Preemptive Genotyping of *CYP2C8* and *CYP2C9* Allelic Variants Involved in NSAIDs Metabolism for Sickle Cell Disease Pain Management

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Abstract

Interindividual variability in analgesic effects of nonsteroidal anti-inflammatory drugs prescribed for sickle cell disease (SCD) pain is attributed to polymorphisms in the *CYP2C8* and *CYP2C9* enzymes. We described *CYP2C8* and *CYP2C9* genotype/phenotype profiles and frequency of emergency department (ED) visits for pain management in an African American SCD patient cohort. DNA from 165 unrelated patients was genotyped for seven *CYP2C8* and 15 *CYP2C9* alleles using the iPLEX ADME PGx multiplexed panel. *CYP2C8*1* (0.806),**2* (0.164), **3* (0.018), and **4* (0.012) alleles were identified. Genotype frequencies were distributed as homozygous wild type (66.7%), heterozygous (27.8%), and homozygous variant/compound heterozygous (5.4%), respectively. *CYP2C9*1* (0.824), **2* (0.027), **3* (0.012), **5* (0.009), **6* (0.009), **8* (0.042), **9* (0.061), and **11*(0.015) were observed with extensive (68.5%), intermediate (18.1%) and poor predicted metabolizers (0.6%), respectively. Fifty-two and 55 subjects, respectively had at least one variant *CYP2C8* or *CYP2C9* allele. Although the distribution of the *CYP2C9* ($p = 0.0515$) phenotypes was marginally significantly in high and low ED users; some *CYP2C8* and *CYP2C9* allelic combinations observed in 15.2% (25) of the cohort are associated with higher risks for analgesic failure. *CYP2C8* and *CYP2C9* preemptive genotyping could potentially enable clinicians to identify patients with impaired metabolic phenotypes. Clin Trans Sci 2015; Volume 8: 272–280

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Introduction

Sickle cell disease (SCD) is one of the most common genetic blood disorders worldwide that affects predominantly people of African ancestry.[1] The hallmark of SCD is the occurrence of painful vaso-occlusive episodes (VOCs) that can start as early as 6 months of age in pediatric patients and continue to occur unpredictably throughout adult life. Severe VOC pain is associated with acute chest syndrome, organ failure and frequent visits to the emergency department (ED) for parenteral opioid treatment.[2] Prodromal signs of VOC pain are treated with a weak opioid such as codeine but more commonly with nonsteroidal anti-inflammatory drugs (NSAIDs) because of their anti-inflammatory, analgesic, and antipyretic effects.[3,4] However, many individuals with SCD display variable response to NSAID treatment and some even fail to achieve adequate analgesia with standard doses of NSAIDs.[2,3]

NSAIDs (e.g., ibuprofen, diclofenac, ketoprofen, naproxen, flurbiprofen, meloxicam, piroxicam, and tenoxicam) are metabolized by two enzymes of the cytochrome P450 superfamily, mainly the *CYP2C8* and *CYP2C9*.[5,6] Polymorphisms in these two genes have been associated with decreased enzyme activity and alteration of NSAIDs pharmacokinetic parameters.[6] Both the *CYP2C8* and *CYP2C9* enzymes are highly polymorphic and various allelic variants reported. More than 16 alleles and over 60 variants have been characterized for the *CYP2C8* and *CYP2C9* enzymes, respectively (http://www.cypalleles.ki.se/). Allelic variants impacts the metabolic activity of the CYP450 enzymes; and previous determinations of enzymatic activity and expression of most CYP450 drug metabolizing enzymes revealed four distinct metabolic phenotypes: ultrarapid metabolizers (UMs), extensive metabolizers (EMs), intermediate metabolizers (IMs) and poor metabolizers (PMs).[5,6] PMs are compound heterozygous for different inactivating alleles or homozygous for an inactivating variant and may display variation in the severity of functional enzyme deficiencies. IMs carry one functional allele and one nonfunctional allele but may demonstrate a wide range of enzymatic activity. EMs have two functional alleles. UMs carry multiple copies of functional alleles. Current NSAIDs dosing strategy in patients with SCD is based on the assumption that the individual patient is an EM. However, accumulated evidence indicates association between decreased or loss of function *CYP2C8* and *CYP2C9* alleles with suboptimal therapeutic response and adverse effects of NSAIDs.[5–8] For SCD patients, suboptimal therapeutic may possibly be linked with higher likelihood of being admitted to hospital for either analgesic drug failure. To date however, relatively few studies have attempted to bridge the concept of pharmacogenetic variability as a determinant of interindividual response to NSAID therapy in SCD patients.[9–12] In this study, we determined the frequency of pharmacologically relevant allelic variants of the *CYP2C8* and *CYP2C9* enzymes in a SCD patient cohort and correlate metabolic phenotypes with frequency of ED visits.

Methods

Human subjects

The study participants were randomly selected patients with SCD receiving care at the Georgia Regents University Comprehensive Sickle Cell Center clinics. The clinics are located in six towns in southeastern Georgia. The study was approved by the Georgia Regents University Institutional Review Board. Written informed consent or assent was obtained from each patient prior to inclusion into the study. Study participants were recruited between January 2011 and January 2013. Medical records of the study participants were reviewed to abstract SCD genotypes, NSAID prescriptions, clinical, and acute care utilization data.

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CYP2C8 and *CYP2C9* genotyping

Whole blood samples (10 mL in tubes containing EDTA) were collected from the study participants in steady state. Genomic DNA was extracted using the Puregene DNA Purification Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. We used the iPLEX ADME PGx multiplex panel (Sequenom, Inc, San Diego, CA, USA) to genotyped seven *CYP2C8* alleles (**1, *2, *3, *4, *5, *7,* and **8*) and 15 *CYP2C9* alleles (**1, *2, *3, *4, *5, *6, *8, *9, *10, *11, *12, *13, *15, *25,* and **27*) across all study participants as previously described.[13] Briefly, the iPLEX® ADME PGx multiplexed panel uses Sequenom Bioscience's iPLEX biochemistry with specific ADME oligo multiplex mixes on the MassARRAY system to simultaneously interrogate 192 biologically relevant polymorphisms in 36 pharmacogenes. After running the reactions, mutations were detected, quantified, and genotype reports automatically created using Sequenom TYPER software (http://bioscience.sequenom.com/iplex-adme-pgx-panel). TYPER software assigns the wild-type (**1*) *CYP2C8* and *CYP2C9* alleles in the absence of other detectable variant alleles The *CYP* allele designations refer to those defined by the Cytochrome P450 Allele Nomenclature Committee.[14]

Statistical analysis

The primary outcome measure was genotype frequencies. The secondary end point compares *CYP2C8* and *CYP2C9* genotypes with the number of ED visits for VOC pain. Descriptive analyses were used for baseline demographic and clinical data and to compare allele frequencies between the study participants and published data of other populations. The *CYP2C8* and *CYP2C9* allele and genotype frequencies were presented as percentage of the study cohort with 95% confidence interval. The observed genotype frequencies were compared with those expected for concordance with Hardy-Weinberg equilibrium using the X^2 test. A p value of less than 0.05 was deemed to represent statistical significance. All statistical analyses were performed using SPSS statistical package version 19.0.

Results

Demographics

This study elucidates the allelic variants of the *CYP2C8* and *CYP2D9* in a SCD cohort. A total of 165 SCD patients (82 males) were recruited. The study participants were all African Americans. Race was self-reported by the subjects. The study participants' demographic features and clinical characteristics are summarized in *Table 1*. The subjects ranged in age from 16 to 61 years and their body mass index ranged from 15.3 to 38.4. SCD genotype frequencies were distributed as SS (97.5%), SB Thal° (1.8%), and S-Los Angeles (0.6%), respectively. Ibuprofen, aspirin and naproxen were the NSAIDs routinely prescribed. One hundred ten subjects (67%) were prescribed hydroxyurea. In terms of treatment of SCD, hydroxyurea is the only FDA approved drug and has been associated with decreased frequency of VOC and morbidity.[2,4]

CYP2C8 alleles and genotype frequencies

Table 2 showed our cohort *CYP2C8* allele and genotype frequencies. The *CYP2C8*1* is considered the wild type with normal enzyme activity. The abnormal *CYP2C8*2* and **3* alleles are the most prevalent alleles associated with decreased enzyme activity, but are unevenly distributed in racial and geographic

Sex	Male Female	No (%) of subjects 82 (49.6%) 83 (50.4%)
Age Median Male Female	 30.5 yrs. (16 – 57) 31.7 yrs. (18 – 61)	
Ethnic origin	African-American	165 (100%)
SCD genotype	SS SB Thal° S-Los Angeles	161 (97.5%) 3 (1.8%) 1 (0.6%)
BMI Median Male Female	 23.1 (15.3 – 35.2) 23.7 (16.5 – 38.4)	
NSAIDs	Ibuprofen (Motrin, Advil) Naproxen (Aleve) Aspirin Acetaminophen (Tylenol)	50 (30.3%) 10 (6.1%) 5 (3%) 20 (12.1%)
Hydroxyurea therapy	Yes	110 (67%)

Table 1. Cohort demographic and clinical characteristics.

populations.[15] The *CYP2C8*4* through *CYP2C8*14* variants alleles are rare and found in less than 1% of racial populations.[16,17] In our cohort, we identified four *CYP2C8* alleles. The *CYP2C8*1* wild-type allele frequency was 0.806. Of the three variant alleles identified, the **2* occurred in the highest frequency (0.164). This was followed by *CYP2C8*3* (0.018) and **4* (0.012), respectively. The *CYP2C8*5, *7,* and **8* rare variants found in less than 1% of populations, mainly Asians, were not detected in our cohort.[18,19]

The *CYP2C8*1, *2, *3,* and **4* frequencies were concordant with Hardy-Weinberg equilibrium. Because of substrate-dependent functional activity of the *CYP2C8* alleles and discrepancies between *in vitro* and *in vivo* data the genotype frequencies were distributed as homozygous wild type, heterozygous and homozygous variant/compound heterozygous, respectively.[17] The *CYP2C8 1/*1* was the most frequent genotype related to NSAID metabolism and it corresponds to an predicted EM phenotype (66.7%); the *CYP2C8 *1/*2, *1/*3,* and **1/*4* genotypes correspond to an IM phenotype and accounted for 27.8% of the study cohort; whereas the PMs *(*2/*2, *3/*3,* and **2/*4* genotypes) accounted for 5.4% of cohort.

CYP2C9 allele, genotypes, and phenotype frequencies

The *CYP2C9* allele, genotype and predicted metabolic phenotype frequencies are summarized in *Table 3*. We surveyed 15 *CYP2C9* alleles (**1, *2, *3, *4, *5, *6, *8, *9, *10, *11, *12, *13, *15, *25,* and **27*) and identified eight alleles (**1, *2, *3, *5, *6, *8, *9, *11*) with the wild-type *1 occurred in the highest frequency (0.824) in our cohort. The *CYP2C9*8* (0.042) and **9* (0.061) were the most common variant alleles. The combined frequency for the reduced activity *CYP2C9*2, *3, *5, *8, *9, *11* and the null **6* variants was 0.176. The *CYP2C9 *4, *12, *13, *15, *25,* and **27* alleles were not detected in our cohort. The high frequency (0.061) of the *CYP2C9*9* allele made **1/*9* the most common genotype with allelic variant in the cohort (9.7%). The observed frequencies for the overall cohort were concordant with Hardy-Weinberg equilibrium. *CYP2C9* phenotypes have been designated extensive (two functional alleles), intermediate (one functional allele/one

Allele	Genetic mutation	Enzyme activity	Number of alleles	Allelic frequency	95% CI (±)	95% CI range
**1*	Wild-type	Normal	266	0.806	0.043	0.763–0.849
**2*	Missense	Decreased	54	0.164	0.040	0.124–0.204
**3*	Missense	Decreased	6	0.018	0.014	0.004–0.033
**4*	Missense	Decreased	4	0.012	0.012	0.000–0.024
**5*	Frame shift	None	0	0.000	0.000	
**7*	Missense	None	0	0.000	0.000	
**8*	Missense	Decreased	0	0.000	0.000	
Genotype	**Number of subjects**	**Observed genotype frequency (%)**	**Expected genotype frequency (%)**	**Predicted phenotype**		
Wild-type						
**1/*1*	110	66.7	65.0	EM		
Heterozygous						
**1/*2*	40	24.2	26.4	IM		
**1/*3*	4	2.4	2.9	IM		
**1/*4*	2	1.2	2.0	IM		
Subtotal	46	27.8				
Homozygous variant/compound heterozygous						
**2/*2*	5	3.0	2.7	PM		
**2/*3*	2	1.2	0.6	PM		
**2/*4*	2	1.2	0.4	PM		
**3/*3*	0	0.0	0.0	PM		
**3/*4*	0	0.0	0.0	PM		
Subtotal	9	5.4				
Total	165	100	99.9			

CI = confidence interval.

Table 2. Allelic, genotypic and phenotype frequencies of *CYP2C8* gene polymorphisms.

dysfunctional), and PMs (two nonfunctional alleles). Based on the observed genotypes and published criteria, we assigned predicted phenotype frequencies for our study participants as follows: extensive (68.5%), intermediate (18.1%), and PMs (0.6%), respectively.[20–24] Because some of the variant *CYP2C9* alleles do not have clear phenotypic consequences, the predicted metabolic phenotype for four *CYP2C9* genotypes (**5/*9, *6/*8, *8/*9,* and **9/*11*) were indeterminate.

Correlation between study participants' genomic and clinical data

Table 4 describes the study participants' *CYP2C8* and *CYP2C9* genomic and ED clinical data. Out of a total of 152 participants with ED visit clinical records, we had 39 high ED users (≥3 ED visits per year) and 113 low ED users (<3 ED visits per year). The distribution for the *CYP2C8* predicted phenotypes was not significantly different in high and low ED users ($p = 0.1668$). However, the distribution of predicted phenotypes was marginally significantly in high and low ED users ($p = 0.0515$) for the *CYP2C9*. *Table 5* presents detailed genomic and clinical data, including NSAIDs prescriptions, number of VOC days and pain score ranges during ED visits or hospital admissions for selected participants with deficient genomic metabolic and clinical risk profiles for NSAIDs therapeutic failure. *Table 6* compares our *CYP2C8* and *CYP2C9* data to allelic data previously reported in other African American and African ethnic populations.[19–25] There is limited published data available from other populations for several minor frequency *CYP2C8* and *CYP2C9* alleles making it somewhat difficult to compare our data with some African ethnic groups with high incidence of SCD.

Discussion

To the best of our knowledge, this study describes the combined *CYP2C8* and *CYP2C9* allelic frequencies, genotypes and predicted phenotypes for the first time in an African American SCD patient cohort. Our study identified twenty five individuals with combined impaired *CYP2C8* and *CYP2C9* genotypes characterized mainly as intermediate and PMs. Interindividual variation in drug response is greatest in IM phenotypic group where it is difficult to determine unequivocally the quantitative/percentage value of altered functionality of allelic variants, except for null alleles.[26–28] Perhaps more significantly, included in this phenotypic group are the six individuals with the delirious *CYP2C8*3* and *CYP2C9*2* allelic combination associated with major ibuprofen clearance impairment and analgesic failure.[28] To the best of our knowledge, no prior studies have identified specific African American subjects with these two genes allelic combination or assess their implications for NSAID analgesic failure.

Allele	Genetic mutation	Enzyme activity	Number of alleles	Allelic frequency	95% CI (±)	95% CI range
**1*	Wild-type	Normal	272	0.824	0.041	0.783–0.865
**2*	Missense	Decreased	9	0.027	0.018	0.010–0.045
**3*	Missense	Decreased	4	0.012	0.012	0.000–0.024
**4*	Missense	NA	0	0.000	0.000	
**5*	Missense	Decreased	3	0.009	0.010	–0.001–0.019
**6*	Frame shift	None	3	0.009	0.010	–0.001–0.019
**8*	Missense	Decreased	14	0.042	0.022	0.021–0.064
**9*	Missense	NA	20	0.061	0.026	0.035–0.086
**10*	Missense	NA	0	0.000	0.000	
**11*	Missense	Decreased	5	0.015	0.013	0.002–0.028
**12*	Missense	Decreased	0	0.000	0.000	
**13*	Missense	Decreased	0	0.000	0.000	
**15*	Missense	NA	0	0.000	0.000	
**25*	Frame shift	None	0	0.000	0.000	
**27*	NA	NA	0	0.000	0.000	
Predicted metabolizer phenotype	**Number of subjects**	**Observed genotype frequency (%)**	**Expected genotype frequency (%)**			
Extensive metabolizer						
**1/*1*	113	68.5	67.9			
Intermediate metabolizer						
**1/*2*	8	4.8	4.5			
**1/*3*	3	1.8	2.0			
**1/*5*	2	1.2	1.5			
**1/*6*	2	1.2	1.5			
**1/*8*	11	6.7	7.0			
**1/*9*	16	9.7	10.0			
**1/*11*	4	2.4	2.5			
Subtotal	46	27.8				
Poor metabolizer						
**2/*3*	1	0.6	0.1			
Unknown metabolizer status						
**5/*9*	1	0.6	0.1			
**6/*8*	1	0.6	0.1			
**8/*9*	2	1.2	0.5			
**9/*11*	1	0.6	0.2			
Subtotal	5	3.0				

CI = confidence interval.

Table 3. Allelic, genotypic, and phenotypic frequencies of *CYP2C9* gene polymorphisms.

CYP2C8	*High	+Low	*CYP2C9*	High	Low
EM	23 (0.61)	83 (0.74)	EM	27 (0.71)	91 (0.81)
IM	15 (0.39)	29 (0.26)	IM	9 (0.24)	10 (0.09)

EM = extensive metabolizer; IM = intermediate metabolizer; ED = emergency department; UNK = Unknown.
*(≥3 ED visits per year); +(<3 ED visits per year).

Table 4. Distribution of *CYP2C8* & *CYP2C9* phenotypes and ED visits.

# of subject	*CYP2C8* genotype	*CYP2C8* phenotype	*CYP2C9* genotype	*CYP2C9* phenotype	Pain range*	# of VOC days	ED visit	Hosp. Adm.	NSAID, number of times prescribed and place	Hu therapy
1	*1/*1	EM	*1/*8	IM	0–8	3	1	2	Ibuprofen 800 mg x14, tylenol 500 mg x10, ibuprofen 800 x17	Y
2	*1/*3	IM	*1/*2	IM	0–7	1	0	1	Naproxen 500 x39	N
3	*1/*3	IM	*1/*2	IM	0–9	67	0	5	N/A	Y
4	*1/*1	EM	*1/*8	IM	0–9	8	2	1	Tylenol 250 x10, ibuprofen 800 mg x 8 (hosp), toradol 30 mg IV x5 (hosp)	Y
5	*1/*1	EM	*1/*8	IM	N/A	N/A	3	4	ASA unknown dose x 26	Y
6	*1/*3	IM	*1/*2	IM	0–5	1	2	5	Toradol 75 mg iv (hosp), acetaminophen 875 (hosp), goody pm x64	Y
7	*2/*3	PM	*1/*2	IM	0–9	1	1	3	Motrin 800 x 2	Y
8	*1/*1	EM	*6/*8	UNK (PM?)		16	6	2	Motrin 800 x 4 (hosp), toradol 60 IM (hosp), theraflu unknown dose x 45, motrin 800 x 72, tylenol 500 x 14	Y
9	*1/*2	IM	*1/*8	IM	0–9	10	0	0	Ibuprofen 800 mg x 24, tylenol 500 mg x4	N
10	*1/*1	EM	*1/*8	IM	N/A	1	0	0	Tylenol 500 mg x1	N
11	*2/*3	PM	*1/*2	IM	0–7	37	3	1	Ibuprofen 200 mg x 85	Y
12	*1/*1	EM	*8/*9	UNK	0–9	0	0	1	N/A	N
13	*1/*2	IM	*1/*6	IM	0–4	4	1	2	N/A	N
14	*1/*1	EM	*1/*8	IM	0–8	0	0	1	N/A	Y
15	*1/*1	EM	*1/*8	IM	0–1	4	0	0	Excedrin unknown dose x 17, advil unknown dose x 1	N
16	*1/*2	IM	*1/*3	IM	0–9	N/A	1	0	N/A	N
17	*1/*2	IM	*1/*8	IM	N/A	N/A	1	0	N/A	Y
18	*1/*3	IM	*1/*2	IM	N/A	N/A	0	2	N/A	N
19	*1/*1	IM	*8/*9	UNK	N/A	N/A	0	0	N/A	Y
20	*1/*2	IM	*1/*8	IM	N/A	N/A	4	0	N/A	Y
21	*1/*1	IM	*1/*3	IM	N/A	0	4	10	N/A	Y
22	*1/*2	IM	*1/*8	IM	N/A	N/A	0	0	N/A	Y
23	*1/*1	EM	*1/*8	IM	N/A	N/A	0	15	Ibuprofen 800 x 11	N
24	*1/*4	IM	*1/*2	IM	0–9	3	8	12	N/A	Y
25	*1/*1	EM	*2/*3	PM	N/A	N/A	3	3	N/A	Y

ADM = admission; EM = extensive metabolizer; IM = intermediate metabolizer; PM = poor metabolizer; Hosp = hospital; ED = emergency department; HU = hydroxyurea; N/A = not available; UNK = unknown; Y = yes; N = no; *Pain range during ED or Hospital admission.

Table 5. Genomic and clinical data for selected SCD participants with compromised metabolic genotypes.

Racial and ethnic group	*CYP2C9* alleles frequency percentage											
	*1	*2	*3	*4	*5	*6	*8	*9	*11	*12	*13	Ref.
African Americans ($n = 165$)	0.824	0.027	0.012	0.000	0.009	0.009	0.042	0.061	0.015	0.000	0.000	This study
African American ($n = 300$)	0.867	0.028	0.020	0.000	0.015	0.010	0.047	–	0.013	–	0.000	19
Ghanaian ($n = 204$)	–	0.000	0.000	0.000	0.000	–	–	–	0.020	–	–	21
Beninese ($n = 111$)	0.955	0.000	0.000	0.000	0.018	–	–	–	0.027	–	–	22
Mozambican ($n = 206$)	–	0.000	0.010	–	0.019	0.000	0.146	–	0.024	–	–	23
Africans ($n = 250$)	0.810	0.120	0.000	–	0.010	0.012	0.040	0.095	0.022	0.020	–	20
	CYP2C8 alleles frequency percentage											
	*1	*2	*3	*4	*5	*7	*8	*9	*10	*11	*12	
African American ($n = 165$)	0.806	0.164	0.018	0.012	0.000	0.000	0.000	–	–	–	–	This study
African American ($n = 500$)	0.878	0.100	0.010	0.012	0.000	0.000	0.000	0.000	0.000	–	0.000	24
Mozambican ($n = 360$)	0.160	0.048	0.000	0.005	0.000	–	–	–	–	–	–	25
Ghanaian ($n = 204$)	–	0.170	0.000	0.000	–	–	–	–	–	–	–	21
(–) Allele not screened in study												

Table 6. *CYP2C9* & *CYP2C8* frequencies in previously studied populations.

We did not observe great variations in the frequency of null and reduced function alleles in the *CYP2C8* and *CYP2C9* genes to ranges reported in other African American populations. Interestingly, two previous genotyping studies reported slightly higher frequencies of mutant gene deletion alleles of the *CYP3A5* and *CYP2D6* in SCD patients compared to healthy African Americans.[9,12] These deficient alleles are associated with the PM phenotype and impaired functionality and have also been associated with failure of codeine treatment for VOC in children on hydroxyurea; and linked with higher likelihood of pediatric and adult SCD patients being admitted to the ED for parenteral opioid management.[10,11] Although the distribution of *CYP2C9* predicted phenotypes was marginally significantly in high and low ED users in our study, it was unclear in the above referenced studies whether the individuals at risk for frequent ED visits also had deficient *CYP2C8* and *CYP2C9* genotypes. Nonetheless, in our cohort, ten out of twelve of the combined alleles of the *CYP2C8* and *CYP2C9* enzymes identified contribute to deficient metabolic genotypes. Fifty-two subjects had at least one variant *CYP2C9* allele (**2, *3,*5, *6, *8, *9,* and **11*) associated with either the IM, poor, or indeterminate phenotype. Fifty-five subjects had at least one *CYP2C8* variant allele (**2, *3,* and **4*) that contributes to impaired metabolic genotypes, and nine subjects were homozygous or compound heterozygous for deficient metabolic genotypes as determined in previous pharmacokinetic and pharmacodynamics studies.[26–34]

Pharmacokinetics and pharmacodynamics effects of *CYP2C8* and *CYP2C9* alleles

The influence of some *CYP2C8* and *CYP2C9* genotypes on analgesic response and therapeutic outcomes of a number of NSAIDs including ibuprofen and naproxen have been established in previous pharmacokinetics studies.[26–28] Ibuprofen pharmacokinetics data is strongly related to variant *CYP2C8* and *CYP2C9* genotypes: heterozygous and homozygous carriers of the *CYP2C8*3* allele display ibuprofen metabolic clearance reduction of approximately 62% and 10%, respectively when compared to individuals homozygous for the *CYP2C8*1* and *CYP2C9*1* genotype.[25] Metabolic clearance values in subjects heterozygous and homozygous for *CYP2C9*2* but not carrying any other allelic mutations were 96% and 84%, respectively when compared to individuals with *CYP2C8*1* and *CYP2C9*1* wild-type alleles.[27] The *CYP2C9*2* allele when linked with the *CYP2C8*3*, translates into a major impairment on ibuprofen clearance as indicated above.[28] Individuals carrying *CYP2C9*3* variant alleles display a mean reduction of clearance of approximately 65% and 17% for heterozygous and homozygous carriers, respectively.[27] In a recent study of 130 healthy individuals who received a single oral dose of 400 mg ibuprofen, the oral clearance of ibuprofen was 4.43, 3.26, 2.91, 2.05, 1.83, and 1.13 1/hour for individuals with *CYP2C9*1/*1*, **1/*2*, **1/*3*, **2/*2*, **2/*3*, and **3/*3* genotypes, respectively.[28] The effects of the variant *CYP2C9* alleles are dissimilar for all NSAIDs.[29] Studies with tenoxicam, however, indicated that oral clearance among carriers of *CYP2C9*2* and *CYP2C9*3* decreases to approximately 70% and 55%[30]; while oral clearance of meloxicam in individuals with the *CYP2C9*1/*13* genotype was significantly decreased by 62% compared to individuals with the *CYP2C9*1/*1* genotype.[31]

Pharmacodynamics studies have also implicated *CYP2C8* and *CYP2C9* genotypes in gastrointestinal toxicity of NSAIDs. Martinez and colleagues found the *CYP2C9*2* and **3* associated with a two-and-a-half fold increased risk of gastric bleeding

episode after dosing with NSAIDs such as celecoxib, diclofenac, ibuprofen, indomethacin, lornoxicam, piroxicam, or naproxen. The increased risk was attributed to the **2* allele which was detected in 23.4% of the study subjects with gastric bleeding episode compared with 13.7% of the control subjects.[32] In another study of gastrointestinal bleeding in NSAIDs users, the frequencies of the *CYP2C8*3* and *CYP2C9*2* alleles were higher in NSAIDs user who experienced a bleed versus those who did not experience a bleed (*CYP2C8*3,* odds ratio: 2.4, $p < 0.002$; *CYP2C9*2*, odds ratio: 2.7, $p < 0.013$).[33] Pilotto et al. found that a significantly higher frequency of *CYP2C9*1/*3* and **1/*2* genotypes were identified in patients with endoscopically documented NSAID-related gastroduodenal bleeding lesions compared to a matched control group. In the study described, the presence of the *CYP2C9*3* allelic variant was associated with a significant high risk of bleeding (OR: 7:3).[34]

Clinical utility of *CYP2C8* and *CYP2C9* preemptive genotyping

Currently, data on the association of NSAIDs treatment with severe drug side effects or analgesic failure are limited in SCD patient population.[4] Interestingly, recent epidemiologic data associates higher doses of some traditional and nontraditional NSAIDs with double risk of congestive heart failure, increased risk of peptic ulcer complications, gastrointestinal bleeding, and increased risk of major vascular events (nonfatal myocardial infarction, nonfatal stroke, or vascular death.[8,35–38] Though these risks fall quickly after drug cessation, nonetheless, with chronic NSAID use, these risks do not wane over years of use.[37–40] For SCD patients, however, due to the ubiquity and chronicity of pain experience, NSAIDs therapy is initiated very early in life and throughout the lifespan. Indeed, as *Table 5* illustrates, NSAIDs are routinely prescribed to SCD with compromised metabolic profiles. Preemptive genotyping to identify patients with *CYP2C8* and *CYP2C9* intermediate and poor predicted metabolic phenotypes could potentially facilitate early prediction of NSAID treatment outcomes both in terms of efficacy and possible development of adverse events in SCD patients. Preemptive genotyping anticipates current and future medication prescription needs of patients as opposed to current practice whereby genotyping is performed only when clinically indicated.[41] With the preemptive genotyping model, a single blood sample is used to genotype specific patient populations for polymorphisms of pharmacogenetic significance in relevant drug metabolizing enzymes and transporters with regards to specific medication classes.[42] *CYP2C8* and *CYP2C9* preemptive genotyping data could potentially facilitate quantification and clinical assessment of pharmacogenetic risk and longitudinal accumulation of NSAIDs risk burden in SCD patients. As depicted in *Table 5*, the identification of an impaired drug metabolic profile in a patient would alert the clinician that special attention should be given to NSAIDs drug response of the patient and would provide explanation for those individuals with unsatisfactory drug response or side effects profiles enabling clinicians to make distinctions between a medication compliance problem and a metabolic defect. More importantly, since the genetic makeup of an individual is virtually invariable, the determination of SCD patients' drug metabolic genotypes provides lifelong applicable information for analgesic pharmacotherapy.[40]

Ultimately, pharmacogenetic screening for SCD patients' *CYP2C8* and *CYP2C9* genotypes would be useful only if it will facilitate development of NSAIDs dosing algorithms similar to those developed for warfarin and tamoxifen dosing with the *CYP2C9/VKORC1* and *CYP2D6* genes, respectively.[43–45] However, this would require additional functionality studies of novel *CYP2C8* and *CYP2C9* variant alleles, as well as the effect of SCD on *CYP2C8* and *CYP2C9* enzyme expressions and the role of environment factors. Perhaps more immediate is the need for appropriate PK and PD studies to determine the effects of allelic variants present in the SCD population. As shown in *Tables 2* and *3*, the *CYP2C8*2* and *CYP2C9*5,*6,*8, *9*, and **11* variant alleles are common in populations with genetic susceptibility for SCD, mostly populations of African ancestry. However, while the relationships between the *CYP2C8*3, CYP2C9*2*, and **3* alleles and metabolic indexes of several NSAIDs are well delineated, to the best of our knowledge, the pharmacokinetic effects of the *CYP2C8*2* and *CYP2C9*5,*6,*8, *9*, and **11* alleles on NSAIDs metabolism has not been evaluated in African American populations. This lack of pharmacokinetics data not only precluded us from assigning predicted metabolic phenotype for four genotypes (i.e., **5/*9, *6/*8, *8/*9, *9/*11*), but also represents a crucial knowledge gap in the use of genetic genotype to inform pharmacogenetic prescribing practice for NSAIDs dosing or for other drugs metabolized by the *CYP2C8* (i.e., amiodarone, fluvastatin, simvastatin, verapamil, montelukast, amodiaquine and chloroquine, morphine and methadone) and the *CYP2C9* (i.e., losartan, tolbutamide and torsemide) enzymes often prescribed for SCD patients in various parts of the world.

Our study has limitations. Though we used a multiplex genotyping panel to determine *CYP2C8* and *CYP2C9* genotypes, the assignment of predicted metabolic profiles in our cohort was based on allelic combinations and associated activity levels reported in the literature. Ultimately, pharmacokinetics study remains the gold standard for discerning SCD patients' metabolic phenotypes and for reporting individuals' analgesic drug response profile for specific substrates. Another study limitation is that with the iPLEX ADME PGx panel, the *CYP2C9*3* and **18* are indistinguishable haplotypes. Consequently, we arbitrarily reported the *CYP2C9*3* allele frequency in our cohort. Additionally, the cross-sectional nature of our study precluded reporting of daily or annual NSAIDs usage by genotypes, pain scores and analgesic response which we reserve for another project. These limitations notwithstanding, to the best of our knowledge, no study attempts to bridge the concept of pharmacogenetic variability as a determinant of interindividual response to NSAID therapy in SCD patients.

Conclusions

In summary, our study determined allele frequencies and genotypes in the *CYP2C8* and *CYP2C9* enzymes in a SCD patient cohort. Ten of 12 of the combined alleles of the *CYP2C8* and *CYP2C9* enzymes identified in our cohort contribute to deficient metabolic genotypes with fifty-two subjects having at least one variant *CYP2C9* allele associated with either the IM, poor, or indeterminate phenotype; while 55 subjects had at least one *CYP2C8* variant allele that contributes to impaired metabolic genotypes. Several of the impaired function variant alleles are being reported for the first time among SCD patients. The *CYP2C8* and *CYP2C9* variant alleles play a significant role in the analgesic effects and toxicity of NSAIDs. These drugs used to treat VOC pain at the prodromal stage in SCD patients are associated

with vascular risks, adverse effects on the gastrointestinal tract, and possibly frequent ED visits for analgesic failure.[7,8] Our study highlights the concept of pharmacogenetic variability as a determinant of interindividual response to NSAIDs therapy in SCD patients. *CYP2C8* and *CYP2C9* preemptive genotyping could potentially enable clinicians to identify patients with impaired metabolic phenotypes. In tandem with preemptive genotyping, additional and appropriate pharmacokinetics and pharmacodynamics studies are required to potentially enable clinicians to tailor NSAIDs dosing accordingly to achieve optimal analgesic response.

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Conflict of Interest

The authors have no financial disclosures or conflicts of interest to declare.

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