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Pharmacogenetic Gene–Drug Associations in Pediatric Burn and Surgery Patients

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Management of critically ill patients requires simultaneous administration of many medications. Treatment for patient comorbidities may lead to drug–drug interactions which decrease drug efficacy or increase adverse reactions. Current practices rely on a one-size-fits-all dosing approach. Pharmacogenetic testing is generally reserved for addressing problems rather than used proactively to optimize care. We hypothesized that burn and surgery patients will have one or more genetic variants in drug metabolizing pathways used by one or more medications administered during the patient's hospitalization. The aim of this study was to determine the frequency of variants with abnormal function in the primary drug pathways and identify which medications may be impacted. Genetic (19 whole exome and 11 whole genome) and medication data from 30 pediatric burn and surgery patients were analyzed to identify pharmacogene–drug associations. Nineteen patients were identified with predicted altered function in one or more of the following genes: *CYP2C9*, *CYP2C19*, *CYP2D6*, and *CYP3A4*. The majority had decreased function, except for several patients with *CYP2C19* rapid or ultrarapid variants. Some drugs administered during hospitalization that rely on these pathways include hydrocodone, oxycodone, methadone, ibuprofen, ketorolac, celecoxib, diazepam, famotidine, diphenhydramine, and glycopyrrolate. Approximately one-third of the patients tested had functionally impactful genotypes in each of the primary drug metabolizing pathways. This study suggests that genetic variants may in part explain the vast variability in drug efficacy and suggests that future pharmacogenetics research may optimize dosing regimens.

Variability in drug efficacy is a major contributor to the difficulties in managing critically ill patients, especially in special populations (eg, burns, trauma, pediatric). Special populations may have more adverse consequences as a result of poor efficacy or adverse effects of drugs due to their altered metabolic states, a narrow therapeutic window and increased sensitivity to adverse effects. Pediatric burn and surgery patients are of particular interest given the larger number of medications administered simultaneously during their long hospitalizations. Pain medications, and particularly opioids, frequently demonstrate enormous variability in efficacy in these children, with resultant increased concerns regarding adverse effects. Opioids, such as fentanyl, hydromorphone, oxycodone, morphine, and methadone, are a mainstay for pain control in critically ill patients; however, the unpredictable response and toxic effects do not allow for a one-size-fits-all dosing approach, and there have been increasing concerns for other analgesics such as nonsteroidal anti-inflammatories (NSAIDs) and acetaminophen. Additional medications used to treat pediatric burn and surgery patients include but are not limited to serotonin 5-HT3 receptor antagonist, anticholinergic, *N*-methyl-d-aspartate receptor antagonist, corticosteroids, antimicrobials, antacids, and antiemetics.

Variability in drug efficacy is largely attributed to genetic variants in drug metabolism pathways. The cytochrome P450 enzymes (CYP) are the largest class of enzymes responsible for drug metabolism accounting for the primary route of metabolism for 70% to 80% of clinically used drugs.[1] These enzymes are in highest abundance in the liver but are also present in other organs such as the gastrointestinal tract, and biotransform endogenous and exogenous compounds to facilitate elimination. Most compounds are inactivated by CYPs, however, some, like morphine, are converted into active metabolites. The activity of CYPs may be altered during hypermetabolism, while coadministration of particular drugs can also cause induction or inhibition of CYPs' activity.[2,3] Numerous CYP subtypes have been identified, with *CYP2D6* and *CYP3A4* being the most clinically significant.[4–6] *CYP2D6* and *CYP3A4* are responsible for the metabolism of 25% and >50%, respectively, of medications in clinical use.[6] Genetic variants associated with CYPs can result in reduced function. This can cause toxic accumulation of drug. Other variants have increased activity resulting in poor efficacy from low levels of drug in circulation. *CYP2D6* polymorphisms occur more frequently and are associated with treatment failures and toxicity for several medications (eg, tamoxifen, propranolol, opioids).[7]

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The reported allele frequency of *CYP2D6* slow-metabolizing variants is reported to be disproportionate among different populations, with increased frequencies in Caucasians and Hispanic-Latinos, compared to African and Asian populations.[8] Several other CYPs and genes (eg, *CYP2B6*, *CYP2C9*, *CYP2C19*, *UGT1A*, *SULT1A1*, transporters, receptors, and channels) are gaining more importance as knowledge regarding their clinical impact is discovered. The goal of pharmacogenetics is to study pharmacogene–drug associations to provide a molecular understanding of patient variability. To this end, genetic screening for not only CYPs but also other gene variants that have a high likelihood of influencing drug efficacy in patients would likely inform personalized dosing of medications.

Clinical decisions based in part on an individual's genetic variants are a classic example of precision medicine. The majority of CYP clinical applications have involved cancer therapy.[9,10] For example, correlating therapeutic efficacy of tamoxifen in breast cancer patients with *CYP2D6* activity has identified slow-metabolizers with greater risk for tamoxifen-induced toxicity, while fast-metabolizers exhibit increased risk for treatment failure and/or tumor recurrence. Another classic example is patients with human leukocyte antigen mutations developing Steven–Johnson syndrome or drug-induced hypersensitivity syndrome after certain medications are administered.[11] Only recently have studies suggested the use of CYP genotyping to improve dosing of common medications for pain management.[12,13] Pharmacogenetics is still a relatively young field and only in the past one to two decades has it really started to make better progress. Advances in genomic techniques, bioinformatics and a better understanding of molecular biology have allowed for the identification of associations between specific individual genetic variants and drug response. While much progress has been made to identify new genotype–phenotype associations by decoding the genetic variants of clinical importance in both metabolism and response pathways (such as the *CYP2D6*/Codeine relationship and the more recent discovery of *CYP2C9* importance for celecoxib), there has not been widespread clinical implementation of pharmacogenetics guided prescribing. The reason for this lack of translation is multifactorial: 1) lack of knowledge on how to translate genetic information into clinical action, 2) interpretation and availability of genotype testing, and 3) lack of uniform recommendations for selecting drug/gene pairs. The Clinical Pharmacogenetics Implementation Consortium (CPIC) has attempted to fill these knowledge gaps. To date, 26 CPIC gene–drug guidelines have been published. Interestingly, four gene–drug guidelines are for the analgesics codeine (*CYP2D6*), tramadol (*CYP2D6*), oxycodone (*CYP2D6*), and celecoxib (*CYP2C9*).[14,15] To further advance the implementation of pharmacogenetics, and particularly incorporate pain management into precision medicine, we must conduct clinical studies in racially/ethnically diverse patient populations to provide robust data to inform clinicians and institutions of relevant and clinically important gene/drug combinations. This is of vital importance to provide individualized patient care, particularly related to pain management regimens, and decrease adverse outcomes.

As high-risk populations, pediatric severe burn patients and complex pediatric surgical patients require numerous medications during their hospitalization. In addition, they may require higher and more frequent opioid and analgesic doses, making them the ideal human model for pharmacogenetic research. Common medications include opioids (eg, fentanyl, hydromorphone), as well as compounds known to alter CYP activity (eg, propranolol, fluconazole).[9] In a previous study in adult burn patients, we reported that, on average, a patient with >20% TBSA burn receives an average of 40.6 ± 20.2 medications per day.[16] Unfortunately, drug dosing in burn patients does not truly account for pharmacokinetic, pharmacodynamic, and pharmacogenetic variation in this complex population. This may result in decreased efficacy, occurrence of adverse events, or development of tolerance and addiction.

Another important factor is that special populations may be at higher risk of phenoconversion, which is the phenomenon of a person's genetic based phenotype being converted to a different category due to a nongenetic extrinsic factor.[17] An example of phenoconversion is a normal metabolizer being converted to a poor metabolizer due to a disease state, such as children with sepsis being reported to have decreased CYP-mediated drug metabolism, thought to be related in part to an increased inflammatory state.[18] Reduction in CYP activity attributed to inflammation has also been observed in patients following hip surgery, which demonstrated acute inflammation altered CYP activity in an isoform-specific manner.[19] A thorough review by Lenoir et al also discussed numerous other inflammatory-related illnesses resulting in CYP inhibition including, infection, impaired organ function, diabetes, autoimmune disease, surgery, cancer, and administration of immune modulating medications.[20] Drug interactions also contribute to phenoconversion, such as antifungal and antiviral medications causing inhibition in CYP-mediated metabolism, while some antimicrobials and antiepileptics have been responsible for CYP induction resulting in increased metabolism.[21] Moreover, hypermetabolism in burn patients results in severe alterations in organ blood flow, protein binding and synthesis, extreme inflammatory responses, as well as other comorbidities that can greatly impact drug metabolism.[22–24]

Personalized dosing relies on a solid foundation of established pharmacokinetic/pharmacodynamic data, along with a thorough understanding of pharmacogenetics and drug–drug interactions. Thus, published data in burns and other special populations (eg, pediatrics) are insufficient for simulation modeling for projecting personalized dosing regimens in complicated patients. Thus, this study aims to elucidate the frequency of significant pharmacogene–drug associations in pediatric burn and surgery patients, specifically for medications they were administered during their hospitalization.

METHODS

Data utilized in this analysis were obtained from two ongoing studies to investigate fentanyl pharmacogenetics in pediatric patients: 1) samples collected in the intensive care unit (ICU) from pediatric acute burn patients and 2) samples collected in the operating room and post-op (OR) from pediatric surgery patients. Given that both groups are undergoing surgery and anesthesia, they are subjected to many of the same drugs and anesthetic treatment strategies. Both groups are being treated at the same institution in parallel with the same patient care staff and workflows. Hence, we have elected to combine data from the burn and surgical pediatric patients to conduct

this pilot analysis. These studies were conducted under approved Institutional Review Board protocols reviewed by the WCG IRB.

Patient Enrollment

Thirty pediatric patients, ≤18 years old, were enrolled by direct informed consent and when necessary, guardian/parental consent. Inclusion criteria for the ICU study include: 1) TBSA burned ≥10%, 2) ability to consent or surrogate consent, and 3) receiving fentanyl therapy during dressing changes. Inclusion criteria for the OR study include: 1) having a surgical procedure, 2) ability to consent or surrogate consent, and 3) receiving fentanyl at the time of surgery. Exclusion criteria include: 1) nonsurvivable injuries and/or 2) condition where collection of research samples is unsafe (eg, severe anemia).

Sample and Data Collection

Blood specimens for genotyping were collected during the patient's in-patient stay. The blood sample was drawn from an existing arterial or venous catheter line. Patient data were collected from their electronic medical records including demographics (eg, age, weight, ethnicity), all drug administrations, including doses and route, any concurrent identified infections, other medical conditions, as well as vital signs and routine laboratory results, and procedures (eg, surgery, line placement, etc.). All data were deidentified for analysis and patients were tracked by an assigned research study identifier. A list of all medications for each patient was extracted from the electronic medical records. Each list was curated to standardize medication names to generic names from brand names and to remove any duplications. Online databases (DrugBank, Pharmacogenetics Knowledgebase [PharmGKB], CPIC) and other published literature were used to determine primary and minor pathways for each medication.[25–30]

Sequencing

Genomic DNA (gDNA) was isolated from whole blood using the Gentra Puregene Blood Kit (Qiagen, Maryland). DNA samples were assessed for quality and concentration. Isolated DNA samples were submitted to the University of California Sequencing Consortium laboratory for whole-exome sequencing (WES) (ICU Study) or to the Shriner's Hospital for Children Genomics Institute for whole-genome sequencing (WGS) (OR Study) to achieve ≥×60 coverage. Raw sequence data (FASTQ) were mapped to the following human reference (GRCh37/hg19, December 2009) using the DRAGEN (Dynamic Read Analysis for Genomics) Ultra-Rapid Next Generation Sequencing Data Analysis Platform (Illumina).[31–33]

Genomic Data Analysis

Full allelic decomposition, genotyping, and assignment of alleles for each pharmacogene were accomplished by applying the Aldy framework.[34,35] Aldy processes BAM alignment files to identify the genomic variants in the pharmacogenes, including single nucleotide variants (SNVs), insertions/deletions (Indels), copy number variants (CNVs), structural variants (SVs), and pseudogene fusions; star-allele discovery is guided by a database containing all known genomic variations associated with the different genotypes. Online databases, specifically the PharmGKB and Pharmacogene Variation Consortium (PharmVar) were used to assign a clinical function to each star-allele. Based on star-alleles identified, activity scores were assigned to each diplotype and patients were grouped into standardized phenotypic categories: poor, intermediate, normal (historically termed extensive metabolizer), rapid, or ultrarapid metabolizers, or unknown/indeterminate.[36,37]

Statistical Analysis

Shapiro–Wilk test was used to evaluate continuous data (age, hospital length of stay, number of medications administered during hospitalization) for normality using GraphPad Prism v9.3.1 (GraphPad Software, California). Descriptive statistics was used to summarize cohort demographics, medication tallies, and length of hospitalization, with normally distributed data presented as mean ± standard deviation, while nonnormally distributed data are presented as median and range. Allele data are summarized by calculating frequencies within the studied population. As described above, the Aldy software utilizes star-allele databases to predict the most likely genotype for each patient dataset. Each Aldy solution has an associated confidence score with a maximum value of 1.0. By default, Aldy will only report out genotype results with a confidence score of 1.0.

RESULTS

A total of 30 patients were analyzed. Patients included 17 males and 13 females with a mean age of 10.3 ± 4.8 years. The patient ethnicities included 17 Hispanic-Latino (3 reported white, 14 other), 9 Caucasian (not Hispanic), 1 African-American, 1 Asian, and 2 unknown or declined to respond. The median length of stay for hospitalization was 27.5 days with a range of 151 days. Twenty of the patients were acute burn cases, 3 were previously burned patients returning for surgery and 10 were orthopedic spine surgery cases. Of the returning burn patients and orthopedic patients, the total length of stay was between 3 and 14 days. Preliminary analysis in 30 patients identified numerous functionally abnormal variants and several others of unknown clinical importance. Analysis was conducted using 19 WES and 11 WGS datasets and analyzed with the Aldy software to classify CYP star-alleles. Diplotype frequencies and predictions of functional impact are presented in Table 1. The wild-type (**1/*1*) diplotype frequency was observed in approximately two-thirds of patients for both *CYP2C9* and *CYP2C19* and in just over half the patients for *CYP2D6*, whereas 90% of patients were wild-type for *CYP3A4* but only 3.3% for *CYP3A5*. The remaining diplotypes have much lower frequencies apart from **3/*3* for *CYP3A5* being represented in 80% of the patients. The *CYP2D6* gene had the greatest number of different genotypes identified (n = 21), while *CYP2C9* and *CYP2C19* each had six different genotypes, *CYP3A5* had four and *CYP3A4* had three different genotypes. Nineteen patients were identified with predicted altered function in one or more of the following genes: *CYP2C9*, *CYP2C19*, *CYP2D6*, and *CYP3A4* (Table 2). The majority had decreased function, except for several patients with *CYP2C19*

Table 1. Diplotype activity scores and frequencies for *CYP2C9*, *CYP2C19*, *CYP2D6*, *CYP3A4*, and *CYP3A5*

Diplotypes in Study	Activity Score	Number of Subjects with Diplotype	Diplotype Frequency	Phenotype Prediction
CYP2C9				
*1/*1	2	20	66.67	NM
*1/*2	1.5	6	20.00	IM
*1/*11	1.5	1	3.33	IM
*1/*3	1	1	3.33	IM
*2/*2	1	1	3.33	IM
*2/*3	0.5	1	3.33	PM
CYP2C19				
*17/*17	4	1	3.33	UM
*1/*17	3	3	10.00	RM
*1/*1	2	19	63.33	NM
*2/*17	2	1	3.33	IM
*1/*2	1	5	16.67	IM
*1/*35	1	1	3.33	IM
CYP2D6				
*1/*1	2	4	13.33	NM
*1/*2	2	3	10.00	NM
*2/*2	2	2	6.67	NM
*1/*35	2	1	3.33	NM
*2/*35	2	1	3.33	NM
*1/*41	1.5	1	3.33	NM
*2/*41	1.5	4	13.33	NM
*1/*4.021	1	1	3.33	IM
*2/*4	1	1	3.33	IM
*2/*4M+rs1135840	1	1	3.33	IM
*4C/*17	1	1	3.33	IM
*4/*35	1	1	3.33	IM
*4C/*35+rs28371703	1	1	3.33	IM
*41/*41	1	1	3.33	IM
*4.021/*41	0.5	1	3.33	IM
*4.021/*59	0.5	1	3.33	IM
*4+*4N.ALDY/*80	n/a	1	3.33	Indeterminate
*80/*83.ALDY or *61/*80	n/a	1	3.33	Indeterminate
*61/*79 or *79/*83.ALDY	n/a	1	3.33	Indeterminate
*1/*127	n/a	1	3.33	Indeterminate
*4.028/*139	n/a	1	3.33	Indeterminate
CYP3A4				
*1/*1	n/a	27	90	NM
*1/*22	n/a	2	6.67	Indeterminate
*3/*22	n/a	1	3.33	Indeterminate
CYP3A5				
*1/*1	n/a	1	3.33	NM
*1/*3	n/a	4	13.33	Indeterminate
*3/*3	0	24	80.00	Nonfunctional
*3/*7	0	1	3.33	Nonfunctional

IM, intermediate metabolizer; *n/a*, not applicable; *NM*, normal metabolizer; *PM*, poor metabolizer; *RM*, rapid metabolizer; *UM*, ultrarapid metabolizer.

variants resulting in rapid (2) and ultrarapid (1) phenotypes. The *CYP2C19*17* allele, which is responsible for the rapid and ultrarapid phenotypes, was only identified in WGS and not WES datasets. Numerous patients have alleles with unknown/indeterminate functional significance in *CYP2D6* and *CYP3A4*. *CYP2C9*, *CYP2C19*, and *CYP2D6* each have one-third of patients with an abnormal variant.

Patients received an average of 43.9 ± 18.2 medications during their length of stay with a minimum of 19 and a maximum of 94. A total of 180 different medications were evaluated. Thirty-nine of the medications are reported to utilize CYPs for primary and/or minor routes of metabolism (Table 3). For the medications evaluated in this study, the *CYP3A4* pathway is responsible for the largest number of medications for primary metabolism, followed by the *CYP2D6* pathway, which had the greatest number of variants with predicted functional impact. Both *CYP2C9* and *CYP2C19* had approximately half the number of medications impacted as *CYP2D6*, however, had a similar number of patients, approximately a third, with variants with predicted functional

Table 2. Summary of abnormal phenotypes for *CYP2C9*, *CYP2C19*, *CYP2D6*, and *CYP3A4*

Patient	*CYP2C9*	*CYP2C19*	*CYP2D6*	*CYP3A4*	Patients With One or More Abnormal Function
1	**IM**	NM	NM	NM	A
2	NM	NM	NM	NM	
3	NM	NM	**IM**	NM	A
4	**IM**	**IM**	**IM**	NM	A
5	NM	NM	NM	NM	
6	**PM**	NM	**UK**	NM	A
7	NM	**IM**	**UK**	NM	A
8	**IM**	**IM**	**IM**	NM	A
9	NM	NM	NM	NM	
10	NM	NM	NM	NM	
11	**IM**	NM	NM	NM	A
12	NM	NM	NM	NM	
13	**IM**	NM	NM	NM	A
14	NM	NM	NM	NM	
15	NM	NM	NM	NM	
16	**IM**	NM	**IM**	NM	A
17	NM	NM	**IM**	**UK**	A
18	NM	NM	NM	NM	
19	NM	**IM**	**IM**	NM	A
20	NM	**RM**	NM	NM	A
21	NM	**RM**	NM	NM	A
22	NM	**IM**	NM	NM	A
23	NM	NM	NM	NM	
24	**IM**	**IM**	**IM**	**UK**	A
25	NM	**UM**	**IM**	**UK**	A
26	**IM**	NM	NM	NM	A
27	NM	NM	**UK**	NM	
28	NM	**RM**	**UK**	NM	A
29	**IM**	**IM**	**IM**	NM	A
30	NM	NM	**UK**	NM	
Totals	10	11	9	3	19

A, abnormal phenotype; *IM*, intermediate metabolizer; *NM*, normal metabolizer; *PM*, poor metabolizer; *RM*, rapid metabolizer; *UM*, ultrarapid metabolizer.

impact (Table 2). The *CYP3A5*3* allele was present in the majority of the patients and accounted for 53 of the 60 alleles analyzed. The *CYP1A1*, *CYP1A2*, *CYP1E1*, *CYP2A6*, *CYP2B6*, and *CYP2C8* were identified in the medication metabolism review as playing a primary role in metabolism of 12 of the medications, however, variant evaluation to predict allele assignment and functional impact was not performed in this investigation.

The medications with the highest number of variants (seven or more) with predicted functional impact in the primary metabolism pathways included propofol (*CYP2C9*), oxycodone (*CYP2D6*), methadone (*CYP2C19*), and ibuprofen (*CYP2C9* and *CYP2C19*). The medications with the greatest number of patients (11 or more) impacted in either the primary or minor metabolism pathways include hydromorphone, methadone, acetaminophen, oxycodone, ondansetron, ketamine, diphenhydramine, and glycopyrrolate.

DISCUSSION

Genetic variants impacting drug efficacy contribute to the interpatient variability that challenges patient care, yet the extent of this impact has yet to be fully understood. In this study, we have demonstrated the extensive array of variants present in significant drug metabolizing enzymes in a small cohort of patients. This is one of the first studies to report the pharmacogene–drug associations in pediatric burn and surgery patients. We found that these patients had a high frequency of genotypes with potential functional impact on clinically administered medications received during their hospitalization. Special populations, such as burn and surgery patients, may be at higher risk of experiencing the consequences of abnormal pharmacogene variants due to the large number of medications they receive not only during their hospitalization, but also after discharge. Thus, to improve pharmacologic prescription efficacy, we will need to evaluate patient genetic variants, identify the variant effects on medications, and identify potential drug–drug interactions due to genetic variants.

Analgesic and anesthetic drugs are among the most widely used medications in burn and surgery patients, as balanced anesthesia and pain management utilize a combination of medications from several drug classes. Approximately half of the drugs identified in this study that utilize CYP metabolism are analgesic and/or anesthetic compounds, with the medications being impacted the most by variants with functional changes all being in this category. This exemplifies the

Table 3. Medications metabolized by CYPs and total anormal variants in primary and minor metabolism pathways

	Totals for CYP2C9, 2C19, 2D6, and 3A4								
Medication	**P**	**P-UV**	**M**	**M-UV**	**N**	**Drug Class**	**Primary Metabolism CYPs**	**Minor Metabolism CYPs**	**Reference(s)**
Acetaminophen	NA	NA	8	7	13	Analgesic	2E1	1A2, 3A4, 2D6, 2A6	Krasniak et al (2014),[27] DrugBank
Budesonide	0	0	NA	NA	0	Corticosteroid	3A4	3A5, 3A7	DrugBank
Buprenorphine	0	1			1	Partial opioid agonist	3A4		DrugBank
Celecoxib*	4	0	2	6	8	NSAID, COX-2 inhibitor	2C9	3A4, 2C8, 2D6	PharmGKB, DrugBank
Ciprofloxacin	NA	NA			NA	Second-generation fluoroquinolone	1A2		DrugBank
Clindamycin	0	0	NA	NA	0	Lincosamide	3A4	3A5	DrugBank
Clonidine	2	0			2	Alpha-2 adrenergic agonist	2D6, 1A2, 3A4, 3A5, 1A1		DrugBank
Dexamethasone	0	3	NA	NA	3	Corticosteroid–glucocorticoid	3A4	3A5	DrugBank
Dexmedetomidine	NA	NA			NA	Alpha 2 adrenergic agonist	2A6		Chidambaran et al[38]
Diazepam	5	2			5	Benzodiazepine	3A4, 2C19		PharmGKB, DrugBank
Diphenhydramine	4	3	14	0	12	Histamine H1-receptor antagonist	2D6	1A2, 2C9, 2C19	DrugBank
Duloxetine	1	0	1	0	1	Serotonin norepinephrine reuptake inhibitor	1A2, 2D6	2C9	DrugBank
Famotidine	4	0			4	H2 receptor antagonist	2C19		PharmGKB, DrugBank
Fentanyl	0	3			3	Opioid	3A4		PharmGKB, DrugBank
Glycopyrrolate	4	4	6	2	11	Muscarinic antagonist	2D6	1A2, 2B6, 2C9, 2C18, 2C19, 3A4	PharmGKB, DrugBank
Hydrocodone*	3	1			4	Opioid	2D6, CYP 3A4		PharmGKB, DrugBank
Hydrocortisone					0	Glucocorticoid	3A4, 3A5		DrugBank
Hydromorphone*			19	5	15	Opioid		2D6, 2C9, 3A4, 3A5	PharmGKB, DrugBank
Hydroxyzine			2	0	2	Histamine H1-receptor antagonist	3A4, 3A5		DrugBank
Ibuprofen*	8	0			6	NSAID, nonselective COX inhibitor	2C8, 2C9, 2C19		PharmGKB, DrugBank
Ketamine	0	1	12	0	12	NMDA antagonist	3A4	2B6, 2C9	Hijazi et al[26]
Ketorolac	3	0			3	NSAID, COX inhibitor	2C8, 2C9		DrugBank
Lidocaine	0	0			0	Local anesthetic	1A2, 3A4		P PharmGKB, DrugBank
Loratadine	2	1	3	0	3	Second-generation antihistamine	3A4, 2D6, 1A1, 2C19	1A2, 2B6, 2C8, 2C9, 3A5	DrugBank
Melatonin	NA	NA	6	0	5	Endogenous hormone	1A2	2C9, 2C19	Facciolá et al[29]
Methadone*	7	3	15	2	15	Opioid	3A4, 2B6, 2C19	2C9, 2C8, 2D6	DrugBank
Metoclopramide					0	Antiemetic, dopamine D2 antagonist	2D6, 3A4	1A2	DrugBank
Metoprolol	1	9			1	Beta-blocker	2D6	3A4	DrugBank
Midazolam	0	3			3	Benzodiazepine	3A4		PharmGKB, DrugBank
Minocycline			0	0	0	Tetracycline analog	3A4		Wehring et al[28]
Morphine*			1	0	1	Opioid		3A4	PharmGKB, DrugBank
Ondansetron*	0	3	8	4	12	Serotonin receptor antagonist	3A4	1A2, 2D6	PharmGKB, DrugBank
Oxycodone*	8	7	1	0	13	Opioid	3A4, 2D6		PharmGKB, DrugBank
Pantoprazole	2	0	0	0	2	Proton pump inhibitor	2C19	3A4	DrugBank
Propofol	9	0			9	GABA agonist	2B6, 2C9		PharmGKB, DrugBank
Risperidone	1	0			1	Second-generation antipsychotic	2D6		DrugBank
Ropivacaine	NA	NA	0	0	0	Local or regional anesthesia	1A2	3A4	Arlander et al (1998)[39]
Scopolamine	1	0	1	0	2	Belladonna alkaloid (anticholinergic)	3A4 suspected		DrugBank

COX, cyclooxygenase; *CYP*, cytochrome P450; *GABA*, gamma-aminobutyric acid; *M*, minor; *N*, total number of different patients with variants identified; *NA*, not analyzed; *NMDA*, *N*-methyl-d-aspartate receptor; *NSAID*, nonsteroidal anti-inflammatory drug; *P*, primary; *UV*, variant identified with unknown functional impact; *X*, variant identified in patient with predicted functional impact.
*Denotes the medications listed in CPIC guidelines related to CYPs.

need for more focused research in pharmacogenetics specifically concentrating on analgesics/anesthetics in special populations to determine the functional impact in a clinical setting. As the initiative to drive precision medicine continues, pharmacogenetic studies are being published at exponential rates. Balyan et al recently reported significantly greater oxycodone exposure in pediatric surgical patients with poor and intermediate *CYP2D6* phenotypes compared to normal and extensive metabolizers.[40] A study evaluating propofol in children found the drug distribution was higher in children with a *UGT1A9* C allele and carriers of *CYP2B6* T allele received significantly lower propofol doses, however, they could not conclude that *CYP2C9* nor *CYP2B6* polymorphisms could be used as independent predictors for propofol pharmacokinetics. This study concluded that further research needs to be conducted in these genes as well as other to explain the large interpatient variability observed with propofol.[41] Additionally, several other studies have recently been published in children related to analgesic pharmacogenetics, including acetaminophen,[27,38] morphine,[42] and fentanyl.[43] However, each of these studies only investigated one drug and focused on a small number of genes. Cohn et al conducted a broader study investigating pharmacogenetic implementation in pediatrics in a tertiary care setting and reported the drug–gene interactions associated with pain medications were identified in the opioids codeine, hydrocodone, oxycodone, and tramadol related to *CYP2D6* and the NSAIDs celecoxib, flurbiprofen, ibuprofen, meloxicam, and piroxicam related to *CYP2C9*.[44] These findings are consistent with many of the medications and variants identified in our patient population.

CYP2C9

Our study demonstrated that one-third of the patients had abnormal alleles (**2*, **3*, **11*) in *CYP2C9*, resulting in nine patients with intermediate and one patient with poor metabolizer phenotypes. The *CYP2C9* pathway is estimated to biotransform approximately 15% of CYP-related drug metabolism and is responsible for the primary metabolism of NSAIDs such as celecoxib, ibuprofen, ketorolac, as well as propofol, voriconazole, and warfarin.[45,46] There is a CPIC guideline specifically for NSAIDs recommending a decreased dose for poor metabolizers, due to the risk of increased adverse effects such as gastrointestinal bleeding, with reduced elimination.[15] There are currently 75 alleles reported in the PharmVar database. The most common variants investigated for *CYP2C9* include the **2* and **3* alleles, which are reported to have decreased and null function, respectively.[1,45] Their frequencies are believed to be low in African and Asian populations, however, the **2* allele has been reported to be in approximately 10% to 16% of Caucasian populations, while the **3* allele is reported to be in 3% to 8% of Caucasian, Hispanic, and East Asian populations.[1,47] The *CYP2C9*11* allele also has reduced function, has been reported in less than 1% of the population, has been identified in all ethnicities, and been associated with impaired warfarin metabolism.[45]

CYP2C19

A third of our patients also had abnormal phenotypes in *CYP2C19* as a result of the **2*, **35*, and **17* alleles. *CYP2C19* is responsible for the metabolism of multiple analgesics, proton pump inhibitors, and other drugs including diazepam, famotidine, ibuprofen, loratadine, methadone, pantoprazole, and voriconazole.[44,48,49] There are two CPIC guidelines related to gene–drug associations identified in our study for proton pump inhibitors[50] and voriconazole.[51] There are currently 36 alleles listed in the PharmVar database. The **2* and **35* alleles are both reported to be null in function, while the **17* allele has increased function. The **2* allele is the most common loss of function allele with frequencies approximately 15% in Caucasians and Africans, and 29% to 35% in Asians.[52] The **35* allele has been reported primarily in African populations at a frequency of 1% to 3%. Interesting the one individual heterozygous for this allele was the only African-American patient enrolled in our study. The **17* allele is the only variant reported in *CYP2C19* to have increased function, which is the result of a C > T transition in the promotor region resulting in increased expression and activity. The presence of **17* has been observed throughout many ethnic groups with frequencies ranging from 3% to 21%.[52] In the current study, the **17* allele was only identified in WGS datasets. While WES is one of the most common approaches used in genomic studies and is much more cost effective compared to WGS, thus allowing a greater number of patients to be included in studies, it does not capture important variants in nonexonic regions, which is the case for *CYP2C19*17*.[53] Thus, it is highly likely we missed identifying numerous *CYP2C19* rapid and ultrarapid metabolizers in our patients analyzed using WES.

CYP2D6

We have also identified several abnormal alleles in the *CYP2D6* gene in this study. It is well known that the *CYP2D6* gene is the most polymorphic pharmacogene. In the current study, the drugs most affected by variants in *CYP2D6* include clonidine, diphenhydramine, duloxetine, glycopyrrolate, hydrocodone, loratadine, metoprolol, oxycodone, and risperidone. Related to the drugs identified in this study, there are two CPIC guidelines discussing several opioids[54] and ondansetron,[14] however, guidance is limited due to a lack of definitive research. The PharmVar database currently lists 142 different alleles for *CYP2D6*. *CYP2D6* is responsible for metabolizing approximately 25% of clinically used drugs, which includes analgesics (eg, opioids), antidepressants, antihistamines, and beta blockers.[49] Translation of *CYP2D6* genotypes into phenotypic categories is complex and has been challenging due to the lack of standardization across clinical and research laboratories, resulting in discordant phenotype assignments. A recent publication from CPIC and the Dutch Pharmacogenetics Working group has addressed this issue recruiting several international experts to establish a standardized method for translating *CYP2D6* genotypes to metabolizer phenotypes.[37] These refined activity score categories have been used in our study, along with defining wild-type equivalent variants as normal metabolizers rather than the historical term of extensive metabolizers.

The *CYP2D6*2* allele has slightly reduced function and ranges from approximately 12% to 28% in most populations. The **41* allele also has slightly reduced function and is present in approximately 2% to 15% of the population. While **2* and **41* are reported to have slightly decreased function, the overall clinical impact does not appear significant and thus both are assigned as

normal function, along with **35*, which was also identified in our study. The **4* allele is nonfunctional and is the most common cause of poor metabolizers. It has been reported to be in approximately 19% of European populations, 12% of Latinos, less than 5% in African populations, and only about 1% of Asians. In the current study, the **4* allele was responsible for the majority of the intermediate metabolizer phenotypes. The **17* allele is reported to have reduced function and is typically observed in African populations with frequencies of 17% to 19%, while most other populations range from less than 1% to 3%. *CYP2D6*17* was identified in the only African-American patient in our study cohort. While the **59* allele is reported to have normal function, when in combination with the **4* allele the resulting phenotype is an intermediate metabolizer. Numerous other *CYP2D6* alleles were identified in our patient population that lack known functional impact. Reported population frequencies of some of the other alleles presented in our study can be found on the PharmGKB *CYP2D6* frequency table (https://api.pharmgkb.org/v1/download/file/attachment/CYP2D6_frequency_table.xlsx).

CYP3A4/CYP3A5

Our study identified the *CYP3A4 *3* allele in one patient and **22* allele in three patients. *CYP3A4* and *CYP3A5* are the predominate cytochrome P450s expressed in the liver. It has been reported that *CYP3A4* is predominant in Caucasians, while *CYP3A5* is predominant in Africans. *CYP3A4* is responsible for 50% to 60% of clinical drug metabolism,[55,56] spanning a large range of drug classes, including but not limited to the opioids fentanyl, hydrocodone, methadone, and oxycodone, anesthetics clonidine, diazepam, midazolam, and ketamine, and antimicrobials clindamycin, minocycline, and voriconazole. There are currently 35 different *CYP3A4* alleles listed in the PharmVar database, however, none to date have been assigned a clinical function. The **3* allele has been reported to have a frequency of approximately 1% in Caucasian populations.[56] *CYP3A4*22* has been reported in the literature to have reduced function and has been reported to have a 3% to 5% allele frequency predominately in Europeans and admixed Americans, and has affected the metabolism of midazolam, tacrolimus, cyclosporin, and statins.[55,57] All three patients in our study with the **3* and **22* alleles are Caucasian. Our study identified the *CYP3A5 *1*, **3*, and **7* alleles. The **1* allele is reported to have normal function and both **3* and **7* are null in function. *CYP3A5* has nine alleles reported in the PharmVar database with predicted functions for many. The PharmGKB *CYP3A5* allele frequency table reports the **1* allele to be in approximately 45% of Africans, 25% to 33% of Asians, 17% of Latinos, and only 7% in European populations. The **3* allele is reported in approximately 92% of Europeans, 77% Latinos, 67% to 75% Asians, and 32% of Africans. Interestingly, the **7* has reported frequencies of 12% in Africans and 2% in Latinos but is not reported in Europeans and Asians. The majority of the **1* alleles identified in our study were in Latino patients with the one Asian patient being heterozygous for **1/*3*. Our patient with the **3/*7* genotype was the only African-American in our study.

The present study is limited to a small number of patients included in this analysis along with decreased ethnic diversity. The majority of our patients are Hispanic-Latino and Caucasian, thus the genotype frequencies presented here are not a good representation of the general population. Larger cohorts with greater ethnic diversity, particularly African and Asian, are needed to truly capture the presence of rarer polymorphism. These rarer polymorphisms are also more likely to be classified as "indeterminate" function as we have presented several examples of for *CYP2D6*, *CYP3A4*, and *CYP3A5*. While there is limited research that is suggestive that some of these alleles have decreased function, eg, *CYP3A4*22* or any of the *CYP2D6* diplotypes that include **4*, until there is a greater amount of evidence to support the clinical function, databases will be reluctant to change their classifications. There is a tremendous need to conduct clinical research in racially diverse populations, particularly in minorities, that not only characterizes the frequency of polymorphisms but also collects data to infer the functional impact of these variants. Future research also needs to focus on the impact of illness, comorbidities, and medical procedures on phenoconversion. Investigating the function of CYPs in patients is challenging without invasive techniques such as tissue biopsies, however, newer approaches such as exploring the utility of circulating biomarkers, eg, specific drugs that serve as biomarkers for a singular pathway or other approaches such as exosomes, may hold promise to identify clinical diagnostic approaches to guide precision dosing adjustments in individual patients.

Moreover, as stated above, another limitation is the WES datasets may have not captured the *CYP2C19*17* allele, which is in a promoter region, in addition to other variants that are in nonexonic regions as well as duplicate genes. This limitation should be taken into great consideration for future pharmacogenetic study design. It is understandable that WES may be attractive to use due to its decreased cost, thus allowing more patients in a study, however the fact we are missing vital information for these patients is a disservice to the advancement of pharmacogenetic research and will hinder the progress of improving outcomes in patient management.

CONCLUSION

The high prevalence of functionally abnormal variants in combination with the high number of impacted medications administered to patients in this study demonstrated significant pharmacogene–drug associations in pediatric burn and surgery patients. This study provides robust clinical data that can be used for targeted future clinical pharmacogenomic studies, particularly in special populations.

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REFERENCES

1. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 2013;138:103–41.
2. Zhou S-F, Liu J-P, Chowbay B. Polymorphism of human cytochrome P450 enzymes and its clinical impact. Drug Metab Rev 2009;41:89–295.

3. Kapur BM, Lala PK, Shaw JLV. Pharmacogenetics of chronic pain management. Clin Biochem 2014;47:1169–87.
4. Feierman DE, Lasker JM. Metabolism of fentanyl, a synthetic opioid analgesic, by human liver microsomes. Role of CYP3A4. Drug Metab Dispos 1996;24:932–9; available from http://www.ncbi.nlm.nih.gov/pubmed/8886601; accessed 6 Nov. 2018.
5. Wu S-B, Cai L-N, Yang X-H et al. Impact of CYP2D6 polymorphisms on postoperative fentanyl analgesia in gastric cancer patients. Genet Test Mol Biomarkers 2015;19:248–52.
6. Topletz AR, Dennison JB, Barbuch RJ, Hadden CE, Hall SD, Renbarger JL. The relative contributions of CYP3A4 and CYP3A5 to the metabolism of vinorelbine. Drug Metab Dispos 2013;41:1651–61.
7. Dahabreh I, Terasawa T, Castaldi P, Trikalinos TA. CYP2D6 testing to predict response to tamoxifen in women with breast cancer: pharmacogenomic. PLoS Curr 2010;2:RRN1176.
8. Bernard S, Neville KA, Nguyen AT, Flockhart DA. Interethnic differences in genetic polymorphisms of CYP2D6 in the U.S. population: clinical implications. Oncologist 2006;11:126–35.
9. Steele AN, Grimsrud KN, Sen S, Palmieri TL, Greenhalgh DG, Tran NK. Gap analysis of pharmacokinetics and pharmacodynamics in burn patients. J Burn Care Res 2015;36:e194–211.
10. Singh MS, Francis PA, Michael M. Tamoxifen, cytochrome P450 genes and breast cancer clinical outcomes. Breast 2011;20:111–8.
11. Fernando SL, Broadfoot AJ. Prevention of severe cutaneous adverse drug reactions: the emerging value of pharmacogenetic screening. CMAJ 2010;182:476–80.
12. Agarwal D, Udoji MA, Trescot A. Genetic testing for opioid pain management: a primer. Pain Ther 2017;6:93–105.
13. Chidambaran V, Ngamprasertwong P, Vinks AA, Sadhasivam S. Pharmacogenetics and anesthetic drugs. Curr Clin Pharmacol 2012;7:78–101.
14. Crews KR, Monte AA, Huddart R et al. Clinical Pharmacogenetics Implementation Consortium guideline for CYP2D6, OPRM1, and COMT genotypes and select opioid therapy. Clin Pharmacol Ther 2021;110:888–96.
15. Theken KN, Lee CR, Gong L et al. Clinical Pharmacogenetics Implementation Consortium guideline (CPIC) for CYP2C9 and nonsteroidal anti-inflammatory drugs. Clin Pharmacol Ther 2020;108:191–200.
16. Godwin Z, Lima K, Greenhalgh D, Palmieri T, Sen S, Tran NK. A retrospective analysis of clinical laboratory interferences caused by frequently administered medications in burn patients. J Burn Care Res 2016;37:e10–7.
17. Shah RR, Smith RL. Addressing phenoconversion: the Achilles' heel of personalized medicine. Br J Clin Pharmacol 2015;79:222–40.
18. Carcillo JA, Doughty L, Kofos D et al. Cytochrome P450 mediated-drug metabolism is reduced in children with sepsis-induced multiple organ failure. Intensive Care Med 2003;29:980–4.
19. Lenoir C, Daali Y, Rollason V et al. Impact of acute inflammation on cytochromes P450 activity assessed by the Geneva cocktail. Clin Pharmacol Ther 2021;109:1668–76.
20. Lenoir C, Rollason V, Desmeules JA, Samer CF. Influence of inflammation on cytochromes P450 activity in adults: a systematic review of the literature. Front Pharmacol 2021;12:1–44.
21. Hakkola J, Hukkanen J, Turpeinen M, Pelkonen O. Inhibition and induction of CYP enzymes in humans: an update. Arch Toxicol 2020;94:3671–722 doi:10.1007/s00204-020-02936-7.
22. Grimsrud KN, Ivanova X, Sherwin CM, Palmieri TL, Tran NK. Identification of cytochrome P450 polymorphisms in burn patients and impact on fentanyl pharmacokinetics: a pilot study. J Burn Care Res 2019;40:91–6.
23. Weinbren MJ. Pharmacokinetics of antibiotics in burn patients. J Antimicrob Chemother 1999;44:319–27.
24. Williams FN, Herndon DN, Jeschke MG. The hypermetabolic response to burn injury and interventions to modify this response. Clin Plast Surg 2009;36:583–96.
25. Gaedigk A, Ingelman-Sundberg M, Miller NA, Leeder JS, Whirl-Carrillo M, Klein TE. The Pharmacogene Variation (PharmVar) Consortium: incorporation of the human cytochrome P450 (CYP) allele nomenclature database. Clin Pharmacol Ther 2018;103:399–401.
26. Hijazi Y, Boulieu R. Protein binding of ketamine and its active metabolites to human serum. Eur J Clin Pharmacol 2002;58:37–40.
27. Krasniak AE, Knipp GT, Svensson CK, Liu W. Pharmacogenomics of acetaminophen in pediatric populations: a moving target. Front Genet 2014;5:1–8.
28. Wehring HJ, Elsobky T, McEvoy JP et al. Adjunctive minocycline in clozapine-treated patients with schizophrenia: analyzing the effects of minocycline on clozapine plasma levels. Physiol Behav 2018;89:73–80.
29. Facciolá G, Hidestrand M, Von Bahr C, Tybring G. Cytochrome P450 isoforms involved in melatonin metabolism in human liver microsomes. Eur J Clin Pharmacol 2001;56:881–8.
30. Relling MV, Klein TE. CPIC: Clinical Pharmacogenetics Implementation Consortium of the pharmacogenomics research network. Clin Pharmacol Ther 2011;89:464–7.
31. DePristo MA, Banks E, Poplin R et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 2011;43:491–8.
32. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 2009;25:1754–60.
33. Cibulskis K, Lawrence MS, Carter SL et al. Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. Nat Biotechnol 2013;31:213–9.
34. Lee S-B, Wheeler MM, Thummel KE, Nickerson DA. Calling star alleles with stargazer in 28 pharmacogenes with whole genome sequences. Clin Pharmacol Ther 2019;106:1328–37.
35. Numanagić I, Malikić S, Ford M et al. Allelic decomposition and exact genotyping of highly polymorphic and structurally variant genes. Nat Commun 2018;9:828. doi:10.1038/s41467-018-03273-1
36. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. Elsevier Enhanced Reader, accessed 26 Jul. 2020; available from https://reader.elsevier.com/reader/sd/pii/S0163725813000065?token=C3AB70EB928AC6A6E68295B3016647E1D17E4684F8C2AD8E7B49E3434A6072EE38745F3BF5AE242D904E60707475C7A0
37. Caudle KE, Sangkuhl K, Whirl-Carrillo M et al. Standardizing CYP2D6 genotype to phenotype translation: consensus recommendations from the Clinical Pharmacogenetics Implementation Consortium and Dutch Pharmacogenetics Working Group. Clin Transl Sci 2020;13:116–24.
38. Chidambaran V, Sadhasivam S. Pharmacogenomics. In: Coté CJ, Lerman J, Anderson BJ, editors. A practice of anesthesia for infants and children. 6th ed. Philadelphia, PA: Elsevier; 2019. p. 81–99.e89.
39. Arlander E, Ekström G, Alm C et al. Metabolism of ropivacaine in humans is mediated by CYP1A2 and to a minor extent by CYP3A4: an interaction study with fluvoxamine and ketoconazole as in vivo inhibitors. Clin Pharmacol Ther 1998;64:484–91. doi:10.1016/S0009-9236(98)90131-X
40. Balyan R, Mecoli M, Venkatasubramanian R et al. CYP2D6 pharmacogenetic and oxycodone pharmacokinetic association study in pediatric surgical patients. Pharmacogenomics 2017;18:337–48.
41. Pavlovic D, Budic I, Stoimenov TJ et al. The effect of UGT1A9, CYP2B6 and CYP2C9 genes polymorphism on propofol pharmacokinetics in children. Pharmgenomics Pers Med 2020;13:13–27.
42. Zuppa AF, Benitez GR, Zane NR et al. Morphine dose optimization in critically ill pediatric patients with acute respiratory failure: a population pharmacokinetic-pharmacogenomic study. Crit Care Med 2019;47:e485–94.
43. Horvat CM, Au AK, Conley YP et al. ABCB1 genotype is associated with fentanyl requirements in critically ill children. Pediatr Res 2017;82:29–35.
44. Cohn I, Manshaei R, Liston E et al. Assessment of the implementation of pharmacogenomic testing in a pediatric tertiary care setting. JAMA Netw Open 2021;4:e2110446.
45. Pratt VM, Cavallari LH, Del Tredici AL et al. Recommendations for clinical CYP2C9 genotyping allele selection: a joint recommendation of the Association for Molecular Pathology and College of American Pathologists. J Mol Diagn 2019;21:746–55.
46. Cohn I, Paton TA, Marshall CR et al. Genome sequencing as a platform for pharmacogenetic genotyping: a pediatric cohort study. npj Genomic Med 2017;2:1–7.
47. Jaja C, Patel N, Scott SA, Gibson R, Kutlar A. CYP2C9 allelic variants and frequencies in a pediatric sickle cell disease cohort: implications for NSAIDs pharmacotherapy. Clin Transl Sci 2014;7:396–401.
48. Zhou S, Skaar DJ, Jacobson PA, Huang RS. Pharmacogenomics of medications commonly used in the intensive care unit. Front Pharmacol 2018;9:1–15.
49. MacKenzie M, Hall R. Utilisation de la pharmacogénomique et de la pharmacogénétique à l'unité de soins intensifs: un compte rendu narratif. Can J Anesth 2017;64:45–64.
50. Lima JJ, Thomas CD, Barbarino J et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2C19 and proton pump inhibitor dosing. Clin Pharmacol Ther 2021;109:1417–23.
51. Moriyama B, Obeng AO, Barbarino J et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for CYP2C19 and voriconazole therapy. Clin Pharmacol Ther 2017;102:45–51.
52. Scott SA, Sangkuhl K, Gardner EE et al. Clinical Pharmacogenetics Implementation Consortium guidelines for cytochrome P450-2C19 (CYP2C19) genotype and clopidogrel therapy. Clin Pharmacol Ther 2011;90:328–32.
53. Caspar SM, Schneider T, Stoll P, Meienberg J, Matyas G. Potential of whole-genome sequencing-based pharmacogenetic profiling. Pharmacogenomics 2021;22:177–90.
54. Bell GC, Caudle KE, Whirl-Carrillo M et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for CYP2D6 genotype and use of ondansetron and tropisetron. Clin Pharmacol Ther 2017;102:213–8 doi:10.1002/cpt.598

55. Mulder TAM, van Eerden RAG, de With M et al. CYP3A4*22 genotyping in clinical practice: ready for implementation? Front Genet 2021;12:711943. doi:10.3389/fgene.2021.711943
56. Van Schaik RHN, De Wildt SN, Brosens R, Van Fessem M, Van den Anker JN, Lindemans J. The CYP3A4*3 allele: is it really rare? Clin Chem 2001;47:1104–6.
57. De Jonge H, Elens L, De Loor H, Van Schaik RH, Kuypers D. The CYP3A4*22 C>T single nucleotide polymorphism is associated with reduced midazolam and tacrolimus clearance in stable renal allograft recipients. Pharmacogenomics J 2015;15:144–52. doi:10.1038/tpj.2014.49