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The Effect of CYP2D6 polymorphisms on the Response to Pain Treatment for Pediatric Sickle Cell Pain Crisis

David C Brousseau, MD, MS[1], **D Gail McCarver, MD**[2], **Amy L Drendel, DO, MS**[1], **Karthika Divakaran, MS**[2], and **Julie A Panepinto, MD, MSPH**[3]

1 *Department of Pediatrics, Section of Emergency Medicine, Medical College of Wisconsin*

2 *Department of Pediatrics, Section of Clinical Pharmacology, Pharmacogenetics and Teratology, Medical College of Wisconsin*

3 *Department of Pediatrics, Section of Hematology/Oncology/BMT, Medical College of Wisconsin*

Abstract

Objectives—To test the hypothesis that children taking hydroxyurea who fail codeine therapy would have reduced functioning CYP2D6 alleles.

Study design—Children with sickle cell disease presenting to an emergency department with a pain crisis unresponsive to codeine were genotyped. The proportion of children with reduced functioning alleles, and CYP2D6 enzyme activity scores ≤ 1.5, were compared, by chi-square analysis, between children taking hydroxyurea and those with mild disease.

Results—73 children completed the study; 42 possessed reduced functioning alleles. 82% of 27 children taking hydroxyurea had reduced functioning alleles versus 47% of 36 mild children ($p < 0.05$). 78% of children taking hydroxyurea had decreased activity scores versus 44% of mild children ($p<0.05$). The odds ratios (95% CI), of children taking hydroxyurea, were 4.9 (1.5 – 15.9) for having reduced functioning alleles, and 4.4 (1.4 – 13.4) for having a low activity score.

Conclusions—Failing codeine therapy for a pain crisis while taking hydroxyurea is associated with an increase in reduced functioning CYP2D6 alleles. We recommend genetic analysis or trial of a non-CYP2D6 analgesic for these children.

Keywords

genetics; hydroxyurea

Much of the morbidity in sickle cell disease is due to recurrent vaso-occlusive pain crises, which result in emergency department (ED) visits or hospitalizations, and adversely affect quality of life.[1, 2] A subset of children with sickle cell disease are classified as severe based on the frequency of vaso-occlusive crises or hospitalizations.[1–4] These children with frequent healthcare utilization for painful crises are frequently placed on hydroxyurea to decrease the number and severity of painful crises. Previous studies attempting to determine a genetic risk for severe sickle cell disease have focused on single nucleotide polymorphisms (SNPs)

Corresponding author: David C Brousseau MD, MS, Children's Corporate Center, Suite 550, 999 N. 92nd St. Wauwatosa, Wisconsin 53226, Ph: (414) 266-2625, Fax: (414) 266-2635, dbrousse@mcw.edu

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associated with an increased prevalence of specific sickle cell complications such as stroke, gallstones or priapism [5–8], but no study to date has investigated the genetically determined lack of a response to pain medication as a reason for the frequent healthcare utilization that can lead to a classification of severe sickle cell disease.

Many children with painful sickle cell crises take the prescription opioid codeine to relieve their pain. The analgesic effect of codeine depends on metabolic activation by the enzyme cytochrome P450 2D6 (CYP2D6), which is known to exhibit genetic polymorphisms that result in decreased production of active opioid.[9–14] The link between these genetic polymorphisms and a clinically relevant decrease in pain relief after codeine use has also been demonstrated.[15–17] We hypothesized that children with severe sickle cell disease, in particular those children who fail outpatient codeine while taking hydroxyurea, are more likely to possess a reduced functioning allele than children with mild sickle cell disease.

Methods

Population/setting

Children with sickle cell disease (4–18 years) presenting to a Children's Hospital ED for continued painful crisis after taking outpatient oral codeine or acetaminophen with codeine therapy were eligible. There was no threshold dose of codeine required to be eligible; any child who reported taking codeine was eligible. Those taking other CYP2D6 substrates (e.g. tramadol, paroxetine, or dextromethorphan) were excluded as they would potentially interfere with the activation of codeine even with normal functioning alleles. The study was approved by the hospital Institutional Review Board.

Children who met study criteria, were accompanied by a legal guardian, and presented for care when a research assistant was present in the ED (generally 8am to 10 pm weekdays and 8 am to 6 pm weekends) were approached for participation. Enrollment occurred between January, 2004 and June, 2005. There were seven refusals. After written informed consent was obtained from parents, and assent from children greater than or equal to seven years of age, blood was drawn for *CYP2D6* genotyping.

Severity

Children were classified as having "severe" sickle cell disease based strictly on the clinical criteria of having a history of three or more hospitalizations for vaso-occlusive crises in the last three years. In addition, any child placed on hydroxyurea for frequent painful crises was classified as severe.[2] All other children were considered "mild". Most severe children/families had agreed to take hydroxyurea; these children were classified as "severe, taking hydroxyurea." Severity was determined by one of the investigators (DB) through chart review of all enrolled children, excluding the study visit and this investigator worked independently of the personnel who determined the CYP2D6 genotyping. Severity classification was determined before the blood samples were genotyped.

DNA analysis

Blood samples were analyzed for 11 *CYP2D6* SNPs, and *CYP2D6* gene deletion and duplication. The combination of differences in these 11 SNPs accounts for the greater than 40 polymorphisms described in the literature.[18] As an example, CYP2D6*4 was determined by detecting the following three SNPs in the gene: 100C>T, 1846G>A, and 4180G>C. Similarly, other alleles were assigned as defined previously.[18] Genomic DNA was PCR-amplified to produce a 5.1 kb CYP2D6 amplicon. Nine SNPs, 100 C>T, 1023 C>T, 1707 G>A, 1846 G>A, 2549 delA, 2613 delAGA, 2850 C>T, 3183 G>A, and 4180 G>C were identified using this product. A second aliquot was used in a PCR reaction to generate the 418 bp region containing

the SNPs 1659 G>A and 1758 G>A, which was sequenced using amplification primers by Dye Terminator Cycle Sequencing following the Quick Start Kit protocol (Beckman Coulter, Fullerton, CA). Gene duplications and deletions were detected by long-range PCR. [13, 19] The DNA analysis was performed by a single investigator (KD) who was blinded to the identity and severity status of the children.

Outcome measures

The main outcome measure was the proportion of children possessing at least one reduced functioning allele, defined as: *CYP2D6* *4, *5 (deletion allele), *6, *10, *17 and *40.[18] A secondary outcome was the *CYP2D6* activity score.[20, 21] The enzymatic activity of CYP2D6 was not measured in our study subjects, rather the activity score, based on extensive genotype/phenotype comparisons and measurements of enzymatic activity, is used to approximate the phenotype for a given genotype in an individual. The scoring system assigns a value of one for normal functioning alleles, and values of 0, 0.5, or 0.75 for reduced functioning alleles, based on enzymatic activity. A child with a normal functioning allele and an allele with an activity score of 0.5 would therefore have an overall activity score of 1.5. Duplicated alleles have their values counted twice, as determined to be valid in the derivation of the activity score.[20, 21] Phenotypic extensive metabolizers are classified as "high metabolizers" if their activity score is greater than 1.5.[21] "High metabolizers" would more readily convert codeine to its active form, resulting in improved pain control, although children with activity scores ≤1.5 would have decreased activation of codeine and thus decreased pain control.

Statistical analysis

The proportion of children with reduced functioning alleles, and the proportion with activity scores ≤ 1.5, were compared by Chi-square analysis between children with severe disease and those with mild disease. The analysis was repeated in children taking hydroxyurea, as they should have less severe crises, but reduced activation of codeine could cause continued visits for pain crises. Significant interaction terms existed between sickle cell genotype and severity/hydroxyurea status; a subset analysis was therefore performed in children with hemoglobin (Hgb) SS, the only genotype with a significant number of children classified as severe or severe taking hydroxyurea. Odds ratios for the presence of a reduced functioning allele based on severity status, and the odds ratio of being classified as severe given the presence of a reduced functioning allele were calculated, along with 95% confidence intervals.

Results

78 children were enrolled; two children were excluded because of insufficient DNA. Six of the remaining 76 children came from three families; one child from each family was randomly withdrawn to obtain independent allelic frequencies, leaving 73 children. All of these children had reported taking codeine or acetaminophen with codeine. No other opioids had been used in the outpatient setting prior to arrival. The mean age was 11.5 years (s.d. 4.3); 42 (58%) were female. The majority were Hgb SS (47/73, 64%), 15 (21%) were Hgb SC, 3 (4%) were HgbSβ° and 8 (11%) were HgbSβ$^+$. Approximately one-half of children (37/73) were classified as severe, and 27 of those 37 children were taking hydroxyurea.

Within the entire sample, 42/73 children (58%) had a reduced functioning allele, a proportion similar to African American norms.[12, 14] Ten children (14%) had *CYP2D6* duplications and 15 (21%) had deletions. Using univariate testing, children classified as having severe disease were older, more likely to Hgb SS, and more likely to have a reduced functioning allele and an activity score ≤ 1.5 (Table I). A child with severe disease had twice the odds of having a reduced functioning allele and twice the odds of having an activity score ≤ 1.5, although the difference was only statistically significant for the activity score. Among the children taking

hydroxyurea, 82% possessed a reduced functioning allele. Children taking hydroxyurea had 4.9 times the odds of possessing a reduced functioning allele and 4.4 times the odds of having an activity score ≤ 1.5 compared to those with mild disease.

Within the subset of children with HgbSS (Table II), 30/47 (64%) were classified as severe and 22 (47%) of the 47 children were taking hydroxyurea. HgbSS children with severe disease again had twice the odds of having a reduced functioning allele and having an activity score ≤ 1.5 compared to children with mild disease, although in this smaller sample, neither odds ratio was statistically significant. HgbSS children taking hydroxyurea had over five times the odds of having a reduced functioning allele and an activity score ≤ 1.5, both significantly increased over children with mild disease.

Alternatively, for the entire population, a child having a reduced functioning allele had 4.9 times the odds of being on hydroxuyrea, and a child with an activity score ≤ 1.5 had 4.4 times the odds of being on hydroxyurea. Limited to children with HgbSS disease, a child with a reduced functioning allele had 5.6 times the odds of being on hydroxyurea, and a child with an activity score ≤ 1.5 had 5.1 times the odds of being on hydroxyurea.

Discussion

Children taking hydroxyurea who present to the ED with sickle cell pain crises after failing outpatient codeine are significantly more likely to possess a reduced functioning CYP2D6 allele, and to have reduced CYP2D6 activity. Although some children may be classified as severe based on more severe underlying pathology, this study suggests that some children may have more frequent healthcare utilization for sickle cell pain crises due to an inadequate pain response to oral codeine.

Although it may be true that all children with severe disease have an increased risk of reduced functioning alleles, we were only able to show a significant difference for children who failed codeine while taking hydroxyurea. Since hydroxyurea lessens the pain of a sickle cell crisis, those children with an inadequate pain response to oral codeine would continue to present to the ED, whereas children whose now reduced pain is controlled with codeine would not require ED management.

For our study, we chose to analyze children who continued to have pain after oral codeine or acetaminophen with codeine. We chose to analyze codeine metabolism because: 1) codeine is the most commonly prescribed oral opioid for outpatient sickle cell pain at our institution, and 2) the important clinical effects of CYP2D6 polymorphisms have been shown with codeine activation, as well as the activation or metabolism of many commonly used drugs, including tramadol, and many cardiovascular or psychoactive drugs. [9, 20–22]

There are study limitations that need to be addressed. First, we did not measure enzyme activity; however, the calculated activity scores closely approximate phenotype, and account for duplications, providing an accurate assessment of the enzyme activity. A second limitation is the definition of sickle cell severity. We used adapted criteria established to determine eligibility for bone marrow transplant or hydroxyurea. There are other markers of severity in sickle cell disease, including elevated white blood cell count, low fetal hemoglobin, and elevated pulmonary artery pressures. [1, 23, 24] We used previously published clinical criteria because treatment interventions for children with frequent complications from their sickle cell disease has become widely accepted. [2, 4] Finally, we did not ensure that children took the maximum allowable dose of codeine. Although it is possible that there may have been underdosing of codeine, this underdosing would have to be systematically different between the two groups to explain the differences we found in our study.

In conclusion, we believe that children taking hydroxyurea who continue to present to the ED for sickle cell pain crisis after failing oral codeine warrant CYP2D6 genotyping. Alternatively, these children could be given a non-CYP2D6 dependent analgesic such as oxycodone or hydrocodone for pain.

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List of abbreviations

ED
emergency department

SNPs
single nucleotide polymorphisms

CYP2D6
cytochrome P450 2D6

Hgb
hemoglobin

Table 1

Differences in characteristics between children with mild disease, severe disease and severe disease taking hydroxyurea. Results are odds ratios (OR) and 95% Confidence Intervals for having a reduced functioning allele or activity score ≤ 1.5 compared to mild disease.

	Mild (n = 36)	Severe (n = 37)	Severe taking Hydroxyurea (n=27)
Mean age (s.d.)	10.1 (4.3)	**12.9 (3.9)***	**12.9 (4.0)***
Female	18 (50%)	24 (65%)	17 (63%)
Sickle cell type	17 (47%)	**30 (81%)***	**22 (82%)***
Hgb SS	1 (3%)	**2 (5%)**	**2 (7%)**
Hgb Sβ°	12 (33%)	**3 (8%)**	**2 (7%)**
Hgb SC	6 (17%)	**2 (5%)**	**1 (4%)**
Hgb $S\beta^+$			
Possessed a reduced functioning allele	17 (47%)	25 (68%)	**22 (82%)***
2D6 Activity score ≤ 1.5	16 (44%)	**25 (68%)***	**21 (78%)***
O.R. (95% CI) for having reduced functioning allele	referent	2.3 (0.9 – 6.0)	**4.9 (1.5 – 15.9)***
O.R. (95% CI) for activity score ≤ 1.5	referent	**2.6 (1.0 – 6.7)***	**4.4 (1.4 – 13.4)***

*Bold indicates significantly different from "Mild"; $p < 0.05$

Table 2

Differences in characteristics between children with mild disease, severe disease and severe disease taking hydroxyurea, in subset of children with HgbSS. Results are odds ratios (OR) and 95% Confidence Intervals for having a reduced functioning allele or activity score ≤ 1.5 compared to mild disease.

	Mild (n = 17)	Severe (n = 30)	Severe taking Hydroxyurea (n=22)
Mean age (s.d.)	10.1 (4.1)	**12.6 (3.9)***	**12.6 (3.9)***
Female	8 (47%)	21 (70%)	15 (68%)
Possessed a reduced functioning allele	9 (53%)	21 (70%)	**19 (86%)***
2D6 Activity score ≤ 1.5	16/36 (44%)	20 (67%)	**18 (82%)***
O.R. (95% CI) for having reduced functioning allele	referent	2.1 (0.6 – 7.1)	**5.6 (1.2 – 26.4)***
O.R. (95% CI) for activity score ≤ 1.5	referent	2.3 (0.7 – 7.6)	**5.1 (1.2 – 21.4)***

* Bold indicates significantly different from “Mild”; $p < 0.05$