and can move through small blood vessels to carry oxygen. In SCD, the blood cells become hard and sticky and have a C-shaped appearance. As they travel through blood vessels, they get stuck and clog the vessels (vaso-occlusion), disrupting blood flow and leading serious problems such as pain. The sickled blood cells also die early causing a shortage of red blood cells in the SCD patient. (2) It affects millions of people throughout the world and is among those whose ancestors come from sub-Saharan Africa, Spanish-speaking regions of the Western Hemisphere, Saudi Arabia, India and Mediterranean countries such as Turkey, Greece and Italy. In the United States more than 70,000 people have sickle cell disease and it occurs in 1 in 500 of every African American births. 2 million people have sickle cell trait and 1 in 12 African-Americans have sickle cell trait. (3)

One of the most intractable problems encountered by SCD children are the painful episodes associated with vaso-occlusive crisis (VOC). VOC can be triggered by many factors including hypoxia, acidosis, dehydration, or infections. Any one or combination of these events can result in polymerization of deoxygenated Hemoglobin S and sickling or the blood cells. The deformed blood cells enter capillaries and attach to the capillary endothelium, compromising blood flow and resulting in ischemia. The

ischemia leads to tissue damage and cell necrosis that gives rise to pain. This pain is unique due to its unpredictable, recurrent and persistent nature. Vaso-occlusive pain is a principle cause of morbidity in SCD children and accounts for a significant number of Emergency department visits and hospitalizations. The standard treatment for SCD patients is rest, rehydration and analgesia. However pain control for these patients is a difficult and complex process that requires continuous adjustments in pain management. (4)

Clinical manifestation of sickle cell disease varies among the different genotypes (5).

1. Sickle Cell Disease: Patients with the most severe sickle cell disease are those who are homozygous for the HbS and have two copies of the HbS gene. The term sickle cell anemia is generally used to describe these patients.
2. Sickle Cell Trait: These patients are benign carriers with no hematologic manifestations. They have one copy of the HbS gene and one normal hemoglobin gene.
3. Hemoglobin SC Disease: Patients with are heterozygotes with one copy each of HbS and HbC gene. The clinical course is between that of patients with sickle cell anemia and sickle cell trait.
4. Sickle Beta Thalassemia: There is impaired production of beta globin chains leading to an excess of alpha globin chain. The alpha globin chains are unstable, incapable of forming soluble tetramers on their own and precipitate within the cell. The clinical severity of sickle beta thalassemia is an inverse function of the quantity of Hb A. There are two categories of Sickle Beta Thalassemia (sickle cell-beta (0) thalassemia and sickle cell-beta (+)

thalassemia) based upon the absence or presence of reduced amounts of beta globin and therefore Hb A. Patients with sickle cell-beta (0) thalassemia may have less severe disease than those with HbSS disease.

The HbS gene is the common factor in all of these diseases. It is responsible for the sickling of the cells that leads of these patients varying degrees of vaso-occlusive pain. This is why we looked at all of the different variants in this study.

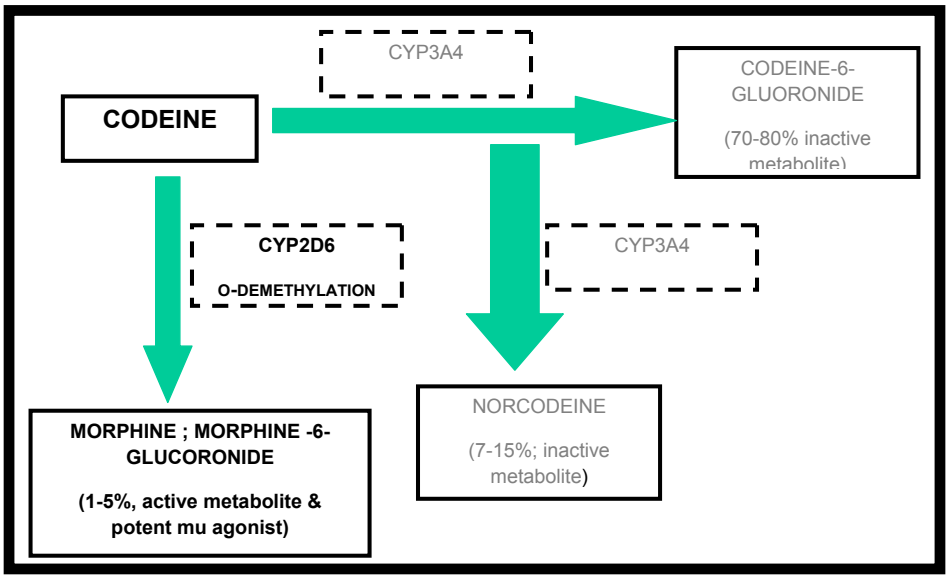
Opiate Metabolism and Cyp450 Polymorphisms:

Opioid analgesics are central to the management of VOC in SCD and exert their effects at specific opioid receptors in the spinal cord and on peripheral afferent nerves. Opioid receptors are classified as either mu **(μ),** delta **(δ)** or kappa **(Ќ)** receptors. Parenteral opiates work by directly binding to opiate receptors after administration and typically their onset of action is short and their analgesic effect is mostly predictable. On the contrary, the clinical response to oral opioids varies widely among individuals. While intravenous opiates such as dilaudid (hydromorphone), morphine and fentanyl are very potent and metabolically “active” analgesics with proven efficacy in the management of inpatient acute pain episodes, the availability of oral opiate formulations that offer consistently quick and sustained pain relief for use in the outpatient setting is critical in this population.

Oral opioid analgesics are used frequently in patients with SCD for treatment of VOC. Codeine (C), hydrocodone, and oxycodone are the three most common oral

opiates used in the ambulatory management of VOC in SCD. They are inactive pro-drugs that require enzymatic modification via the Cytochrome P450 enzyme system to become active analgesic compounds. CYP450 is a large group of mono-oxygenase enzymes responsible for the metabolism of toxic hydrocarbons. The clinical role of the CYP450 enzymes is that of drug metabolism with over 70% of xenobiotic drugs being metabolized by the hepatic cytochrome P450 enzymes. CYP450 enzymes are found in the endoplasmic reticulum, liver, small intestine and mitochondrial membrane. (6,7)

Figure 1: Mechanisms of Codeine Metabolism



Codeine is the most common oral opiate drug used in pediatrics. It is a weak agonist at the mu receptor and its analgesic effect is dependent on conversion to active morphine analogues. O-demethylation of codeine by CYP2D6 to morphine and morphine-6-gluconrinide accounts for less than 10% of total codeine clearance, yet is the sole mechanism by which circulating codeine is converted into active analgesic

substances (Figure 1). (8) Approximately 70% of a dose of codeine is glucoronidated to codeine-6-glucoronide, a poor opioid agonist and 7-15% of the dose is converted by N-demethylation to norcodeine by CYP3A4, (both inactive metabolites). Codeine has a favorable side effect profile in pediatrics and much more is known about the influence of CYP450 on codeine metabolism compared to other oral opiates making it the ideal target for this pilot study.

CYP2D6 Gene Polymorphisms and Metabolic Activity

The CYP2D6 gene is highly polymorphic, with over one-hundred allelic variations discovered to date (http://www.cypalleles.ki.se). These Allelic variants are classified according to the activity of the cognate enzyme into:

1. ***Extensive Metabolizers (EM)*** represent the normal phenotype. Patients have normal (wild type) enzymatic activity due to the presence of normal alleles.
2. ***Intermediate Metabolizers (IM)*** have reduced enzymatic activity due to a combination of active, inactive or partially active alleles. The result is decreased function of the CYP2D6 enzyme making oral medications less effective than the extensive metabolize genotype.
3. ***Poor Metabolizers (PM)*** have very low or no enzymatic activity or analgesic effects after codeine (9,10) This phenotype is the result of either homozygous or double heterozygous non-functional alleles and patients have extremely low concentrations of both plasma and urinary active metabolites morphine and morphine glucoronide (11,12,13)

4. ***Ultra rapid Metabolizers (UM)*** have very rapid enzymatic activity. The active metabolites are produced at rapid rate, with potentially life-threatening side effects as a result. (7,13) The UM phenotype results when there are duplications of functional CYP2D6 alleles. Patients have been reported to have over 45 times the concentration of O-demethylated codeine metabolites when compared to PM's. (9)

Allelic Variation among African Americans

The prevalence of the poor and ultra-rapid metabolizer phenotypes varies among populations of African descent. Figure 2 shows that among black or African American subjects the percentage of poor metabolizers varies from 0 to 20 percent while among subjects from the African Continent the percentage of Poor Metabolizers is smaller at around 2 percent.

Figure 2

Prevalence of Poor Metabolizer & Ultra Extensive Metabolizer Phenotype Cytochrome P450 2D6 Enzymes by Race/Ethnicity							
White		Asian		Black		Ethiopian/Saudi Arabian	
Poor Metabolizer	Ultra rapid Extensive Metabolizer	Poor Metabolizer	Ultra rapid Extensive Metabolizer	Poor Metabolizer	Ultra rapid Extensive Metabolizer	Poor Metabolizer	Ultra rapid Extensive Metabolizer
5-10	1-10	1	0-2	0-20	2	1.8-2	10-29

Table modified from Rogers et al, Am J Medicine 2002, 113 (10)

Only one study specifically looked at the pediatric sickle cell population. (14) The authors of this study hypothesized that children taking hydroxyurea who fail codeine therapy have an increase in reduced-functioning CYP2D6 alleles. The children genotyped in this study were seen in the emergency department after failing codeine therapy and comparison was made between those taking hydroxyurea and those with mild disease. For this study, severe disease is defined as 3 or more hospitalizations for vaso-occlusive pain over a period of three years. All others are considered mild. Of the 73 children completing the study, 42 had reduced-functioning alleles; 82% of the 27 children taking hydroxyurea had reduced-functioning alleles, versus 47% of 36 those with mild disease. Activity scores were decreased in 78% of the children taking hydroxyurea and in 44% of those with mild disease. The authors concluded that children taking hydroxyurea with sickle cell pain crisis after failing outpatient codeine were significantly more likely to have reduced CYP2D6 activity.

III. Methods

Hypotheses:

Our primary hypothesis was that patients with sickle cell disease will have a higher prevalence of the poor metabolizer genotype as determined by specific CYP2D6 alleles. We developed a secondary hypothesis to compare the poor metabolizers to the other metabolizers stating that poor metabolizers would have a greater number of hospitalizations when compared to the combined intermediate metabolizers, extensive metabolizers and ultra-rapid metabolizers.

Our study has two specific aims:

- to determine the prevalence of the CYP2D6 in a large pediatric sickle cell population; and
- to identify pediatric sickle cell patients with a history of hospitalizations due to vaso-occlusive pain crisis AND TO use this information to compare the poor metabolizers to the other genotypes as a group.

Study Design:

This project was a descriptive epidemiologic study using pediatric SCD patients to establish prevalence data of CYP2D6 polymorphic variants. The subgroups include IM (intermediate metabolizers), URM (ultra-rapid metabolizers), EM (extensive metabolizers or wild type / normal metabolism) and PM's (poor metabolizers).

Procedures/Methods

The prevalence study of CYP450 genotype polymorphisms was performed on the first 100 consecutive SCD patients enrolled in study. A sample of each patient's blood was collected in a purple tube using blood left over after routine labs run as part of the patient's clinic visit.

Genotyping was done for isoforms 2D6, with the Multiplex-it kit using the following procedures.

A. Multiplex PCR

Tubes of PCR Primer Mix A, PCR Primer mix B and the 10X LA PCR Buffer were thawed and brought to room temperature. The tubes were vortexed for 2-5 seconds to mix reagents and centrifuged (2-5 seconds) to bring reagents to the bottom of tubes. 76 PCR tubes were labeled 1A to 76A. A second set was labeled 1B to 76B. 1.5 ml microcentrifuge tube was labeled MM. The PCR master mix was prepared as follows:

Master Mix Reagent	**76 Samples**
DNase free water	1233.100 µl
10X LA Taq Buffer	253.080 µl
LA Taq Polymerase HS Version	33.820 µl
Total Volume	1520.000 µl

The PCR Master Mix was vortexed and centrifuged briefly to collect reagents at bottom of tube. One 1.5 mL microcentrifuge tube MMA was labeled and another tube MMB. The Master Mix and Primer Mix A or Primer Mix B were aliquoted as follows:

Master Mix A	
Primer Mix A	228.0 µl
Master Mix	684.0 µl

Master Mix B	
Primer Mix B	228.0 µl
Master Mix	684.0 µl

Master Mixes A and B were vortexed and centrifuged briefly to collect reagents at bottom of tube.

12 µl was aliquoted from tube MMA to each PCR tube labeled 1A to 76A 11. 12 µl was aliquoted from tube MMB to each PCR tube labeled 1B to 76B. 12. For each sample, 3 µl of DNA template was added to PCR A tubes and 3 µl DNA template to PCR B tubes. Each tube was vortexed and centrifuged briefly to collect reagents at bottom of tube and then transferred to thermalcycler and cycled using the following conditions:

Thermalcycler temperature was set as BLOCK Temperature with the heated lid enabled and at max speed.

1 Cycle	35 Cycles	1 Cycle	End
98°C — 3 min	95°C — 60 sec	72°C -- 5min	4°C — HOLD
	66°C — 30sec		
	72°C — 210 sec		

PCR tubes were stored at 2-8°C until ready to use.

B. Amplicon Treatment

Completed PCR reactions were Vortexed for 2-5 sec and centrifuged for 2-5sec. For each sample, corresponding PCR-A and PCR-B reactions were combined. pooled reactions Vortexed and centrifuged for 2-5 sec. In a microcentrifuge tube, Enzyme Mix was made as follows:

Reagents	
SAP	235.125
Exonuclease I	85.5
Total Volume	320.625

Enzyme mix was vortexed and centrifuged for 2-5 sec. 3.75 µl of Enzyme Mix was aliquoted into each PCR tubes containing approximately 30µ1 of pooled PCR reaction. Tubes were vortexed and centrifuged for 2-5secs. Tubes were incubated in a thermalcycler programmed as follows:

Hold	37°C	30 mins
Hold	99°C	5 mins
Hold	4°C	forever

The treated PCR tubes were stored at 2-8°C until ready to use.

C. Multiplex ASPE

The tubes of ASPE Primer Mix in 2D6 kit and the 5X Platinum Tfi Reaction Buffer and 50mM MgCl2 supplied with the Enzyme were thawed and brought to room temperature. The 5X Buffer and 50mM MgCIZ were warmed at 37°C in the incubator for 20 minutes. The tubes were vortexed for 5-7 seconds. The PCR tubes were labeled. A 1.5m1 microcentrifuge tube was labeled as AMM. The ASPE Master Mix was prepared for 76 samples as follows:

Reagents	
Distilled Water	865 µl
5X Platinum Tfi Reaction Buffer	329 µl
50mM MgCl2	49 µl
ASPE Primer Mix	124 µl
PlatinumTfi Exo(-) DNA Polymerase	33 µl
Total Volume	1400 µl

The ASPE master mix was vortexed and centrifuged for 2-5 sec. 17 µl of the ASPE Master Mix was aliquoted into the labeled tubes. Tubes of treated PCR amplicons were vortexed and centrifuged for 2-5sec. 3 µl of treated PCR product was added to the correspondingly labeled tube containing 17 µl of the ASPE Master Mix the tubes were capped after addition of sample. Tubes were vortexed and centrifuged for 2-5 sec. Tubes were placed in thermocyler and cycled using the following conditions:

1 Cycle	40 Cycles	1 Cycle	End
96°C - 2 min	94°C - 30 sec	99°C-15 min	4°C - HOLD
	56°C - 30 sec		
	74°C - 30 sec		

ASPE reaction tubes were stored at 2-8° until ready for use.

D. Bead Hybridization

The Bead Mix and 10X Wash Buffer were thawed and brought to room temperature provided in 2D6 kit. The Wash Buffer was diluted to a 1X concentration before being used by transferring 3.0ml of the 10X Wash Buffer to a 50 ml tube containing 27 ml of distilled water. The contents were vortexed to mix and the. 1X Wash Buffer was stored at 4° C. the appropriate number of wells of a 96 well low level plate Labeled for the hybridization reaction. The tube of Bead Mix vortexed for 10 seconds and then sonicated for 10 seconds to disperse the beads then vortexed for 10 seconds and sonicated for 10 seconds a second time. 45 µl of the bead mix were pipetted into the labeled wells.

The tubes of ASPE reaction were vortexed and centrifuged for 2-5 seconds and 5ul of the ASPE reaction was aliquoted into the correspondingly labeled well. The wells were covered the wells with a plate seal, making sure all wells are covered and plate was placed in a thermocycler programmed as follows:

Hold	96°C	1 minute
Hold	37°C	1 hour

The appropriate number of wells on a Millipore MultiScreen filter plate were labeled and all wells not used were covered with Adhesive Sealing Film or tape to provide a better vacuum for the filtration steps. The Reporter Solution was prepared 5-10 minutes before completion of the one hour incubation. The tube of Streptavidin, R-Phycoerythrin (SA-PE) conjugate was vortexed for 2-5 seconds. 19 µl of SA-PE was added to 19 ml of 1X Wash Buffer, covered with paraffin and vortex for 10 seconds to mix. The filter plate was placed on a manifold attached to a vacuum system able to provide low-pressure vacuum. 66.5 mls of 1X Wash Buffer was transferred into a reservoir basin. Using a multichannel pipette, the filter plate membrane was pre-wet for the required number of wells by transferring 150 µl of 1X Wash and vacuum was applied until all the buffer had been drawn through. This step was repeated using a second 150 µl aliquot of 1X Wash Buffer.Using a multichannel pipette, 100u1 of 1X Wash Buffer aliquoted from reservoir into the hybridized samples. The entire contents of the hybridized specimens and 1X Wash Buffer were transferred into the appropriately labeled wells of the filter plate. Vacuum was applied to completely remove most of the liquid from the samples. Beads were washed by adding 200 µl of the 1X Wash Buffer into the appropriate wells of the filter plate. Vacuum was applied to completely remove all liquid from the samples. The plate from vacuum manifold was removed; the bottom of the plate was blotted dry with paper towel and place plate on a MultiScreen centrifuge alignment frame. The reporter solution was transferred into a reservoir basin. Using a

multichannel pipette, 150 µl of the reporter solution was added into each well. The plate was covered with the plate cover and incubated in the dark at room temperature for 20 minutes. After incubation the contents of each well was transferred into a round bottom plate to be read on the Luminex.

E. DATA ACQUISITION AND ANALYSIS

A new batch for the P450-2D6 Assay using the 'xTAG 2D6 18v' template and the Batch Information was filled in by providing a unique batch Name, Description and Creator. The appropriate patient information, number of samples, and sample Ids was entered. At the end of 15 minutes incubation of the samples, the filter plate was placed on the plate holder and Retract holder. Readings were carried out at ambient temperature. To view the results, TDAS was launched on a PC and the session data file analyzed.

DNA extracted from blood samples will be stored for future evaluation of CYP3A4 isozymes, other important opiate drug transporters and biomarkers as well as opiate mu receptor polymorphisms.

A database was developed both to capture available retrospective clinical and laboratory data as well as to frame a system for prospective tracking of patients. Informed consent and assent documents were drafted and sent with a clinical research protocol to the IRB. IRB approval was obtained prior to commencement of patient enrollment and data collection.

Statistical Plan:

Demographic data for each patient was extracted from hospital charts and electronic database. The data was compiled using Microsoft Access and analyzed using SAS.

Univariate analysis was used to compare Hemoglobin S variants and CYP2D6 phenotype/genotypes. Comparison of poor metabolizers to all other metabolizers as a group (extensive, intermediate and ultra-rapid metabolizers) was made using pooled t-test and Sattherwaite test. The most appropiate test was chosen for analysis. In order for the t test to be valid, the variance of the means between the two groups of each variable had to be equal. The F-test was used to ascertain which of these two tests was most appropriate to use to determine significance. An f statistic with a value greater than .05 means the variance is equal and the appropriate test was the Pooled t Test.

Study Population:

Subjects for this study were recruited from the Comprehensive Sickle Cell Program of Children's Healthcare of Atlanta at Egleston. This program cares for children and adolescents with SCD in the metro-Atlanta area. The study included the commonly occurring sickle cell genotypes HBSS, HBSC and HBS-Beta Thalassemia. Approximately 95% of the patients are of African, Afro-Caribbean and/or African American descent. In contrast, less than 5% of our patients are from other ethnicities including Hispanic, Arabic, Indian and Caucasian descent.

Case Definitions:

A diagnosis if SCD is established based on a positive new born screen for SCD and / or a confirmatory hemoglobin electrophoresis showing the complete absence of any normal hemoglobin (A1) or a reduction in Hgb A1 below 50%. All patients seen in the clinic had documentation of either of these two scenarios as part of standard of care practice.

Participant Inclusion Criteria*:*

Patients were eligible for this pilot study if they had a hemoglobin electrophoresis or new born screen result confirming SCD and were in the age range of 6-21 years. Prevalence estimates and study procedures involved a onetime blood draw.

Participant Exclusion Criteria:

a) Sickle cell trait (Hgb AS)

b) Individuals with chronic pain or on chronic opioid therapy

c) Individuals on long-term (> 3 months) therapy with medications that induce or inhibit CYP 2D6 – including but not limited to the following: beta-blockers, tricyclic antidepressants (TCA), selective serotonin reuptake inhibitors (SSRI), dopamine receptor antagonists (haloperidol, risperidone, etc), stimulant medications (amphetamine, dextromethorophan, MDMA), dexamethasone, rifampin

d) Individuals with chronic hepatitis or liver dysfunction

e) Individuals with chronic renal failure

IV. Results

Of the 108 patients approached in clinic, 101 children and their parents or guardians consented to participate in the study. Blood was collected from each patient for genotyping, however only 76 samples were sufficient to provide results. Two of the patients were excluded from the t test analysis because their number of hospitalization days of 137 and 147 were much greater than that of the other patients.

Demographic and Clinical Characteristics

Table 1 displays the demographic and clinical characteristics of the 76 subjects genotyped in three columns, one representing the entire population and the second third representing those patients who had been hospitalized at least once and those patient who no prior hospitalization and thus unknown pain status.

Of the 76 subjects genotyped 42 (55.26%) were male, 73 (96.05%) were African-American and the mean age was 12.59 years with an interquartile range of 10 -15 years. 54 (71.05%) were diagnosed with HbSS, 14(18.42) with HbSC, and 4 (5.26%) each with HbS-Beta + Thalassemia and HbS-Beta 0 Thalassemia. Genotyping showed 14 (18.42) were EM, 38 (50.00) were IM, 15(19.74) PM and 9 (11.84) were UM.

Forty-three (43) subjects genotyped had at least one hospitalization, 25 (58.14%) were male, 42 (97.26) were African-American and the mean age was 12.18 years with an interquartile range of 9-15 years. 33 (76.34%) were diagnosed with

HbSS, 7(17.07) with HbSC, and 2(4.65%) with HbS-Beta + Thalassemia and 1(2.33) HbS-Beta 0 Thalassemia. Genotyping showed 8 (18.60) were EM, 21 (48.78) were IM, 10(23.26) PM and 4 (9.30) were UM.

Of 33 subjects genotyped with unknown pain status, 17 (51.52%) were male, 31 (93.94) were African-American and the mean age was 13.12 years with an interquartile range of 11-15 years. 21 (63.64%) were diagnosed with HbSS, 7(21.21) with HbSC, and 2(6.06%) with HbS-Beta + Thalassemia and 3(9.09) HbS-Beta 0 Thalassemia. Genotyping showed 6(18.18) were EM, 17(51.52) were IM, 5(15.15) PM and 5(15.15) were UM.

A visual representation of patients based Hemoglobin S variant in Graph 1 showed that when compared to the other three HbS variants, the number of subjects were diagnosed with HbSS 54(71.05) was much greater than the combined total of the other three diagnoses, 22(29.95%), combined. We also found all four metabolizer genotype/phenotypes are only represented within the HbSC and HbSS populations. Very few patients genotyped carried Beta Thalassemia alleles, which may account for none of these patients having the poor or ultra-rapid metabolizer alleles.

Graph 1 presents a visual representation of the data by metabolizer phenotype. Of the four metabolizer groups, only the IM group has a sample representative of all the HbS variants. All patients with the HbS Beta 0 Thalassemia were intermediate metabolizers.

Comparison of Mean Hospitalizations among the Phenotypes

Eight variables were selected for comparison of the Subjects in the PM group to the other three metabolizer groups combined (IM, EM and UM). The variables and what they represented were:

- *Admissions:* total number of admission to the hospital
- *Admission Days*: total number of admission days
- *Admissions*: total number of admission to the hospital for pain
- *Admission Days for Pain*: total number of admission days for pain
- *Admission/Year*: total number of admission to the hospital divided by each patient's age
- *Admission Days/Year*: total number of admission days divided by each patient's age
- *Admissions for pain/Year*: total number of admission to the hospital for pain divided by each patient's age
- *Admission Days for Pain/Year*: total number of admission days for pain divided by each patient's age

Table 2 shows the mean for the PM variables compared to that for the other group then uses the difference between these two means for statistical analysis. Looking at the mean difference, the poor metabolizers have a greater mean in all hospitalizations categories when age is not used in the equation. When age is factored in the difference becomes smaller and only the Mean Difference for Admissions days per year is shown to be greater for the PM group. Of the eight variables, the four using age in the equation all had f-statistic greater than 0.05 making the Pooled t Test appropriate for determining significance for those four variables and the Sattherwaite test for the other

four. All the p-values are greater than alpha of .05 showing no significant difference between the two groups for any of the variables. Also, the confidence interval for each variable is wide indicating that the analysis was imprecise.

Because the majority of the patients genotyped had the intermediate phenotype, the data was further analyzed by combining the poor metabolizers and intermediate metabolizers into one group and the extensive metabolizers and ultra-rapid metabolizers in the second group. The results are shown in Table 3. Since intermediate metabolizers should exhibit reduced enzyme function, these patients would also have more hospitalizations. The mean difference is greater for the poor and intermediate metabolizer group in all categories with the exception of admissions per year. However, again the difference is not statistically significant.

Comparison of Hemoglobin Variant and Phenotype based on Disease Status

We knew that children that had been hospitalized had pain severe enough to fail outpatient drug therapy and therefore have more severe disease. Graph three shows a comparison of the children who had been hospitalized to those whose disease status was unknown. Of the 76 children genotyped, 43(56.58%) were hospitalized at least once and were determined to have severe disease. Disease status could not be determined for the other 33 children because we did know have method to determine how they would react to pain. Among the patients diagnosed with HbSS, 7(63.64%) of the EM, 18(69.23%) of the IM, 6(60.00%) of the PM and 3(42.68%) of the UM had been hospitalized for pain. Among the patients diagnosed with HbSC, 1(50%) of the EM,

1(20) of the IM, 4(80%) of the PM and 2(100%) of the UM had been hospitalized for pain. Among the patients diagnosed with HbS-Beta +Thalassemia , 1(33.33%) of the IM and 1(100%) of the EM had been hospitalized for pain. There were no patients represented with the diagnosis Beta + Thalassemia who had been hospitalized pain and had been genotyped as EM or UM. Among the patients diagnosed with HbS-Beta 0 Thalassemia, 1(25%) of the IM had been hospitalized for pain and. There were no patients represented with the diagnosis of Beta 0 Thalassemia who had been hospitalized and had been genotyped as EM, PM or UM.

Severity of disease among the patients with unknown pain status could not be determined using pain status. In table 4, demographics and other information collected such as surgeries and episodes of acute chest syndrome are used to compare these individuals to those patients with hospitalizations due to pain crisis. In both groups greater than fifty percent of the patients had male gender, the majority of were diagnosed HbSS and genotyped IM. Comparison of two groups mean age showed that the hospitalized patients were slightly younger by one year and had wider Interquartile age range of 9 - 15 compared to the Unknown group of 11 – 15. The number of surgeries for each category was about 30% or less with no patient in the Unknown group having had appendectomies. Acute chest syndrome is a common complication in children with sickle cell disease. A large number of the patients had had at least one episode of acute chest syndrome in the hospitalized group 26 (63.11) and the unknown group 27 (79.41%).

V. Discussion

Sickle cell disease (SCD) is an inherited disorder that can be detected with newborn screening. Early detection allows for treatment and monitoring of these children before complications occur. One major complications of SCD is vaso-occlusive pain crisis caused by the sickle-shaped red blood cells obstructing capillaries leading to restriction of blood flow. The loss of blood flow results in ischemia and pain in the affected areas and in many cases organ damage. Although this is a common complication for children with sickle cell disease, the frequency and severity of these crises varies widely among individual patients. While one child might suffer frequently from VOC pain and require constant observation and treatment, another child might not suffer a single VOC episode making the course of the disease unpredictable once a child is diagnosed at birth.

Once a child experiences VOC pain, the treatment is hydration and analgesics. The standard of care is the use of oral opioids such as codeine for the relief of pain in the outpatient setting. However, as with the course of the disease each child's response to a dose of medication varies. For some children oral medications may be ineffective. These children require hospitalization and treatment with IV pain medications.

The P450 system is responsible metabolizing opioids and studies show individual response to these medications may vary based on the patient's genotype. A reduction in metabolizing function will cause suboptimal response to pain medications. Of the four p450 metabolizer types, poor metabolizers have no ability to metabolize opioids

and would get no pain relief with these drugs. Therefore, we hypothesized that the majority of patients failing drug therapy would have the PM genotype and a nonfunctioning P450 system. Because patients who fail outpatient drug therapy require IV drugs for relief of pain, this study used previous hospitalizations to determine which patients had severe pain.

The data collected and analyzed did not support the hypothesis of this study. Comparison of the poor metabolizers to the intermediate, extensive and ultra-rapid metabolizers did not show a significant difference between the mean for the hospitalization variables. Also, the intermediate metabolizers were the dominant variant among the patients tested rather than the poor metabolizers. The percentage of 19.74 put them at the upper limit of the range given earlier for the black adult population.

Intermediate metabolizers have a reduction of function of the CYP2D6 enzyme, which may contribute to the numbers of patients requiring hospitalization for pain relief. This group was combined with the poor metabolizers to determine if the was a difference between hospitalizations for patients with reduced or nonfunctioning P450 system and all other patients. However, analysis showed no statistically significant difference in hospitalizations due to pain among these patients.

Sickle cell disease severity of illness vs. efficacy of pain treatment is what this study and other studies are helping to elucidate. The metabolizer genotypes, especially just one of multiple polymorphisms that could be involved in pain therapy, cannot solely determine the patient's response to pain treatment during a sickle cell disease vaso-occlusive pain crisis.

Insufficient information was available for the non-hospitalized patients. These patients may have either had pain and oral medications were adequate for treatment or they may have been unchallenged with pain medications. Clinical manifestations of sickle cell disease are variable among individual and the progression of disease cannot be predicted based on genotyping. However, this knowledge can enhance supportive care of these patients.

The limitations for this study were that the study did not include other mechanisms involved in opiate metabolism such as the opiate receptors and patients were included whose pain status was unknown. Also, data regarding Emergency department visits was not collected. Thereby excluding patients from the severe category who may have experienced painful episodes where oral medications failed but were not admitted to the hospital.

The study strengths were the study was not biased based on selection because all patients who came to the clinic were asked to participate in the study and the majority of them enrolled.

The future directions that can be taken from this study are to increase the population sample size to get a better representation of the sickle cell variants.

The study was designed to determine the prevalence of each genotype in the study population. Comparison of the pain among the groups was limited because some of the individuals included in the study had not experienced pain requiring hospitalization. Further information will be obtained through a pharmacokinetics study of codeine and its plasma and urinary metabolites in this pediatric sickle cell population.

This data will help determine which patients do not respond to a standard dose of oral codeine and which patients experience toxicity. The ability to predict drug metabolism in individual patients will allow physician to provide personalized pain management for sickle cell patients improving quality of life and limiting the need for hospitalization.

VI. References

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VII. Tables and Graphs

Table 1: Demographic and Clinical Characteristics for Subjects Genotyped (n=76)

Characteristic	Total Subjects (n=76)	Hospitalized (n=43)	Unknown (n=33)
Gender – no. (%)			
Male sex	42 (55.26)	25 (58.14)	17 (51.52)
Race – no. (%)			
African-American	73 (96.05)	42 (97.26)	31 (93.94)
Caucasian	1 (1.32)	0 (0.0)	1 (3.03)
Other	2 (2.63)	1 (2.33)	1 (3.03)
Age (yrs)			
Mean	12.59	12.18	13.12
Interquartile Range	10 – 15	9 – 15	11 - 15
Diagnosis – no (%)			
HbS-Beta + Thal	4 (5.26)	2 (4.65)	2 (6.06)
HbS-Beta 0 Thal	4 (5.26)	1 (2.33)	3 (9.09)
HbSC	14 (18.42)	7 (16.28)	7 (21.21)
HBSS	54 (71.05)	33 (76.74)	21 (63.64)
Metabolizer – no. (%)			
EM	14 (18.42)	8 (18.60)	6 (18.18)
IM	38 (50.00)	21 (48.78)	17 (51.52)
PM	15 (19.74)	10 (23.26)	5 (15.15)
UM	9 (11.84)	4 (9.30)	5 (15.15)

Abbreviations:
EM – Extensive Metabolizer
IM – Intermediate Metabolizer
PM – Poor Metabolizer
UM – Ultra-rapid Metabolizer

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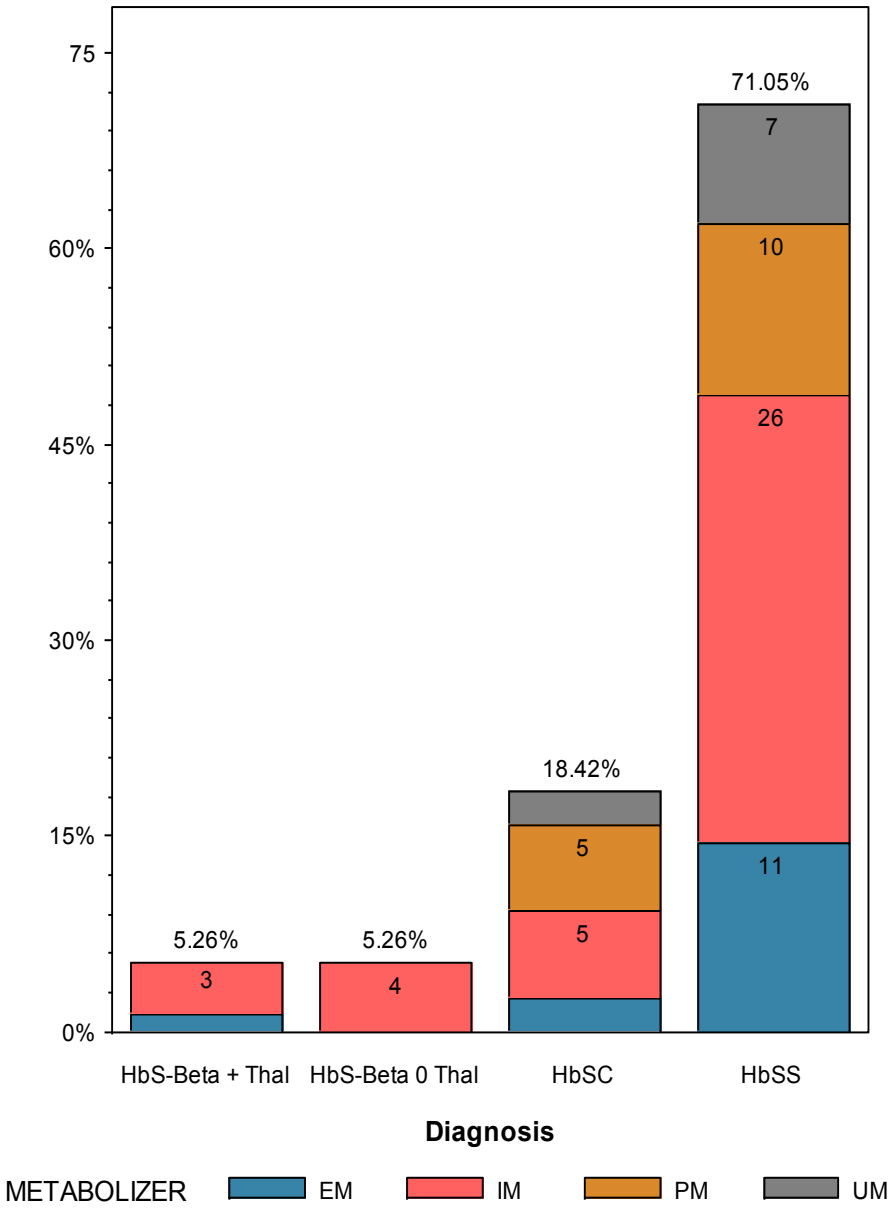
Graph 1: Patients by Hemoglobin S Variant subdivided by Metabolizer Phenotype



Graph 2: Patients by Metabolizer Phenotype subdivided by Hemoglobin S Variant

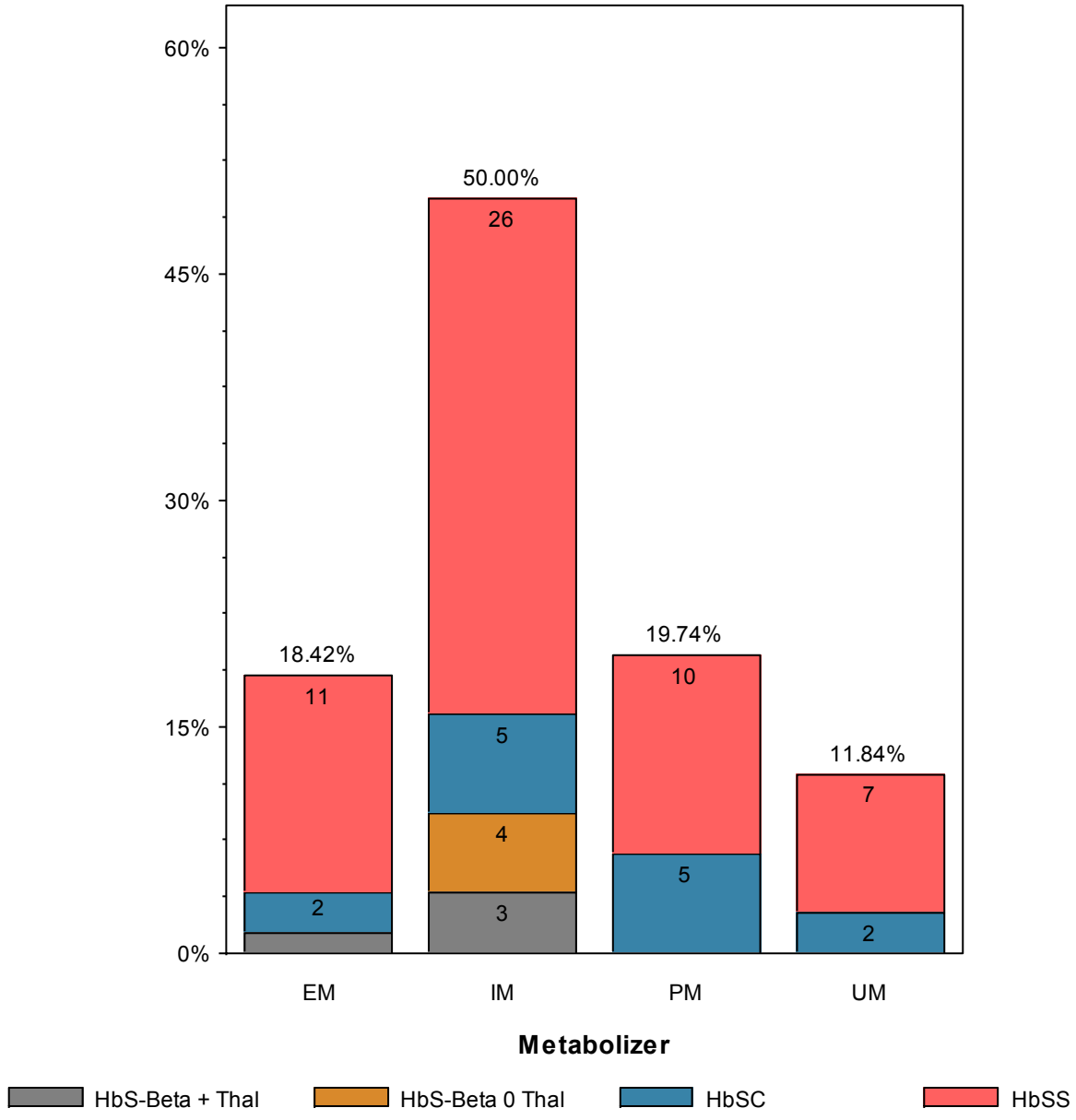


Table 2: Comparison of Mean Hospitalization Variables for Poor Metabolizers and All Other Groups (Intermediate, Extensive and Ultra-Rapid Metabolizers)

Variable	PM Mean	Others Mean	Method	Mean Difference	Mean 95% CL	p-value	f-test
Admissions	10.1333	7.0339	S	3.0994	-2.8977 – 9.0966	0.2908	0.0197
Admission Days	30.667	22.0536	S	8.6131	-11.8660 – 29.0922	0.3878	0.0279
Admissions for Pain	6.5333	2.5763	S	3.9571	-1.2305 – 9.1447	0.1251	< .0001
Admits Days for Pain	19.2143	8.8036	S	10.4107	-7.7976 – 28.6190	0.2418	.0021
Admissions /Year	0.6055	0.6315	P	-0.0260	-0.3652 – 0.3132	0.8790	0.2602
Admissions Days/Year	2.1840	2.0230	P	0.1610	-1.0589 – 1.3808	0.7931	0.3457
Admissions for Pain/Year	0.1935	0.2868	P	-0.0933	-0.3482 – 0.1615	0.4678	0.7673
Admissions Days for Pain/Year	0.5476	0.9216	P	-0.3740	-1.2698– 0.5218	0.4078	0.1910

Method: S = Satherwaite. P = Pooled Ttest

Table 3: Comparison of Mean Hospitalization Variables for Poor and Intermediate Metabolizers versus Extensive and Ultra-Rapid Metabolizers

Variable	PM + IM Mean	EM + UM Mean	Method	Mean Difference	Mean 95% CL	p-value	f-test*
Admissions	7.7885	7.3636	S	0.4248	-3.4877 – 4.3374	0.8292	0.7431
Admission Days	25.3629	20.1000	S	5.2529	-8.7013 – 19.2074	0.4552	0.5570
Admissions for Pain	3.6731	2.6818	S	0.9913	-1.9336 – 3.9161	0.5015	0.2201
Admits Days for Pain	11.8824	8.2105	S	3.6718	-7.4711 – 17.8149	0.5130	0.2252
Admissions /Year	0.5977	0.6922	P	-0.944	-0.3924 – 0.2035	0.5294	0.3279
Admissions Days/Year	2.0713	2.0129	P	0.0584	-0.9639 – 1.0806	0.9096	0.0591
Admissions for Pain/Year	0.2926	0.2098	P	0.0829	-0.0845 – 0.2502	0.3268	< .0001
Admissions Days for Pain/Year	0.9641	0.6305	P	0.3336	-0.2500 – 0.9171	0.2580	< .0001

Graph 3: Comparison of Disease severity between Patients Hospitalized for Pain and Patients with Unknown Pain Status

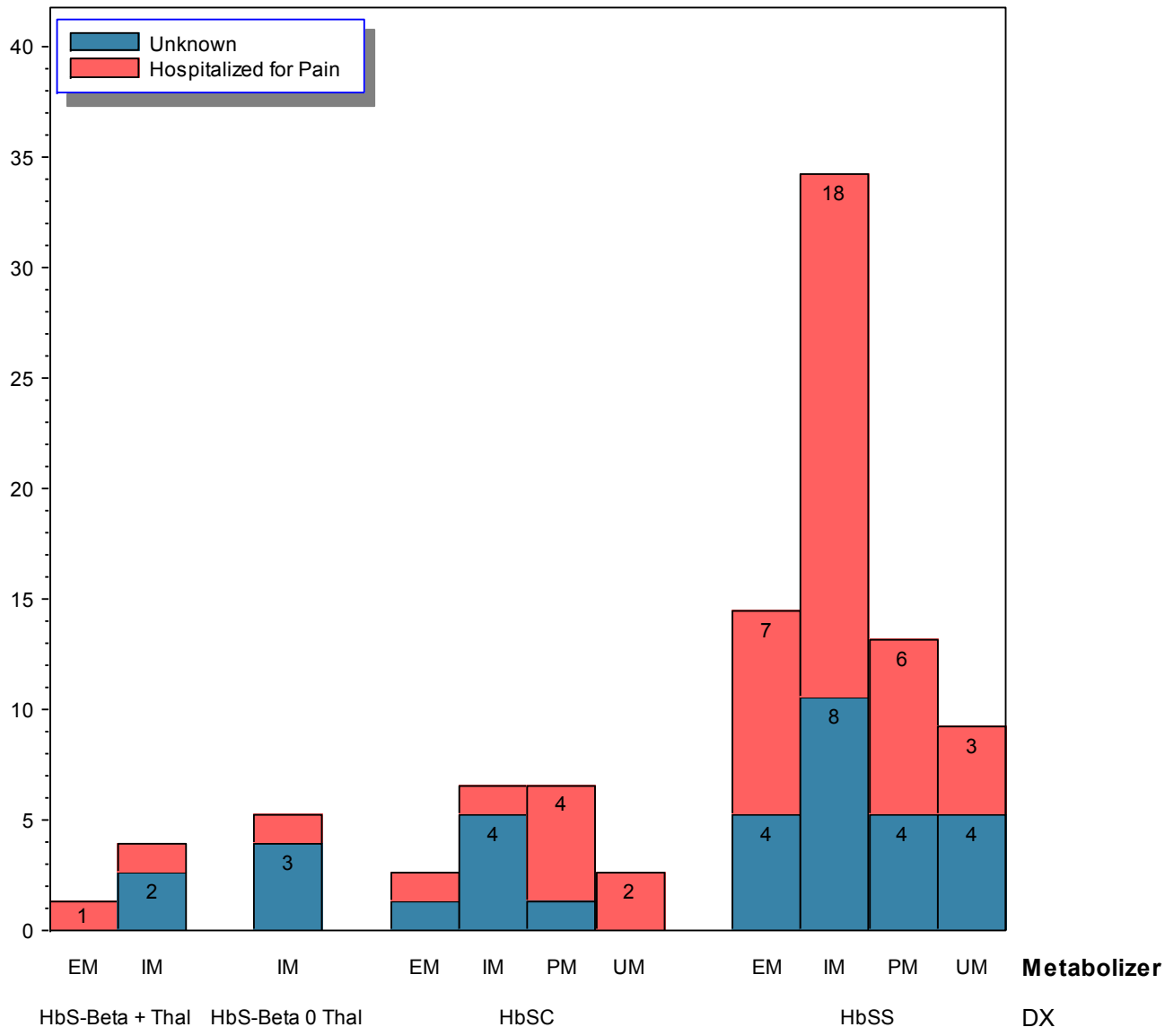


Table 4: Comparison of Clinical Characteristics of Patients Hospitalized for Pain (n=43) to Patients with Unknown Pain Status (n=33)

Characteristic	Hospitalized	Unknown
Gender Male – no. (%)	25 (58.14)	17 (51.52)
Age (yrs)		
Mean	12.18	13.12
Interquartile Range	9 – 15	11 – 15
Diagnosis – no (%)		
HbS-Beta + Thal	2 (4.65)	2 (6.06)
HbS-Beta 0 Thal	1 (2.33)	3 (9.09)
HbSC	7 (16.28)	7 (21.21)
HBSS	33 (76.74)	21 (63.64)
Metabolizer – no. (%)		
EM	8 (18.60)	6 (18.18)
IM	21 (48.84)	17 (51.52)
PM	10 (23.26)	5 (15.15)
UM	4 (9.30)	5 (15.15)
Surgeries – no. (%)		
Appendectomy	2 (4.65)	0 (0.0)
Cholecystectomy	9 (20.93)	4 (6.06)
Splenectomy	10 (30.23)	2 (11.76)
Tonsillectomy	13 (30.23)	6 (18.18)
Acute Chest Syndrome no. (%)	34 (79.07)	27 (79.41)