The purpose of the study was to determine if UDP-glucuronosyltransferase (UGT) 2B7 allelic variants encoding for UGT2B7, primary enzyme responsible for morphine glucuronidation contribute to the variability in the hepatic clearance of morphine in sickle cell disease (SCD). Twenty-four hour PK study of morphine and UGT2B7 variants genotyping was performed in 20 SCD patients in a steady state of health. Presence of the −840G allele (GG and GA) was associated with lower morphine metabolites/morphine AUC ratio compared with AA genotype (1.8 ± 0.5 vs. 3.0 ± 1.8 for M6G/M and 10.1 ± 2.7 vs. 15.7 ± 9.4 for M3G/M) (P = 0.03). Presence of UGT2B7 −840G allele is associated with significantly reduced glucuronidation of morphine and thus contributes to the variability in hepatic clearance of morphine in SCD. Am. J. Hematol. 83:200–202, 2008. © 2007 Wiley-Liss, Inc.
detect any association between genotype $-79G>A$ to AUC M6G/M and M3G/M.

Study design
This study, based on a cross-sectional design, was approved by the Howard University Institutional Review Board and General Clinical Research Center (GCRC) Advisory Committee.

Patients
Twenty sickle cell disease (SCD) patients with normal serum creatinine and hepatic enzymes (alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase) participated in the study. During the study, patients were free of pain and other acute complications of SCD. Patients who used opioid within the week prior to the study were not eligible to participate. After informed consent, patients were admitted to the GCRC inpatient unit. On admission, history, and physical examination was performed and baseline laboratory data were obtained including blood samples for morphine and morphine metabolite assays, and genotyping. A urinary drug screen was used to test for recent use of narcotics. Two indwelling venous catheters (one in each arm) were placed.

Morphine, morphine-3-glucuronide, and morphine-6-glucuronide assays
All patients received a single infusion of morphine sulfate (0.1 mg/kg dose, upper limit 10 mg) over 30 min in one catheter. Blood samples were obtained from the other catheter before beginning, at the end, and 15, 30, 45, 60, 90 min, and 3, 4, 5, 6, 12, 18, and 24 hr after completion of the infusion. Assays of morphine (M), morphine-3-glucuroni- 

d (M3G), and morphine-6-glucuronide (M6G) in the plasma were performed using liquid chromatography/electrospray ionization tandem mass spectrometry (Center for Human Toxicology, University of Utah, Salt Lake City, UT) [10]. A noncompartmental pharmacokinetic analysis was performed calculating area under the curve (AUC) using the linear trapezoidal method.

UGT 2B7 $-840G>A$ and $-79G>A$ genotyping
Genomic DNA was isolated from blood samples using Gentra D-50K Puregene DNA extraction protocol (Gentra Systems Inc. Minneapolis, MN). Genotyping for the UGT2B7 $-840G>A$ and $-79G>A$ allelic variants was performed on 12.5 ng genomic DNA using a TaqMan allelic discrimination assay on an ABI Prism 7000 sequence detection system (Applied Biosystems, Foster City, CA). Polymerase chain reactions (PCR) were performed in a reaction volume of 12.5 µl containing assay-specific primers, allele-specific TaqMan MGB probes, TaqMan Universal PCR Master Mix No AmpErase UNG (2X) and genomic DNA. The thermal profile was 2 min 50 C, 10 min 95 C, followed by 40 cycles of 15 sec 92 C and 1 min 60 C incubations. Genotypes were scored by measuring allele-specific fluorescence using allelic discrimination SDS 1.2.3 software (Applied Biosystems). The assay was validated by direct sequencing, confirming wild type, heterozygote, and homozygote samples.

Statistical analysis
Analysis employed t tests and ANCOVA to evaluate morphine AUC, as well as the AUC ratios of M3G/M, and M6G/ M by genotype (GG or GA vs. AA). In preliminary analyses, none of these measures were normally distributed, therefore normalizing transformations were applied before parametric analyses were conducted. Results were considered to be statistically significant at a $P<0.05$. The data were analyzed using STATA 9.1 software (STATA Corp, College Station, TX).

Discussion
We report the evidence linking the presence of UGT2B7 $-840G$ allele to reduced glucuronidation of morphine in patients with SCD. The study was carried out in the steady state of health to exclude possible confounding effects of painful episodes or other complications of SCD on hepatic clearance of morphine. Additionally, we used AUC ratios for comparison among the genotypes to remove sampling bias. The difference in metabolite/morphine AUC ratio remained significant when controlled for creatinine clearance and hemoglobin level indicating that confounding by anemia or altered renal clearance was not present. Previ-ous studies of this UGT2B7 allelic variant in Norwegian cancer patients observed a lower frequency of variant allele (0.35) and did not detect similar association with morphine metabolites utilizing mean morphine glucuronide to morphine serum ratios [9]. Observed frequency of the variant allele in our population was comparable with the Yorubas in Ibadan Nigeria as reported in the International HapMap project. In our study, while the metabolites/mor- 

phine AUC ratios were significantly different among the genotypes, the differences in AUCs itself did not reach sig- 

nificance, possibly due to the limited number of subjects and/or confounding effect of other elimination pathways on raw AUCs. We utilized metabolite/morphine ratios instead of raw AUC values for comparing hepatic clearance among the genotypes with control for other competing elimination pathways.

Evidence of reduced morphine glucuronidation in the presence of UGT2B7 $-840G$ allele may have important clinical implications for morphine therapy in patients with SCD. The high frequency of the variant allele (0.7) and detection of its significant effect on morphine metabolism in a relatively small random population of SCD patients makes this variant a suitable candidate for future genotype–pheno-

type studies of morphine metabolism in this population.

Acknowledgments
Center for Human Toxicology, University of Utah, Salt Lake City, UT, for subsidizing the cost of morphine assays, Adriana Malheiro and Mezbah Faruque for DNA isolation,
References


