Progressing Preemptive Genotyping of CYP2C19 Allelic Variants for Sickle Cell Disease Patients

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Aims: Interindividual variability in drug response and adverse effects have been described for proton pump inhibitors, anticonvulsants, selective serotonin reuptake inhibitors, tricyclic antidepressants, and anti-infectives, but little is known about the safety and efficacy of these medications in patients with sickle cell disease (SCD). We genotyped the *CYP2C19* gene which has been implicated in the metabolism of these drugs in an SCD patient cohort to determine the frequencies of reduced function, increased function, or complete loss-of-function variants. **Materials and Methods:** DNAs from 165 unrelated SCD patients were genotyped for nine *CYP2C19* (*2, *3, *4, *5, *6, *7, *8, *12, and *17) alleles using the iPLEX[®] ADME PGx multiplex panel. **Results:** Three *CYP2C19* alleles (*2, *12, and *17) were detected with the following frequencies: 0.209, 0.006, and 0.236, respectively. The predicted phenotype frequencies were distributed as extensive (31.5%), intermediate (24.8%), poor (5.5%), ultrarapid (30.3%), and unknown metabolizers (7.9%). **Discussion:** Prognostic genotyping is potentially useful for identifying SCD patients with allelic variants linked to proven clinical pharmacokinetic consequences for several drugs metabolized by the *CYP2C19* gene. However, the main challenge to implementing a genetics-guided prescribing practice is ensuring concordance between *CYP2C19* genotypes and metabolic phenotypes in SCD patients.

Keywords: pharmacogenetics, drug metabolizing enzyme, sickle cell disease, precision medicine, preemptive genotyping

Introduction

SICKLE CELL DISEASE (SCD) is a genetic disorder affecting largely populations of African ancestry worldwide. Painful, acute vaso-occlusive crisis is the hallmark symptom of SCD and is the precursor of diverse clinical comorbidities affecting various organ systems, including cerebrovascular events, seizure, inflammation, infection, chronic pain, and chronic depression (Lee, 2013; Elmariah *et al.*, 2014). Proton pump inhibitors, anticonvulsants, anti-infectives, selective serotonin reuptake inhibitors, and tricyclic antidepressants are drugs commonly prescribed for SCD patients for these SCD-related morbidities. Interindividual variability in pharmacologic response and adverse drug response has been described for these drugs, but little is known about the safety and efficacy of these medications in patients with SCD (Gardiner and Begg, 2006; Lee, 2013). The highly polymorphic *CYP2C19* enzyme is involved in the metabolism and variability in response for these drug classes. Of the 36 allelic variants reported for the *CYP2C19* enzyme, at least 12 variants have no enzymatic activity (www.cypalleles.ki.se/cyp2c19.htm, accessed: May 10, 2016). Based on the activity levels of these allelic variants, four distinct metabolic phenotypes are identified: ultrarapid metabolizers (UMs), extensive metabolizers (EMs), intermediate metabolizers (IMs),

and poor metabolizers (PMs). 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 levels of enzyme activity. EMs have two functional alleles. Ultrarapid metabolizers carry multiple copies of functional alleles.

Preemptive genotyping of allelic variant functional activity level could be used to determine SCD patients' metabolic profiles for *CYP2C19* drugs. Preemptive genotyping anticipates current and future medication prescription needs of patients as opposed to this practice, whereby genotyping is performed only when clinically indicated (He and McLeod, 2012; Scott *et al.*, 2012). Indeed, preemptive pharmacogenetic genotyping programs linked to patients' electronic health records are available for oncologic, cardiovascular, and psychiatric disorders in major academic medical centers (Verschuren *et al.*, 2012; Rasmussen-Torvik *et al.*, 2014; Birdwell *et al.*, 2015; Dunnenberger *et al.*, 2015; Hicks *et al.*, 2015). The frequency of *CYP2C19* polymorphic alleles and predicted metabolic profiles displays distinct interracial and ethnic variation (Dandara *et al.*, 2001; Hamdy *et al.*, 2002; Allabi *et al.*, 2003; Luo *et al.*, 2006; Matimba *et al.*, 2009; Babalola *et al.*, 2010; Kearns *et al.*, 2010; Man *et al.*, 2010;

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Martis *et al.*, 2013). African Americans with SCD constitute a patient group, for whom the preemptive determination of *CYP2C19* metabolic genotypes may potentially provide lifelong applicable information for selection of appropriate dosages of *CYP2C19* drugs and identification of individuals pharmacogenetically prone to unsatisfactory drug response or side effects (He and McLeod, 2012; Scott *et al.*, 2012). *CYP2C19* data could facilitate quantification and clinical assessment of pharmacogenetic risk in SCD patients. However, to our knowledge, *CYP2C19* allelic frequency and genotype data for African American patients with SCD are unavailable (Babalola *et al.*, 2010). Therefore, the primary purpose of this study was to describe the frequency of *CYP2C19* allelic variants, genotypes, and predicted metabolic phenotypes in an African American SCD patient cohort and check for correspondence with previous studies in populations of African ancestry.

Materials and Methods

Human subjects

The study participants were randomly selected patients with SCD receiving care at the Georgia Regents University Comprehensive Sickle Cell Center outreach clinics 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 before inclusion into the study. Study participants were recruited between January 2011 and January 2013. Medical records of the study participants were reviewed to assess SCD genotype, clinical, and medical data.

CYP2C19 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) according to the manufacturer's instructions. We used the iPLEX[®] ADME PGx multiplex panel (Sequenom, Inc.) to genotyped *CYP2C19* *2, *3, *4, *5, *6, *7, *8, *12, and *17 alleles across all study participants. The genotype profiles were reported as heterozygous, homozygous, and homozygous variants, or no "call." 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 TYPER software. TYPER software assigns the wild-type (*1) *CYP2C19* alleles in the absence of other detectable variant alleles (<http://bioscience.sequenom.com/iplex-adme-pgx-panel>). The CYP allele designations refer to those defined by the Cytochrome P450 Allele Nomenclature Committee (Sim and Ingelman-Sundberg, 2006).

Statistical analysis

The primary outcome measure was genotype frequencies. *CYP2C19* allele frequencies were presented with 95% confidence interval. 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 χ^2 test.

Results

Demographic, clinical, and medical characteristics

This study elucidates the allelic variants of the *CYP2D19* in an 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, clinical characteristics, and disease comorbidities 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^o (1.8%), and S-Los Angeles (0.6%), respectively. Ten subjects died due to disease complications during the course of the study.

TABLE 1. DEMOGRAPHIC, MEDICAL, AND CLINICAL CHARACTERISTICS OF SCD PATIENT COHORT

	No. (%) of subjects, n (%)
Sex	
Male	82 (49.6)
Female	83 (50.4)
Age, years	
Median	
Male	30.5 (16–57)
Female	31.7 (18–61)
Ethnic origin	
African American	165 (100)
SCD genotype	
SS	161 (97.5)
SB Thal ^o	3 (1.8)
S-Los Angeles	1 (0.6)
BMI	
Median	
Male	23.1 (15.3–35.2)
Female	23.7 (16.5–38.4)
Disease comorbidities	
Avascular necrosis	31 (18.1)
Pulmonary hypertension	15 (9.09)
Cardiomyopathy	15 (9.09)
Acute chest syndrome	13 (7.98)
Stroke	11 (6.66)
COPD	10 (6.06)
Chronic pain	10 (6.06)
Renal disease	10 (6.06)
Leg ulcer	8 (4.84)
Psychiatric disorders	6 (3.63)
Deep vein thrombosis	6 (3.63)
Liver disease	5 (3.03)
Dysrhythmia	3 (1.81)
Neurologic complications	3 (1.81)
Asthma	2 (1.21)
Congestive heart failure	2 (1.21)
Diabetes	1 (0.6)
Other complications	8 (4.84)
Deceased from disease complications	10 (6.06)

BMI, body mass index; COPD, chronic obstructive pulmonary disease; SCD, sickle cell disease.

CYP2C19 alleles and genotype frequencies

Table 2 showed our cohort *CYP2C19* allelic frequencies, genotypes, and predicted metabolic phenotype frequencies. We genotyped nine *CYP2C19* alleles (*2, *3, *4, *5, *6, *7, *8, *12, and *17) across all study subjects. The *CYP2C19**1 is considered the wild type with a normal enzyme activity. The abnormal *CYP2C19**2 (splicing defect) and *3 (premature stop codon) alleles are the most prevalent alleles associated with the PM profile across racial populations (Scott *et al.*, 2012). Other defective (loss-of-function) alleles include the *CYP2C19* *4, *5, *6, *7, *8, *9, and *10. The function of the *CYP2C19**12-*16, *18-*26 alleles is unknown or indeterminate, but the *17 allele is deemed an increased function allele (Scott *et al.*, 2012). Regarding the *CYP2C19*-predicted metabolic phenotypes, individuals are classified as EM (homozygous for the *CYP2C19**1); IM (heterozygous with either *1 or *17 plus a reduced or absent enzyme function allele); PM (two loss-of-function alleles); and ultrarapid metabolizer (or homozygous genotype for the *17 or heterozygous for the *1 and *17) (Scott *et al.*, 2012). We identified three variant alleles and seven genotypes of the *CYP2C19* in our cohort. The *CYP2C19**1 wild-type allele frequency was 0.545. Of the three variant alleles identified,

the *17 occurred in the highest frequency (0.236). This was followed by *2 (0.209) and *12 (0.006), respectively. The *CYP2C19**1, *2, *12, and *17 frequencies were in concordance with Hardy–Weinberg equilibrium (p -value 0.261). Fifteen subjects (9.0%) were homozygous for the *17/*17 genotype and 35 subjects (21.2%) were heterozygous for the *1/*17. Thirty-nine subjects (23.6%) had the *1/*2 genotype, two subjects (1.2%) were heterozygous for the *1/*12 allele, and nine subjects (5.4%) were homozygous for the *2/*2 allele. The *CYP2C19**3, *4, *5, *6, *7, and *8 alleles were not detected. Based on genotype frequencies, the assigned predicted metabolic phenotypes in the cohort were distributed as ultrarapid (UM/30.3%/50 subjects), extensive (EM/31.5%/52 subjects), intermediate (IM/24.8%/41 subjects), and poor (PM/5.5%/9 subjects) metabolizers, respectively. Because the phenotypic consequences of the *2/*17 genotype may fall between the EM and IM phenotype dependent on the *CYP2C19* substrate, we assigned this combination to the unknown phenotype category (UNK/7.9%/12 subjects), together with one subject with the indeterminate *17/UNK genotype.

Since the frequency of *CYP2C19* polymorphic alleles displays distinct interracial and ethnic variation, naturally, we expected our cohort *CYP2C19* allelic frequencies to mirror that of healthy African Americans (Dandara *et al.*,

TABLE 2. CYP2C19 ALLELIC AND GENOTYPE FREQUENCIES

<i>Allele</i>	<i>Genetic alteration</i>	<i>Enzyme activity</i>	<i>Number of alleles</i>	<i>Allelic frequency</i>	<i>95% CI range</i>
*1	Wild type	Normal	180	0.545	0.483 to 0.590
*2	Splicing defect	None	69	0.209	0.165 to 0.253
*3	Stop Codon	None	0	0.000	0.000 to 0.000
*4	Missense	None	0	0.000	0.000 to 0.000
*5	Missense	None	0	0.000	0.000 to 0.000
*6	Missense	None	0	0.000	0.000 to 0.000
*7	Splicing defect	None	0	0.000	0.000 to 0.000
*8	Missense	None	0	0.000	0.000 to 0.000
*9	Missense	Decreased	0	0.000	0.000 to 0.000
*12	Missense	Unknown	2	0.006	–0.002 to 0.014
*17	Increased function	Increased	78	0.236	0.191 to 0.282
UNK	—	—	1	0.003	–0.003 to 0.009
Total			330	1.0	

<i>Metabolizer phenotype</i>	<i>Genotype</i>	<i>Number of subjects (%)</i>	<i>Observed frequency (%)</i>	<i>Predicted frequency (%)</i>
Ultrarapid metabolizer				
Heterozygous	*1/*17	35 (21.2)	21.2	25.4
Homozygous	*17/*17	15 (9.1)	9.1	5.6
	Sub total	50 (30.3)		
Extensive metabolizer	*1/*1	52 (31.5)	31.5	29.8
Intermediate metabolizer				
	*1/*2	39 (23.6)	23.6	23.8
	*1/*12	2 (1.2)	1.2	0.7
	Subtotal	41 (24.8)		
Poor metabolizer	*2/*2	9 (5.4)	5.5	4.4
Unknown	*2/*17	12 (7.2)	7.3	9.9
	*17/UNK	1 (0.6)	0.6	0.1
	Subtotal	13 (7.8)		
	Total	165		

CI, confidence interval; UNK, unknown.

TABLE 3. CYP2C19 FREQUENCIES IN PREVIOUSLY STUDIED POPULATIONS

Racial and ethnic group	N	Allele frequencies															Ref.
		*1	*2	*3	*4	*5	*6	*7	*8	*9	*10	*12	*13	*15	*17	UNK	
African American	165	0.545	0.209	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.006	0.000	0.000	0.236	0.003	This study
African American	250	0.594	0.194	0.004	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.012	0.014	0.182	—	Martis <i>et al.</i> (2013)
African American	114	—	—	—	—	—	—	—	—	—	—	—	—	—	0.210	—	Kearns <i>et al.</i> (2010)
African American	236	—	0.182	0.008	—	—	—	—	—	—	—	—	—	—	—	—	Luo <i>et al.</i> (2006)
African American	250	0.769	0.192	0.002	0.000	0.000	0.000	0.000	0.014	0.060	0.060	0.000	0.016	0.000	0	0.012	Man <i>et al.</i> (2010)
African ethnic group																	
Luo	30	—	0.070	0.000	—	—	—	—	—	—	—	0.040	0.030	0.050	—	—	Matimba <i>et al.</i> (2009)
Ibo	20	—	0.330	0.000	—	—	—	—	—	0.000	—	0.000	0.040	0.000	—	—	Matimba <i>et al.</i> (2009)
Shona	15	—	0.230	0.000	—	—	—	—	—	0.000	—	—	0.030	0.000	—	—	Matimba <i>et al.</i> (2009)
Venda	9	—	0.170	0.000	—	—	—	—	—	0.060	—	—	0.000	0.000	—	—	Matimba <i>et al.</i> (2009)
African national group																	
Nigerian	158	0.845	0.155	0.000	—	—	—	—	—	—	—	—	—	—	—	—	Babalola <i>et al.</i> (2010)
Nigerian SCD	115	0.843	0.157	0.000	—	—	—	—	—	—	—	—	—	—	—	—	Babalola <i>et al.</i> (2010)
Tanzanian	106	0.821	0.179	0.000	—	—	—	—	—	—	—	—	—	—	—	—	Dandara <i>et al.</i> (2001)
Beninese	119	0.870	0.130	0.000	—	—	—	—	—	—	—	—	—	—	—	—	Allabi <i>et al.</i> (2003)
Egyptian	247	0.888	0.110	0.002	—	—	—	—	—	—	—	—	—	—	—	—	Hamdy <i>et al.</i> (2002)

(—), not screened; UNKN, undetermined.

2001; Hamdy *et al.*, 2002; Allabi *et al.*, 2003; Luo *et al.*, 2006; Matimba *et al.*, 2009; Kearns *et al.*, 2010; Man *et al.*, 2010; Martis *et al.*, 2013). However, as depicted in Table 3, the frequency data for variant *CYP2C19* alleles beyond those normally tested (*2, *3, and recently *17) for African Americans are very limited. To our knowledge, only a single study conducted in an African population has published data on the prevalence of the *2 and *3 alleles in SCD patients (Babalola *et al.*, 2010). Recently, Martis *et al.* (2013) reported the *CYP2C19**15 variant in a large African American cohort. Man *et al.* (2010) reported the *CYP2C19**9, *10, and *13 alleles in African subjects. However, none of these alleles were observed in our cohort. The frequency (23%) for the *17 allele is the highest, to date, reported in African Americans (Martis *et al.*, 2013). Our cohort exhibited a high frequency of the *17/*17 ultra-rapid genotype (9.1%) compared to (2.8%) reported in Martis *et al.* (2013). However, no differences were detected for the *1/*17 (UM), *2/*2 (PM), and *2/*17* (UNK) genotypes in the cohort (21.2%, 5.5%, and 7.3%) and healthy African Americans (20.4%, 4.8%, and 9.2%), respectively (Martis *et al.*, 2013). Our study reports the frequency of the *CYP2C19**12 allele for the first time in an African American cohort. As depicted in Table 3, the limited studies suggest that to date, the *CYP2C19* *2, *3, *9, *12, *13, *15, and *17 are the variants of potential interest for preemptive genotyping in populations of African descent (Alessandrini *et al.*, 2013).

Discussion

To the best of our knowledge, this study is the first to identify the frequency of genetic polymorphisms in the *CYP2C19* gene in an African American SCD patient cohort. This information is useful for the proper design of pharmacogenetic study of *CYP2C19* substrates for rational prescribing practice in this patient population. The *CYP2C19* metabolizes several psychotropics, proton pump inhibitors, anti-infectives, and anticonvulsants often prescribed for SCD comorbidities (Bertilsson, 1995; Furuta *et al.*, 2004; Sim *et al.*, 2006; Simon *et al.*, 2009; Sibbing *et al.*, 2010; Hodgson *et al.*, 2014; Luk *et al.*, 2014). For the antiplatelet drug, clopidogrel, genotype-directed guidelines have been provided by the Clinical Pharmacogenetics Implementation Consortium (Robert *et al.*, 2012; Scott *et al.*, 2013).

Preemptive genotyping and clinical application of functional information of *CYP2C19* allelic variants could empower clinicians to communicate pharmacologic risk and drug response prediction to SCD patients using biological evidence, as opposed to explaining statistical risk without biological significance, and possibly inform drug-dose adjustments according to the genotype (Schildcrout *et al.*, 2012; Sparkenbaugh and Pawlinski, 2013). For instance, with regard to treatment of peptic ulcer disease with standard doses of proton pump inhibitors, we could extrapolate that 30% of our cohort who are UMs (*1/*17 and *17/*17) would experience reduced acid-inhibitory effect, which may result in therapeutic failure. Also, with our cohort being 31.5% EM and 30.3% UM, greater than half of our subjects will theoretically have significantly decreased drug exposure for *CYP2C19* substrates such as, diazepam, citalopram, amitriptyline, and clomipramine as the concentrations of these drugs may decrease by rapidly converting into inactive

metabolites with a possible reduction of therapeutic effects. Interestingly, for the 8% of our cohort with unknown predicted metabolic phenotypes, the pharmacologic risks for *CYP2C19* substrates could not be determined, making these individuals candidates for clinical vigilance and alternative drug choices. Thus, for SCD patients prone to multiorgan disease-related comorbidities and polypharmacy, preemptive genotyping provides readily available genetic information to guide therapy (Leeder, 2015). Even normal *CYP2C19* metabolic genotype result has clinical utility for SCD patients. Clinicians could use normal metabolic profile results to reassure patients of the safety of the drugs prescribed and likelihood to work for them, thereby potentially promoting adherence. In addition, SCD patients could share their pharmacogenetic results with their other health provider to ensure optimal prescribing for future medications (Haga and Mills, 2015).

To be sure, the utility of genotype-guided prescribing practice for *CYP2C19* substrates in SCD patients will depend on *CYP2C19* enzyme expression, and genotype-metabolic phenotype concordance, which may be influenced by SCD. Generally, the amount of active *CYP2C19* depends on the level of gene expression. Acute pain crisis in SCD is the quintessential inflammatory state (Sparkenbaugh and Pawlinski, 2013). Interestingly, recent reports have associated inflammation response with a decreased *CYP2C19* activity in patients with severe inflammatory disease. The decrease in drug metabolism can be up to 70% and is mediated by proinflammatory cytokines (Vet *et al.*, 2011). Thus, independent of metabolic genotype, systematic inflammation associated with SCD pain may downregulate *CYP2C19* enzyme expression in SCD patients and lead to “acquired” PM status in patients either with homozygous (**1/*1*) or heterozygous (**1/*2*) genotype. Downregulation of the *CYP2C19* enzyme has been reported in solid tumor and multiple myeloma cancer patients due to tumor-associated inflammatory factors (Williams *et al.*, 2000; Helsby *et al.*, 2008; Vet *et al.*, 2011; Burns *et al.*, 2014). Thus, it is likely that during vaso-occlusive pain crisis, SCD patients may have increased exposure to drugs because of decreased clearance and an increased risk of adverse drug effects (Shah and Smith, 2015). Furthermore, inflammation could also affect the expression and activity of drug transporters, influence protein binding, capillary permeability, cardiac output, and liver blood flow. Collectively, these processes may have important pharmacokinetic and pharmacodynamic consequences and corresponding phenotypic disposition (Shah and Smith, 2015). Thus, given the numerous variables that could cause discordance in drug metabolic genotype-phenotype relationship, extrapolating allelic frequencies and genotypes of the *CYP2C19* enzyme from healthy African Americans to SCD patients is not recommended.

Interestingly, our study indicated a significantly higher percentage of African American EM (31.3%) compared to 23.2% EM in the Martis study, raising the possibility that within African American patient subgroups, some *CYP2C19* alleles may be underrepresented or overrepresented because of founder effect (Martis *et al.*, 2013). For instance, SCD patients are reported to have a slightly higher frequency of the *CYP2D6* gene deletions compared to healthy African Americans (Yee *et al.*, 2013). *CYP2D6* gene deletions are associated with the PM phenotype and impaired ability to convert opioids such as codeine and hydrocodone into their

active analgesic forms and as such are implicated in SCD pain phenotype and analgesic drug response. Undoubtedly, to understand variability in drug response linked to *CYP2C19* enzyme in SCD patients, further work should focus on measurement of *CYP2C19* activity, especially for psychotropic medications frequently used as adjuvant analgesics for SCD pain (Darbari *et al.*, 2011). These medications have been shown to have infrequent, but potentially important hematologic side effects in SCD patients (Jerrell *et al.*, 2011).

There are limitations to our study that we acknowledged. The method used to genotype the *CYP2C19* alleles does not differentiate between the *CYP2C19**1B, *1C, and *9. Thus, it is likely that some individual classified at EM may harbor the reduced-function *CYP2C19**9 allele. Also, genotyping only allows identification of the *CYP2C19* allelic status and is independent of environmental influences. This feature could likewise be considered a drawback as this approach provides no information on the actual level of enzyme activity that may depend on numerous modulating influences as noted above. Phenotyping on the other hand, that is, the direct analysis of metabolite ratios using appropriate probes would take into account such factors. Thus, pharmacokinetic study remains the gold standard for discerning individual SCD patients' drug metabolic phenotypes. Nonetheless, preemptive *CYP2C19* genotyping aided by appropriate pharmacokinetics and pharmacodynamics studies brings into sharp relief the potential for genomic-based dosing strategies in SCD patients in the same way as pharmacogenetics guides oncological use of biological agents (Anwar *et al.*, 2015).

Conclusion

In conclusion, preemptive genotyping is potentially useful for identifying SCD patients with allelic variants linked to proven clinical pharmacokinetic consequences for several drugs metabolized by the *CYP2C19* gene. Indeed, guidelines and treatment algorithms have been developed to assist clinicians in genotype-directed anti-platelet therapy (Roberts *et al.*, 2012) and other prescribing algorithms are currently underway (Hicks *et al.*, 2015). Such guidelines, primarily driven by an increasing evidence base, categorize patients based on their *CYP2C19* genotypes to guide drug dosing. However, the main challenge to implementing genetics-guided prescribing practice for SCD patients is ensuring concordance between *CYP2C19* genotypes-metabolic phenotypes.

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Authors' Contributions

Conceived and designed the experiments: C.J., M.L., and A.K. Participated in research design: C.J., N.B., and A.K.; performed the experiments: N.P. Analyzed the data: C.J. and H.X. Wrote or contributed to the writing of the article: C.J., N.B., A.K., and M.L.

Author Disclosure Statement

No competing financial interests exist.

References

- Alessandrini M, Asfaha S, Dodgen TM, *et al.* (2013) Cytochrome P450 pharmacogenetics in African populations. *Drug Metab Rev* 45:253–275.
- Allabi AC, Gala JL, Desager JP, *et al.* (2003) Genetic polymorphisms of CYP2C9 and CYP2C19 in the Beninese and Belgian populations. *Br J Clin Pharmacol* 56:653–657.
- Anwar MS, Iskadar MZ, Parry HM, *et al.* (2015) The future of pharmacogenetics in the treatment of heart failure. *Pharmacogenomics* 16:1817–1827.
- Babalola CP, Adejumo O, Ung D, *et al.* (2010) Cytochrome P450 CYP2C19 genotypes in Nigerian sickle-cell disease patients and normal controls. *J Clin Pharm Ther* 35:471–477.
- Bertilsson L (1995) Geographic/interracial differences in polymorphic drug oxidation: current state of knowledge of cytochrome P450 (CYP) 2D6 and 2C19. *Clin Pharmacokinet* 29:192–209.
- Birdwell KA, Decker B, Barbarino JM, *et al.* (2015) Clinical pharmacogenetics implementation consortium (CPIC) guidelines for CYP3A5 genotype and tacrolimus dosing. *Clin Pharmacol Ther* 98:19–24.
- Burns KE, Goldthorpe MA, Porteus F, *et al.* (2014) CYP2C19 genotype-phenotype discordance in patients with multiple myeloma leads to an acquired loss of drug-metabolising activity. *Cancer Chemother Pharmacol* 73:651–655.
- Dandara C, Masimirembwa CM, Magimba A, *et al.* (2001) Genetic polymorphism of CYP2D6 and CYP2C19 in east- and southern African populations including psychiatric patients. *E J Clin Pharmacol* 57:11–17.
- Darbari DS, Neely M, van den Anker J, *et al.* (2011) Increased clearance of morphine in sickle cell disease: implications for pain management. *J Pain* 12:531–538.
- Dunnenberger HM, Crews KR, Hoffman JM, *et al.* (2015) Preemptive clinical pharmacogenetics implementation: current programs in five US medical centers. *Annu Rev Pharmacol Toxicol* 55:89–106.
- Elmariam H, Garrett ME, De Castro LM, *et al.* (2014) Factors associated with survival in a contemporary adult sickle cell disease cohort. *Am J Hematol* 89:530–535.
- Furuta T, Shirai N, Sugimoto M, *et al.* (2004) Pharmacogenomics of proton pump inhibitors. *Pharmacogenomics* 5:181–202.
- Gardiner SJ, Begg EJ (2006) Pharmacogenetics, drug-metabolizing enzymes, and clinical practice. *Pharmacol Rev* 58:521–590.
- Haga SB, Mills R (2015) Nurses' communication of pharmacogenetic test results as part of discharge care. *Pharmacogenomics* 16:251–256.
- Hamdy S, Hiratsuka M, Narahara K, *et al.* (2002) Allele and genotype frequencies of polymorphic cytochromes P450 (CYP2C9, CYP2C19, CYP2E1) and dihydropyrimidine dehydrogenase (DPYD) in the Egyptian population. *Br J Clin Pharmacol* 53:596–603.
- He YJ, McLeod HL (2012) Ready when you are: easing into preemptive pharmacogenetics. *Clin Pharmacol Ther* 92:412–414.
- Helsby NA, Lo WY, Sharples K, *et al.* (2008) CYP2C19 pharmacogenetics in advanced cancer: compromised function independent of genotype. *Br J Cancer* 99:1251–1255.
- Hicks JK, Bishop JR, Sangkuhl K, *et al.* (2015) Clinical pharmacogenetics implementation consortium (CPIC) guideline for CYP2D6 and CYP2C19 genotypes and dosing of selective serotonin reuptake inhibitors. *Clinical pharmacogenetics implementation consortium*. *Clin Pharmacol Ther* 98:127–134.
- Hodgson K, Tansey K, Dernovsek MZ, *et al.* (2014) Genetic differences in cytochrome P450 enzymes and antidepressant treatment response. *J Psychopharmacol* 28:133–141.
- Jerrell JM, Tripathi A, Stallworth JR (2011) Pain management in children and adolescents with sickle cell disease. *Am J Hematol* 86:82–84.
- Kearns GL, Leeder JS, Gaedigk A (2010) Impact of the CYP2C19*17 allele on the pharmacokinetics of omeprazole and pantoprazole in children: evidence for a differential effect. *Drug Metab Dispos* 38:894–897.
- Lee SJ (2013) Clinical application of CYP2C19 pharmacogenetics toward more personalized medicine. *Front Genet* 3:318.
- Leeder JS (2015) Meaningful use and clinical utility of preemptive pharmacogenetic testing (Re)View from a CYP2D6 poor metabolizer. *Clin Pharmacol Ther* 97:119–121.
- Luk S, Atayee RS, Ma JD, *et al.* (2014) Urinary diazepam metabolite distribution in a chronic pain population. *J Anal Toxicol* 38:135–142.
- Luo HR, Poland RE, Lin KM, *et al.* (2006) Genetic polymorphism of cytochrome P450 2C19 in Mexican Americans: a cross-ethnic comparative study. *Clin Pharmacol Ther* 80:33–40.
- Man N, Farne M, Dumaual C, *et al.* (2010) Genetic variation in metabolizing enzymes and transporter genes: comprehensive assessment in 3 major East Asian subpopulations with comparison to Caucasians and African. *J Clin Pharmacol* 50:929–940.
- Matimba A, Jurgen DF, Van Broeckhoven C, *et al.* (2009) Novel variants of major drug-metabolising enzyme genes in diverse African populations and their predicted functional effects. *Hum Genomics* 3:169–190.
- Martis S, Peter I, Hulot J-S, *et al.* (2013) Multi-ethnic distribution of clinically relevant CYP2C genotypes and haplotypes. *Pharmacogenomics J* 13:369–377.
- Rasmussen-Torvik LJ, Stallings SC, Gordon AS, *et al.* (2014) Design and anticipated outcomes of the eMERGE-PGx project: a multicenter pilot for preemptive pharmacogenomics in electronic health record systems. *Clin Pharmacol Ther* 96:482–489.
- Roberts JD, Wells GA, Le May MR, *et al.* (2012) Point-of-care genetic testing for personalisation of antiplatelet treatment (RAPID GENE): a prospective, randomised, proof-of-concept trial. *Lancet* 379:1705–1711.
- Schildcrout JS, Denny JC, Bowton E, *et al.* (2012) Optimizing drug outcomes through pharmacogenetics: a case for preemptive genotyping. *Clin Pharmacol Ther* 92:235–242.
- Scott SA, Sangkuhl K, Shuldiner AR, *et al.* (2012) PharmGKB summary: very important pharmacogene information for cytochrome P450, family 2, subfamily C, polypeptide 19. *Pharmacogenet Genomics* 22:159–165.

- Scott SA, Sangkuhl K, Stein CM, *et al.* (2013) Clinical pharmacogenetics implementation consortium clinical pharmacogenetics implementation consortium guidelines for CYP2C19 genotype and clopidogrel therapy: 2013 update. *Clin Pharmacol Ther* 94:317–323.
- Shah RR, Smith RL (2015) Inflammation-induced phenocconversion of polymorphic drug metabolizing enzymes: hypothesis with implications for personalized medicine. *Drug Metab Dispos* 43:400–410.
- Sibbing D, Koch W, Gebhard D, *et al.* (2010) Cytochrome 2C19*17 allelic variant, platelet aggregation, bleeding events, and stent thrombosis in clopidogrel-treated patients with coronary stent placement. *Circulation* 121:512–518.
- Sim SC, Ingelman-Sundberg M (2006) The human cytochrome P450 allele nomenclature committee web site: submission criteria, procedures, and objectives. *Methods Mol Biol* 320:183–191.
- Sim SC, Risinger C, Dahl ML, *et al.* (2006) A common novel CYP2C19 gene variant causes ultrarapid drug metabolism relevant for the drug response to proton pump inhibitors and antidepressants. *Clin Pharmacol Ther* 79:103–113.
- Simon T, Verstuyft C, Mary-Krause M, *et al.* (2009) Genetic determinants of response to clopidogrel and cardio-vascular events. *N Engl J Med* 360:363–375.
- Sparkenbaugh E, Pawlinski R (2013) Interplay between coagulation and vascular inflammation in sickle cell disease. *Br J Haematol* 162:3–14.
- Verschuren JJ, Trompet S, Wessels JA, *et al.* (2012) A systematic review on pharmacogenetics in cardiovascular disease: is it ready for clinical application? *Eur Heart J* 33:165–175.
- Vet NJ, de Hoog M, Tibboel D, *et al.* (2011) The effect of inflammation on drug metabolism: a focus on pediatrics. *Drug Discov Today* 16:435–442.
- Williams ML, Bhargava P, Cherrouk I, *et al.* (2000) A discordance of the cytochrome P450 2C19 genotype and phenotype in patients with advanced cancer. *Br J Clin Pharmacol* 49: 485–488.
- Yee M, Josephson C, Hill C, *et al.* (2013) Cytochrome P450 2D6 Polymorphism and Predicted Metabolism in African American Children with Sickle Cell Disease. *J Pediatr Hematol Oncol* 7:e301–e305.

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