PRECISION MEDICINE: IMPACT OF CYTOCHROMES P450 AND TRANSPORTERS GENETIC POLYMORPHISMS, DRUG-DRUG INTERACTIONS, DISEASE ON SAFETY AND EFFICACY OF DRUGS

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Topic Editors:
Caroline Flora Samer, Geneva University Hospitals (HUG), Switzerland
Youssef Daali, Geneva University Hospitals (HUG), Switzerland
Amin Rostami-Hodjegan, The University of Manchester, United Kingdom

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EDITORIAL
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Editorial: Precision Medicine: Impact of Cytochromes P450 and Transporters Genetic Polymorphisms, Drug-Drug Interactions, Disease on Safety and Efficacy of Drugs

Youssef Daali[1,2,3]*, *Amin Rostami-Hodjegan*[4,5] *and Caroline F. Samer*[1,3]

[1]Division of Clinical Pharmacology and Toxicology, Department of Anesthesiology, Pharmacology, Intensive Care and Emergency Medicine, Geneva University Hospitals, Geneva, Switzerland, [2]Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of Geneva, Geneva, Switzerland, [3]Laboratory of Clinical Pharmacology, Faculty of Medicine, University of Geneva, Geneva, Switzerland, [4]Centre for Applied Pharmacokinetic Research, School of Health Sciences, University of Manchester, Manchester, United Kingdom, [5]Certara, Princeton, NJ, United States

Keywords: pharmacokinetics, personalized & precision medicine (PPM), pharmacogenenomics and personalised medicine, drug-drug interactions, PBPK

Editorial on the Research Topic

Precision Medicine: Impact of Cytochromes P450 and Transporters Genetic Polymorphisms, Drug-Drug Interactions, Disease on Safety and Efficacy of Drugs

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José A. G. Agúndez,
University of Extremadura, Spain

***Correspondence:**
Youssef Daali
youssef.daali@hcuge.ch

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The idealistic concept "one-size fits all" in drug treatment has faced the challenge of variable patient response over several decades. The concept has gradually been replaced by precision dosing, under the umbrella of precision medicine, where patients receive individually tailored therapy to minimize the risk of potential adverse drug effects or inefficacy (Darwich et al., 2021). A large interindividual variability in response to treatment is frequently observed due to the variety of genetic and environmental factors. The covariates determining the variable pharmacodynamics are not adequately studied. However, the individual attributes defining pharmacokinetic variability are well established. These play an important role in precision dosing and involve factors such as genetic polymorphisms of phase I and phase II drug metabolizing enzymes, drug-drug interactions (DDI), the modulating effect of diseases itself on function, and activity of enzymes and transporters.

Identification of the main factors influencing the activity of enzymes and transporters at the individual level is essential for tailored therapy. However, the complex interaction between multiple factors leading to complex drug-drug-gene-disease interactions is difficult to predict, with occasionally fatal consequences (Storelli et al., 2018). Therefore, there is an urgent need to increase applications of the knowledge gathered so far regarding these sources of variability into clinical practice. The use of model informed precision dosing (MIPD) alongside better patient characterization are powerful tools that help clinicians in individualized patient care. These computer-based modeling and simulation techniques can integrate information on individual capacity for enzymes and transporters alongside many other factors to predict a drug dose for a given patient and manage complex drug-drug-gene-disease scenarios (Polasek et al., 2019). Hence, the current issue of the journal is devoted to the Research Topic of precision dosing and the impact of cytochromes P450 (CYPs), transporters genetic polymorphisms, drug-drug interactions, and disease, on the safety and efficacy of drugs.

In oncology, the development of tyrosine kinase inhibitors (TKI) has revolutionized anticancer targeted therapy by increasing patient survival rates, particularly in hematologic neoplasms. However, treatment failure is observed in 20–25% of chronic myeloid leukemia (CML) patients

due to mutations in the BCR-ABL1 gene or other factors not related to the fusion gene such as CYP3A4/5 and transporters (OCT1, ABCB1, ABCG2) as reported by Kaehler and Cascorbi. The authors reviewed all pharmacogenetic aspects related to impaired TKI response in CM, discuss BCR-ABL1-dependent mechanisms as well as mutations in PK pathways (CYPs and transporters), and the role of alternative signaling pathways. The majority of these targeted drugs are metabolized by CYP3A4/5 leading to DDI with inhibitors or inducers. Molenaar-Kuijsten et al. reviewed all potential DDIs with oral targeted anticancer drugs and provided recommendations for clinical practice on how to deal with DDIs. The authors pointed out the large interindividual variability in the PK of the studied drugs with a range of 23–78%, which was reflected in the variability of the effects of CYP3A4/5 inhibitors and inducers. This variability in exposure could partly be explained by the highly variable CYP3A4/5 activity, which is 60–90% genetically determined (Ozdemir et al., 2000). Moreover, the importance of the metabolic pathway and the presence of active metabolites should be considered in the interpretation and recommendation in the clinical setting. Therefore, more complex drug-drug-genetic (DDGI) interactions should be considered.

Yang et al. also discuss the DDGI between tacrolimus and nifedipine, CYP3A5 competitive inhibitor, and the influence of CYP3A5 genotype. The co-administrated nifedipine in renal transplant patients carrying CYP3A5*3/*3 allele significantly increased tacrolimus concentrations. The authors conclude that tacrolimus personalized therapy, accounting for CYP3A5 genotype detection as well as therapeutic drug monitoring, is necessary when there is a risk of DDI. In line with these findings, Srinivas et al. evaluated the effect of CYP3A5, CYP3A4, and ABCB1 gene polymorphisms on tacrolimus trough concentrations in South Indian renal transplant patients and developed a genotype-based dosing equation to calculate the required starting daily dose of tacrolimus. The authors also investigated the effect of these genes on toxicity and organ rejections after tacrolimus administration. Transporters are also an important determinant in the variability of drugs response. They are expressed in many vital organs such as the liver and kidneys and barriers like brain-blood-barrier and intestines. Like metabolic enzymes, transporters' activity (influx or efflux) is affected by genetic polymorphisms and DDI. El Biali et al. nicely demonstrated, using PET imaging, the influence of ABCB1 and ABCG2 and the ABCG2 c.421C > A genotype on the distribution of substrates of these transporters to the human retina. Subjects undergoing treatment with potentially retinotoxic drugs need to be carefully genotyped for this SNP and any DDI checked to avoid the potential accumulation of toxic drugs because of these complex gene-drug-drug interactions.

Villapalos-García et al. studied the effects of CYPs and transporters polymorphisms on the variability of dutasteride and tamsulosin, a combination therapy for the management of prostatic hyperplasia. Tamsulosin pharmacokinetics were very sensitive to the activity of CYP2D6 and significant associations were observed between dutasteride exposure and CYP3A4 and CYP3A5 genotypes and between tamsulosin and ABCG2, CYP3A5, and SLC22A1 genotypes. Lenoir et al. conducted a systematic review to assess another source of variation in CYP activity, inflammation. In total, 218 studies and case reports were retrieved and classified into 14 different sources of inflammation. They found that the impact of inflammation on CYP activities was isoform-specific and dependent on the nature and severity of the underlying disease causing the inflammation.

There are two approaches available for CYPs activity assessment. One involves the projection of activity based on established links either to genotyping (very common) or liquid biopsy (only started recently) (Achour et al., 2021). The other approach is a direct assessment of activity (phenotyping) using validated probes singularly or as part of a cocktail. CYPs phenotyping gives information on enzymes activity not only related to genetic variation and abundance but also on environmental and endogenous variables affecting the activity of a given level of expression for certain genotypes (Samer et al., 2013).

Ing Lorenzini et al. evaluated the concordance in CYPs activity between genotype and phenotype in the clinical setting using a Geneva micrococktail. A total of 241 patients underwent simultaneous genotyping and phenotyping and except for poor metabolizers where a perfect correlation between phenotype and genotype was observed, discrepancies were observed for the other phenotypic groups (intermediate, normal ad ultra-rapid) and not always explained by DDI. Other personal factors such as disease, inflammation, or environmental factors like food or exposition to toxins could affect CYPs activity. Interestingly, genotype and/or phenotype results explained the clinical event in 44% of cases, demonstrating the clinical utility of these tests.

MIPD using PBPK or POPPK is increasingly used in a clinical setting to help in the management of complex drug-drug-gene-disease interactions. Abouir et al. reviewed all PBPK models available for predicting DDIs and integrating intrinsic and extrinsic factors such as genetic polymorphisms and pathologies.

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BRIEF RESEARCH REPORT
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ABCB1 and ABCG2 Together Limit the Distribution of ABCB1/ABCG2 Substrates to the Human Retina and the *ABCG2* Single Nucleotide Polymorphism Q141K (c.421C> A) May Lead to Increased Drug Exposure

*Myriam El Biali[1], Rudolf Karch[2], Cécile Philippe[3], Helmuth Haslacher[4], Nicolas Tournier[5], Marcus Hacker[3], Markus Zeitlinger[1], Doreen Schmidl[1], Oliver Langer[1,3] and Martin Bauer[1]**

[1]Department of Clinical Pharmacology, Medical University of Vienna, Vienna, VIE, Austria, [2]Centre for Medical Statistics, Informatics, and Intelligent Systems, Medical University of Vienna, Vienna, VIE, Austria, [3]Division of Nuclear Medicine, Department of Biomedical Imaging and Image-guided Therapy, Medical University of Vienna, Vienna, VIE, Austria, [4]Department of Laboratory Medicine, Medical University of Vienna, Vienna, VIE, Austria, [5]Laboratoire d'Imagerie Biomédicale Multimodale (BioMaps), CEA, CNRS, Inserm, Service Hospitalier Frédéric Joliot, Université Paris-Saclay, Orsay, France

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Edited by:
Youssef Daali, Geneva University Hospitals (HUG), Switzerland

Reviewed by:
Vera Ribeiro, University of Algarve, Portugal
Amit Kumar Mitra, Auburn University, United States

***Correspondence:**
Martin Bauer
martin.m.bauer@meduniwien.ac.at

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The widely expressed and poly-specific ABC transporters breast cancer resistance protein (ABCG2) and P-glycoprotein (ABCB1) are co-localized at the blood-brain barrier (BBB) and have shown to limit the brain distribution of several clinically used ABCB1/ABCG2 substrate drugs. It is currently not known to which extent these transporters, which are also expressed at the blood-retinal barrier (BRB), may limit drug distribution to the human eye and whether the *ABCG2* reduced-function single-nucleotide polymorphism (SNP) Q141K (c.421C > A) has an impact on retinal drug distribution. Ten healthy male volunteers (five subjects with the c.421CC and c.421CA genotype, respectively) underwent two consecutive positron emission tomography (PET) scans after intravenous injection of the model ABCB1/ABCG2 substrate [^{11}C]tariquidar. The second PET scan was performed with concurrent intravenous infusion of unlabelled tariquidar to inhibit ABCB1 in order to specifically reveal ABCG2 function.In response to ABCB1 inhibition with unlabelled tariquidar, *ABCG2* c.421C > A genotype carriers showed significant increases (as compared to the baseline scan) in retinal radiotracer influx K_1 (+62 ± 57%, p = 0.043) and volume of distribution V_T (+86 ± 131%, p = 0.043), but no significant changes were observed in subjects with the c.421C > C genotype. Our results provide the first evidence that ABCB1 and ABCG2 may together limit the distribution of systemically administered ABCB1/ABCG2 substrate drugs to the human retina. Functional redundancy between ABCB1 and ABCG2 appears to be compromised in carriers of the c.421C > A SNP who may therefore be more susceptible to transporter-mediated drug-drug interactions at the BRB than non-carriers.

Keywords: ABCG2, ABCB1, blood-retinal barrier, c421C>A, single-nucleotide polymorphism, PET, human, tariquidar

INTRODUCTION

The transport of specific molecules across lipid membranes is an essential function of all living organisms (Liu, 2019). In humans, the widespread expression and poly-specificity of the adenosine triphosphate-binding cassette (ABC) family efflux transporter breast cancer resistance protein (ABCG2) makes it an important determinant of the pharmacokinetics of a variety of drugs (Mao and Unadkat, 2015). Many ABCG2 substrates are additionally substrates of another ABC-transporter, P-glycoprotein (ABCB1), so that the net effect on the disposition of drugs which are dual ABCB1/ABCG2 substrates may be attributed to the combined action of both transporters. These two transporters have been recognized by the International Transporter Consortium to be involved in clinically relevant transporter-mediated drug-drug interactions (DDIs) given their impact on the disposition of their substrates (International Transporter et al., 2010). The co-localization of ABCG2 and ABCB1 at several blood-tissue barriers suggests a crucial role in protecting key vulnerable and/or target tissues, e.g., the brain or the placenta, from xenobiotics and harmful metabolites. It is, however, difficult to predict the functional impact of ABCB1- and ABCG2-mediated efflux on tissue exposure from conventional plasma pharmacokinetic data (Wijaya et al., 2017).

ABCB1 and ABCG2 are co-expressed at the luminal membrane of brain capillary endothelial cells contributing to the protective function of the blood-brain barrier (BBB) (Uchida et al., 2011; Kalvass et al., 2013; Billington et al., 2019; Li and Zhu, 2020). Studies in *Abcb1a/b* and *Abcg2* knockout mice have provided evidence for functional redundancy between ABCB1 and ABCG2 in limiting the distribution of dual ABCB1/ABCG2 substrate drugs to the brain. In absence of either ABCB1 alone or ABCG2 alone (*Abcb1a/b*$^{(-/-)}$ mice or *Abcg2*$^{(-/-)}$ mice) the remaining transport capacity of the other transporter was largely sufficient to restrict brain distribution of ABCB1/ABCG2 substrates, for which brain distribution was only substantially increased in absence of both transporters (*Abcb1a/b*$^{(-/-)}$*Abcg2*$^{(-/-)}$ mice) (Kodaira et al., 2010; Wijaya et al., 2017; Robey et al., 2018). A comparable functional redundancy between ABCB1 and ABCG2 has been confirmed *in vivo* at the human BBB (Bauer et al., 2016). Transporter-mediated DDIs at the BBB may potentially result in cerebral uptake and toxicity of medications that normally are not targeted to the brain without significant changes in drug plasma concentrations (Sasongko et al., 2005; Eyal et al., 2009; Bauer et al., 2017), although the risk for their occurrence in clinical practice is considered relatively low (Kalvass et al., 2013).

The eye, just like the brain, is a vulnerable organ as it requires the strict maintenance of a stable inner environment to insure neuro-retinal homeostasis and its sensory function (Fujii et al., 2014). The protection of the posterior segment of the eye, especially the retina, from systemically circulating phototoxic endogenous and exogenous substances and the regulation of the influx transport of vital molecules are essentially provided by the blood-retinal barrier (BRB) (Asashima et al., 2006; Agrahari et al., 2016), in a similar way as the BBB does for the brain (Fujii et al., 2014). The BRB is divided in two layers: (a) the inner BRB consisting of endothelial cells of the retinal capillaries (ECRC) and (b) the outer BRB composed of the retinal pigmental epithelial (RPE) cells, located between the neural retina and choriocapillaris.

There is evidence from preclinical studies that both, ABCB1 and ABCG2, are expressed at the BRB. ABCG2 was identified in mouse and rat retina and in the conditionally immortalized rat ECRC cell line TR-iBRB (Asashima et al., 2006) as well as concomitantly with ABCB1 at the luminal side of ECRC in rabbit and mouse eyes (Chapy et al., 2016; Pascual-Pasto et al., 2017). The data concerning the expression of ABCG2 and ABCB1 at the outer BRB are contradictory (Liu and Liu, 2019). A dominant protein expression of ABCG2 in pig eyes at the inner BRB over the outer BRB (22.8 fmol/μg protein and 2.76 fmol/μg protein respectively) was demonstrated (Zhang et al., 2017). The same study revealed that ABCG2 expression at the inner porcine BRB is 2.6-fold higher than that of ABCB1 and that the transporter expression pattern is positively correlated between the BBB and the inner BRB in pigs (Zhang et al., 2017). These findings are consistent with absolute quantification data at the human BBB, which showed a higher expression of ABCG2 than of ABCB1 (Uchida et al., 2011; Billington et al., 2019; Li and Zhu, 2020).

For the human BRB, ABCG2 and ABCB1 protein expression has been corroborated with a predominance of ABCG2 through RNA expression profiling and immunohistochemistry (Dahlin et al., 2013). All in all, it is currently not known to which extent these two efflux transporters limit the distribution of systemically administered drugs to the human eye.

The nonsynonymous *ABCG2* single-nucleotide polymorphism (SNP) Q141K (c.421C > A), which affects the stability of the ABCG2 protein in the endoplasmic reticulum and enhances its susceptibility to proteosomal degradation (Furukawa et al., 2009), has been shown to lead to reduced transporter expression in different tissues (Kobayashi et al., 2005; Prasad et al., 2013; Tanaka et al., 2015). It has been reported that c.421AA carriers have an *in vivo* intestinal ABCG2 function approximately 23% of that in c.421CC subjects (Tanaka et al., 2015). The efficacy and the toxicity of diverse ABCG2 substrates, e.g., statin drugs, chemotherapy or allopurinol, has been found to be affected by the c.421C > A variant (Chen et al., 2019). Our previous data indicated that carriers of the c.421C > A SNP had diminished activity of ABCG2 at BBB, leading to increased susceptibility to ABCB1 inhibition (Bauer et al., 2016). It remains to be explored whether the c.421C > A SNP has an impact on ABCG2 function at the human BRB.

Positron emission tomography (PET) with radiolabelled transporter substrates allows to directly and non-invasively assess the influence of transporters at the BBB on drug distribution to the human brain (Bickel, 2005; Tournier et al., 2018). Next to measuring ABCB1 function at the BBB (Bauer et al., 2012; Bauer et al., 2015; Bauer et al., 2017), PET with the radiolabelled ABCB1 substrate (*R*)-[^{11}C]verapamil has also been used to measure ABCB1 function at the human BRB (Bauer et al., 2017). PET with the dual ABCB1/ABCG2 substrate [^{11}C] tariquidar (Bankstahl et al., 2013) with concurrent infusion of

a high dose of unlabelled tariquidar to inhibit ABCB1 was successfully employed to reveal and measure for the first time the transport activity of ABCG2 at the human BBB (Bauer et al., 2016). In the present study, we aimed to extend the analysis of data from our previously published study in healthy volunteers (Bauer et al., 2016) to assess the impact of ABCB1 and ABCG2 and the *ABCG2* c.421C > A genotype on the distribution of [^{11}C] tariquidar to the human retina.

METHODS

The study was registered with EUDRACT (number 2012-005796-14), approved by the Ethics Committee of the Medical University of Vienna, and conducted in accordance with the Declaration of Helsinki. The reported data are from an extended analysis of the previously published study of Bauer et al. (Bauer et al., 2016). Eleven out of 52 screened subjects were identified as carriers of the *ABCG2* c.421C > A SNP by means of probe-based polymerase chain reaction as previously described (Bauer et al., 2016). The sample management and the SNPgenotyping was performed at the MedUni Wien Biobank according to standard operating procedures (Haslacher et al., 2018). In total, five male subjects who were non-carriers (c.421CC) and five male subjects who were heterozygous carriers (c.421CA) of the *ABCG2* c.421C > A SNP and who were judged to be medication free and healthy based on the screening examinations, were enrolled into the [^{11}C] tariquidar study arm. A summary table of the human subjects included is available in the **Supplementary Table S1**. The volunteers (mean age: 30 ± 9 years) underwent two consecutive 60 min PET scans on an Advance scanner (General Electric Medical Systems, Milwaukee, WI, United States) after intravenous injection of [^{11}C]tariquidar (injected radioactivity amount: 388 ± 18 MBq). Serial arterial blood samples were drawn as previously described during the imaging sessions (Bauer et al., 2013). The second PET scan was performed with concurrent intravenous infusion of unlabelled tariquidar (AzaTrius Pharmaceuticals, Mumbai, India) to inhibit ABCB1 only and reveal ABCG2 function as previously described (Bauer et al., 2016). Tariquidar infusion was started 1 h before and continued until the end of the image acquisition (total infusion length: 120 ± 4 min). The total administered dose of unlabelled tariquidar was 5.8 ± 1.0 mg/kg body weight (mean subject weight: 80 ± 12 kg).

Region of interest (ROI) analysis was conducted for the retina on magnetic resonance (MR)-to-PET co-registered images based on individual T1-weighted MR images with PMOD software (version 3.6; PMOD Technologies Ltd., Zürich, Switzerland). Probabilistic atlas-based, whole brain grey matter (WBGM) data were already reported in (Bauer et al., 2016). A standard 2-tissue-4-rate constant compartmental (2T4K) model (see **Supplementary Figure S1**) was fitted to the time–activity curves (TACs) of [^{11}C]tariquidar in the retina and in WBGM from 0 to 60 min after radiotracer injection (Bauer et al., 2013) using an arterial plasma input function which was not corrected for radiolabelled metabolites of [^{11}C]tariquidar (due to the low percentage of radiolabelled metabolites in plasma). Modelling outcome parameters were the radiotracer transfer rate constants across the BRB and BBB between plasma and the first tissue compartment (influx rate constant K_1 and efflux rate constant k_2) as well as between the first and second tissue compartments (influx rate constant k_3 and efflux rate constant k_4) (see **Supplementary Figure S1**). The fractional arterial blood volume in tissue (V_b) was included as a fitting parameter. Logan graphical analysis was used to estimate the total volume of distribution (V_T) in a model-independent manner (Logan et al., 1990). V_T equals the tissue-to-plasma concentration ratio at steady state. All data are given as arithmetic mean ± standard deviation (SD). Differences in the outcome parameters between scan 1 and 2 were tested using the Wilcoxon signed rank test and between groups using the Mann-Whitney test (Statistica 6.1, StatSoft, Tulsa, OK, United States). To assess correlations, the Spearman rank correlation coefficient r_s was calculated. A p value of less than 0.05 was considered statistically significant.

We additionally performed an extended data analysis of a (*R*)-[^{11}C]verapamil PET data set previously published by our group (Wagner et al., 2009) in which 5 healthy volunteers underwent two consecutive (*R*)-[^{11}C]verapamil PET scans before and after administration of tariquidar at a dose which only partially inhibits ABCB1 function at the BBB (2 mg/kg) in order to investigate the effect of this tariquidar dose on ABCB1 function at the BRB. The methods used are described in the **Supplementary Material**.

RESULTS

During the experiments only mild or moderate adverse events were recorded and are listed in reference (Bauer et al., 2016).

Figure 1 shows representative [^{11}C]tariquidar PET images for scans without and with ABCB1 inhibition of one c.421CA carrier with the outlined retina ROI. Mean modelling outcome parameters for the retina and the brain for the two PET scans in c.421CC and c.421CA carriers are given in **Table 1**. In **Figure 2**, selected modelling outcome parameters for the baseline scan and the scan with ABCB1 inhibition for the retina and the brain in individual c.421CC and c.421CA subjects are displayed.

For the baseline scans, K_1 and V_T values of [^{11}C]tariquidar were 4 to 5-fold higher for the retina than for WBGM. Baseline distribution of [^{11}C]tariquidar to the retina as well as to WBGM did not significantly differ between c.421CC and c.421CA subjects. In response to ABCB1 inhibition with unlabelled tariquidar, *ABCG2* c.421C > A genotype carriers showed significant increases as compared to the baseline scan in retinal radiotracer influx (K_1: +62 ± 57%, p = 0.043), and $V_{T\ Logan}$ (+86 ± 131%, p = 0.043) (**Figure 2**). No significant changes in any of the modelling outcome parameters were observed in subjects with the c.421CC genotype (**Figure 2**). The BRB findings were in good agreement with those for the BBB, for which K_1 (+72 ± 35%, p = 0.043) and $V_{T\ Logan}$ (+91 ± 82%, p = 0.043) were also significantly and with a similar magnitude increased following ABCB1 inhibition in c.421CA subjects only. In

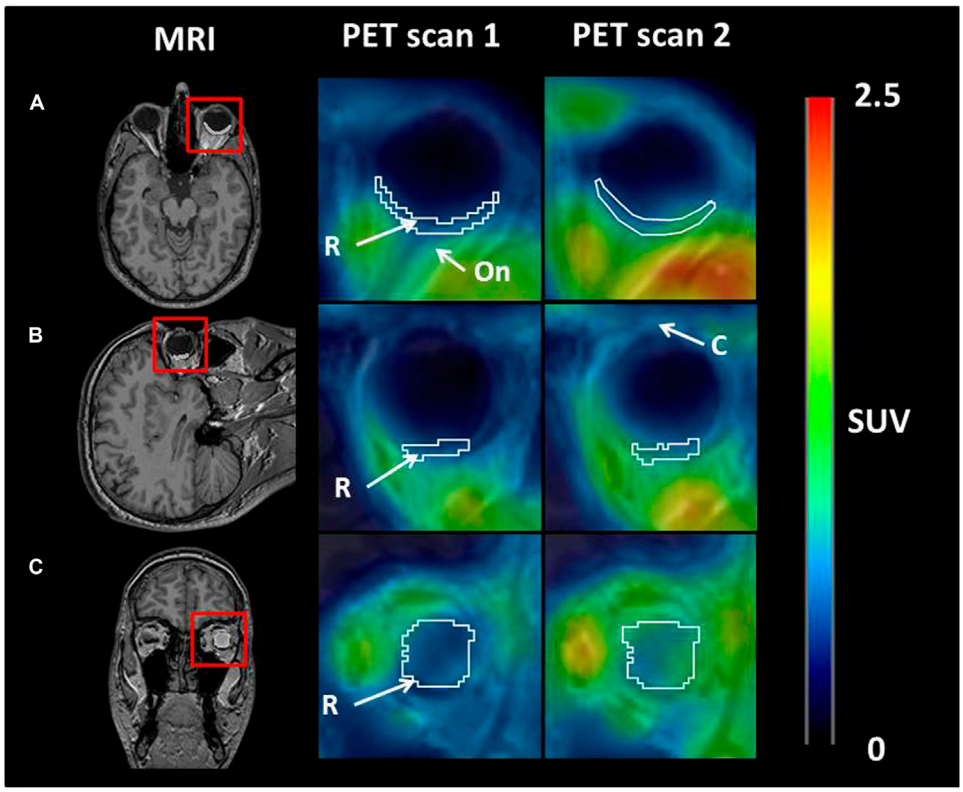

FIGURE 1 | Axial **(A)**, sagittal **(B)**, and coronal **(C)** planes of representative MR and PET average images (0–60 min) at baseline (scan 1) and during ABCB1 inhibition (scan 2) in one c.421CA carrier. Red rectangles on MR images indicate magnified area on PET images. A representative region of interest for retina (white contour) is shown. Anatomical structures are labelled with arrows: R, retina; On, optical nerve; C, cornea. Radiation scale is expressed as standardized uptake value (SUV) and set from 0 to 2.5.

c.421CA subjects, there was a trend towards a positive correlation between the percentage change in $V_{T\ Logan}$ in the retina and in the brain in scan 2 relative to scan 1 ($r_s = 0.8$, $p = 0.133$, not shown). In contrast to the BRB, k_3 values were not significantly increased in the brain of c.421CA subjects following ABCB1 inhibition (**Table 1**).

The mean modelling outcome parameters for the retina and the brain for the (*R*)-[^{11}C]verapamil PET scans without and with ABCB1 inhibition with a lower dose of unlabelled tariquidar (2 mg/kg) are reported in the **Supplementary Table S2**. In contrast to [^{11}C]tariquidar, baseline K_1 and V_T values were for (*R*)-[^{11}C]verapamil comparable for the retina and for the brain. Following ABCB1 inhibition, K_1 and V_T values of (*R*)-[^{11}C] verapamil were significantly increased as compared with the baseline scan, both for the retina and the brain (K_1, retina: +97 ± 100%, $p = 0.043$; K_1, WBGM: +49 ± 36%, $p = 0.043$; $V_{T\ Logan}$, retina: +43 ± 30%, $p = 0.043$; $V_{T\ Logan}$, WBGM: +24 ± 15%, $p = 0.043$).

DISCUSSION

In this study, we used PET imaging to assess the functional impact of the two efflux transporters ABCB1 and ABCG2 at the BRB on controlling the distribution of a model ABCB1/ABCG2 substrate ([^{11}C]tariquidar) to the human retina. Tariquidar is a non-marketed, third-generation ABCB1 inhibitor, which was originally developed to overcome multidrug resistance in cancer patients (Fox and Bates, 2007) and which has been re-purposed to inhibit ABCB1 at the BBB in an experimental setting (Bauer et al., 2012; Bauer et al., 2015; Bauer et al., 2017). Interestingly, non-clinical data indicated that tariquidar highly accumulates in the eye, which has been attributed to binding to melanin (INVESTIGATOR BROCHURE Tariquidar, 2007), which is abundantly expressed in RPE cells (Rimpelä et al., 2018). At tracer doses [^{11}C]tariquidar is transported by both ABCB1 and ABCG2 and has been employed as a PET tracer to measure the activity of ABCB1 and ABCG2 at the BBB (Bankstahl et al., 2013; Bauer et al., 2016). In the present study, we extend for the first time previous *ex vivo* investigations on the impact of ABCB1 on the distribution of diverse model ABCB1 substrates to the mouse and rat eye (Hosoya et al., 2010; Toda et al., 2011; Fujii et al., 2014; Chapy et al., 2016) to a dual ABCB1/ABCG2 substrate examined *in vivo* in humans. We further investigated the effect of the *ABCG2* c.421C > A genotype on the retinal distribution of [^{11}C]tariquidar. We performed two consecutive [^{11}C]tariquidar PET scans in five subjects who were carriers of the fully functioning *ABCG2* allele (c.421CC) and in five subjects with

TABLE 1 | [^{11}C]Tariquidar modelling outcome parameters for the retina and whole brain grey matter in c.421CC and c.421CA subjects for the baseline scan and the scan during ABCB1 inhibition with unlabelled tariquidar.

Region of interest	Group	K_1 (mL/(cm^3·min))	k_2 (1/min)	k_3 (1/min)	k_4 (1/min)	$V_{T\ Logan}$ (ml/cm^3)	V_b
Retina	c.421CC baseline	0.046 ± 0.018 (28)	0.590 ± 0.382 (138)	0.593 ± 0.554 (134)	0.037 ± 0.050 (158)	1.730 ± 0.995 (53)	0.009 ± 0.002 (67)
	c.421CC ABCB1 inhibition	0.057 ± 0.022 (32)	0.503 ± 0.347 (180)	0.294 ± 0.099 (501)	0.151 ± 0.294 (119)	1.742 ± 0.490 (7)	0.010 ± 0.006 (31)
	c.421CA baseline	0.035 ± 0.007 (29)	0.239 ± 0.154 (95)	0.150 ± 0.091 (95)	0.041 ± 0.029 (75)	1.130 ± 0.939 (10)	0.010 ± 0.003 (27)
	c.421CA ABCB1 inhibition	0.056 ± 0.015 (45)*	0.503 ± 0.252 (118)	0.390 ± 0.236 (63)*	0.113 ± 0.163 (54)	1.556 ± 0.890 (19)*	0.011 ± 0.007 (81)
Whole brain grey matter	c.421CC baseline	0.009 ± 0.004 (26)	0.340 ± 0.209 (57)	0.152 ± 0.037 (34)	0.012 ± 0.006 (23)	0.430 ± 0.102 (8)	0.047 ± 0.005 (8)
	c.421CC ABCB1 inhibition	0.008 ± 0.002 (16)	0.176 ± 0.103 (46)	0.119 ± 0.050 (32)	0.014 ± 0.005 (25)	0.408 ± 0.090 (7)	0.046 ± 0.006 (8)
	c.421CA baseline	0.008 ± 0.002 (32)	0.193 ± 0.074 (70)	0.116 ± 0.055 (38)	0.010 ± 0.001 (41)	0.417 ± 0.112 (13)	0.055 ± 0.008 (9)
	c.421CA ABCB1 inhibition	0.013 ± 0.004 (13)*	0.195 ± 0.097 (34)	0.140 ± 0.040 (22)	0.015 ± 0.006 (18)	0.738 ± 0.196 (8)*	0.047 ± 0.008 (10)*

*Values are reported as arithmetic mean ± standard deviation. The value in parentheses represents the precision of the parameter estimates (expressed as their mean standard error in percent). K_1 (mL/(cm^3.min)), rate constant for radiotracer transfer from plasma into the first tissue compartment; k_2 (1/min), rate constant for radiotracer transfer from the first tissue compartment into plasma; k_3 (1/min), rate constant for radiotracer transfer from the first tissue compartment into the second tissue compartment; k_4 (1/min), rate constant for radiotracer transfer from the second tissue compartment into the first tissue compartment; $V_{T\ Logan}$ (ml/cm^3), total volume of distribution estimated with Logan graphical analysis; V_b, fractional arterial blood volume in the eyes/brain. *$p < 0.05$ for comparison with baseline scan using the Wilcoxon signed rank test.*

the c.421CA genotype which presumably results in reduced ABCG2 abundance and function. Following the baseline PET scan, a second scan was performed with a concurrent high dose infusion of unlabelled tariquidar to achieve significant ABCB1 inhibition and thereby specifically reveal ABCG2 function at the human BRB (Bauer et al., 2015; Bauer et al., 2016). Data obtained with [^{11}C]tariquidar for the BRB were compared with data previously obtained with [^{11}C]tariquidar for the BBB (Bauer et al., 2015; Bauer et al., 2016) and with data obtained with the ABCB1-selective substrate radiotracer (*R*)-[^{11}C]verapamil.

One first important finding of our study was that [^{11}C] tariquidar distribution across the BRB to the retina (K_1, V_T) was considerably higher (4 to 5 fold) than its distribution across the BBB to the brain, regardless of the genotype and ABCB1 inhibition condition (see **Table 1**). This is largely consistent with the results from studies in rodents, which revealed a higher distribution of prototypical ABCB1 substrates (e.g., verapamil, quinidine and digoxin) to the retina than to the brain (Hosoya et al., 2010; Toda et al., 2011; Fujii et al., 2014; Chapy et al., 2016). This has been interpreted in a way that ABCB1-mediated efflux is quantitatively less important at the rodent BRB than at the rodent BBB, while passive transcellular permeability appears to be similar at the BRB and BBB for lipophilic compounds (Hosoya et al., 2010). However, our results obtained with [^{11}C]tariquidar differed from those obtained with (*R*)-[^{11}C]verapamil in humans, for which K_1 and V_T values were comparable for the retina and the brain (see **Supplementary Table S2** and reference (Bauer et al., 2017). This may point to species differences between rodents and humans with regards to the retinal distribution of verapamil, which may be possibly related to the presence of an unidentified uptake transporter which mediates verapamil uptake across the BRB (Hosoya et al., 2010; Kubo et al., 2013; Chapy et al., 2016).

A second important finding of our study was that the BRB efflux transport function in c.421CC carriers remained unaltered during ABCB1 inhibition which confirms for the first time the presence of functional ABCG2 at the human BRB. This suggests that a similar functional redundancy between ABCB1 and ABCG2 as described for the rodent and human BBB (Kodaira et al., 2010; Bauer et al., 2016) exists at the human BRB in controlling the retinal distribution of dual ABCB1/ABCG2 substrates.

Our results showed that carriers of the c.421C > A SNP had significant increases in retinal distribution of [^{11}C]tariquidar following ABCB1 inhibition, while c.421CC subjects did not. This supports that the investigated *ABCG2* SNP decreased the function of ABCG2 at the BRB, just as previously reported for the BBB (Bauer et al., 2016). This may suggest that SNP carriers may be more susceptible to transporter-mediated DDIs at the BRB than non-carriers because the functional redundancy between ABCB1 and ABCG2 is compromised.

The percentage increase in retinal distribution of [^{11}C] tariquidar in c.421CA subjects following complete ABCB1 inhibition was of similar magnitude as the percentage increase in its brain distribution (**Table 1**). This again contrasted with results obtained with (*R*)-[^{11}C]verapamil for which the increase in brain uptake was considerably higher than the increase in retinal uptake (3.8- *vs.* 1.5-fold increase in V_T) following complete

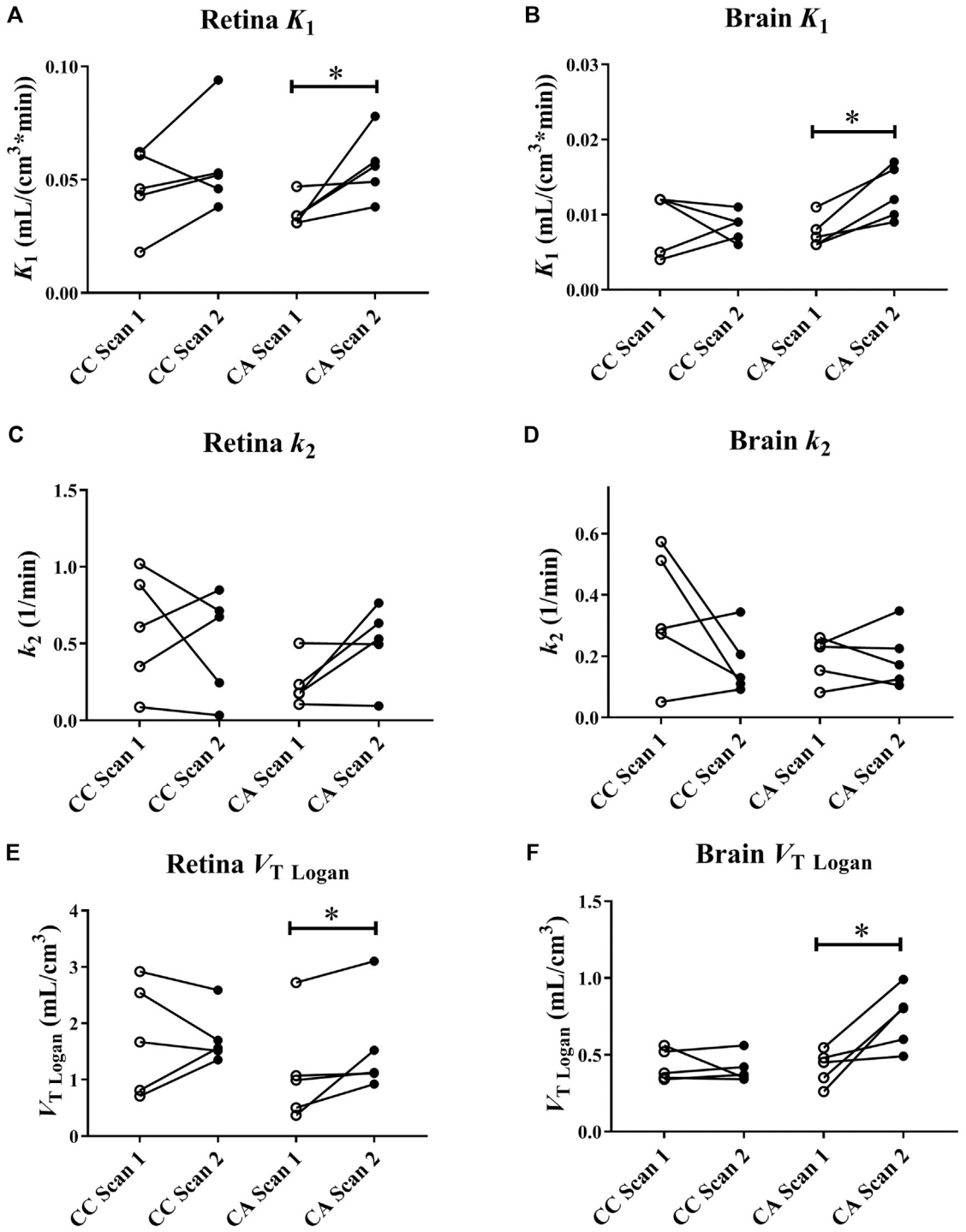

FIGURE 2 | [^{11}C]tariquidar modelling outcome parameters (K_1 and k_2 estimated from 2T4K model and V_T estimated with Logan graphical analysis) for retina **(A, C, and E)** and whole brain grey matter **(B, D, and F)** in c.421CC (CC) and c.421CA (CA) subjects for baseline scan (scan 1) and scan during ABCB1 inhibition (scan 2). Brain data are taken from Bauer et al. (Bauer et al., 2016). *$p < 0.05$, Wilcoxon signed rank test.

ABCB1 inhibition by employing the same tariquidar infusion protocol as in the present study (Bauer et al., 2017). This could mean that the functional impact of ABCG2 is comparable at the human BRB and BBB, while the functional impact of ABCB1 is lower at the human BRB than at the human BBB. However, the presence of an uptake transport system for verapamil at the BRB, which effect was revealed only when ABC efflux transport was abolished, complicates the comparison of the response to ABCB1

inhibition between (*R*)-[^{11}C]verapamil and [^{11}C]tariquidar (Chapy et al., 2016).

The percentage increase in (*R*)-[^{11}C]verapamil K_1 and V_T values at the BRB following administration of a tariquidar dose (2 mg/kg) which only partially blocks ABCB1 at the human BBB (Wagner et al., 2009; Bauer et al., 2015) (see **Supplementary Table S2**) was comparable to the percentage increase following administration of a tariquidar dose which almost completely blocks ABCB1 at the human BBB (see reference (Bauer et al., 2017). This indicated that that the low dose of tariquidar (2 mg/kg) may have already led to complete inhibition of ABCB1 at the BRB. This may suggest that ABCB1 at the human BBB is less susceptible to inhibition than ABCB1 at the human BRB. Consequently, lower doses of ABCB1 inhibitors may lead to significant changes in retinal distribution of ABCB1 substrates than those needed for inhibition of ABCB1 at the BBB. This could suggest that there is an overall higher risk for ABCB1-mediated DDIs to occur at the BRB than at the BBB. These findings are in line with those of a study in mice which revealed the need of only half of the dose of an ABCB1 inhibitor (elacridar) to achieve full ABCB1 inhibition at the BRB than for the BBB (Chapy et al., 2016). The authors linked this finding to lower activity of ABCB1 at the BRB as compared to the BBB.

On one hand, transporter-mediated DDIs at the BRB due to concomitant treatment with drugs which inhibit ABCB1 and/or ABCB2 could contribute to enhanced ocular and in particular retinal toxicity of drugs that normally penetrate poorly into the eye, such as imatinib (Ho et al., 2013), other tyrosine kinase inhibitors (Williamson and Reddy, 2021), ciprofloxacin (Ramirez et al., 2011), tamoxifen (Griffin and Garnick, 1981; Noureddin et al., 1999; Grzybowski et al., 2015) and methotrexate (Balachandran et al., 2002; Iqbal et al., 2005; Sbeity et al., 2006; Sharma and Sharma, 2011; Grzybowski et al., 2015) (see **Supplementary Table S3**). On the other hand, DDIs could generate therapeutic benefits by enhancing the ocular penetration of systemic treatments for retinal disorders struggling to cross the BRB, which represents so far a major challenge for ocular drug delivery (Jordán and Ruíz-Moreno, 2013; Agrahari et al., 2016; Pascual-Pasto et al., 2017; Kim and Woo, 2021). This may be the useful for an improved treatment of diseases, such as neovascular age-related macular degeneration, diabetic retinopathy, and retinal vascular disorders, which are the leading causes of vision deterioration in most developed countries (Bickel, 2005). There is therefore a need for methodology, such as PET imaging, to measure the ocular disposition of drugs in humans.

Just as for the BBB (Kalvass et al., 2013; Bauer et al., 2016), the likelihood of clinically relevant DDIs at the human BRB for dual ABCB1/ABCG2 substrate drugs is likely to be low if ABCB1 and ABCG2 function is preserved, since both transporters possess mutual functional redundancy. Nevertheless, some physiological or pathological conditions have been associated with a reduction in the abundance of ABCB1 and ABCG2 at the BBB as for instance healthy ageing or Alzheimer's disease (Kannan et al., 2017; Storelli et al., 2020), which may raise the risk for ABCB1-mediated DDIs at the BBB and central side effects in the elderly (Bauer et al., 2017; Bauer et al., 2021). Consequently, a clinically achievable degree of ABCB1 inhibition in the brain and retina could be sufficient to lead to significantly higher tissue exposure in *ABCG2* c.421C > A genotype carriers and thereby increase the risk of side effects for ABCB1/ABCG2 substrate drugs with a narrow therapeutic index. The expression of the c.421C > A SNP, which is one of the most common reduced-function variants of ABCG2, is highly dependent on ethnicity. The heterozygous (c.421CA) and homozygous (c.421AA) variants occur with a frequency of 40–45% and 8–12%, respectively, in the East Asian population (Chinese, Japanese, Korean), whereas c.421CA and c.421AA carriers are rare in Caucasians (frequency of 17 and 1%, respectively) and even rarer in Africans (combined frequency of 1.3%) (Lai et al., 2012; Li and Barton, 2018; Chen et al., 2019). The enhanced risk for transporter-mediated DDIs under certain pathological conditions and in SNP carriers may play a clinical role for subjects undergoing treatment with potentially retinotoxic drugs such as tamoxifen or methotrexate (Grzybowski et al., 2015). Selected ABCG2 substrates from references (Mao and Unadkat, 2015; Fohner et al., 2017) with potential retinotoxicity or ocular therapeutic applications are listed in **Supplementary Table S3**.

One limitation of our study was that we did not measure effective retinochoroidal blood flow (RCBF) in study participants. Retinal uptake of [^{11}C]tariquidar and (*R*)-[^{11}C]verapamil may partly depend on RCBF and may thus need correction for RCBF to specifically reveal the function of ABCB1 and ABCG2 at the BRB. While previous data suggested that administration of a pharmacological dose of tariquidar does not affect cerebral blood flow in humans (Kreisl et al., 2010), its effect on RCBF currently remains unknown. The choroidal circulation provides blood supply for the outer retina and particularly the photo-receptors while the central retinal artery irrigates the inner retinal layers (Vaghefi & Pontre, 2016). This dual source of retinal blood supply complicates the estimation of the impact of RCBF variation on [^{11}C]tariquidar and (*R*)-[^{11}C]verapamil distribution to the retina in particular in case of inhomogeneous expression of ABCG2/ABCB1 between the inner and outer BRB. Hence it is not possible to exclude that tariquidar-induced increases in RCBF could have at least partly contributed to the observed increases in retinal [^{11}C]tariquidar and (*R*)-[^{11}C]verapamil uptake.

Another limitation of the study is that we could only include heterozygous (c.421CA) but no homozygous carriers (c.421AA) of the *ABCG2* c.421C > A SNP owing to the rarity of this polymorphism in the Caucasian population.

In conclusion, our study highlights the potential utility of PET imaging to non-invasively assess ocular disposition of drugs in humans. We provide the first evidence that, in analogy to the BBB, ABCB1 and ABCG2 may together limit at the BRB the distribution of systemically administered ABCB1/ABCG2 substrate drugs to the human retina. Carriers of the c.421C > A SNP may be more susceptible to transporter-mediated DDIs at the BRB than non-carriers. This may play a role for subjects undergoing treatment with potentially retinotoxic drugs such as tamoxifen or methotrexate.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics committee of the Medical University of Vienna. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

MB, OL, NT, DS, and MEB wrote the article; MEB, OL, RK, MH, HH, and MZ designed the research; MB, RK, CP, HH, and OL performed the research; MB, RK, MZ, DS, NT, MEB, and OL analyzed the data.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.698966/full#supplementary-material

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REVIEW
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Pharmacogenomics of Impaired Tyrosine Kinase Inhibitor Response: Lessons Learned From Chronic Myelogenous Leukemia

*Meike Kaehler and Ingolf Cascorbi**

Institute of Experimental and Clinical Pharmacology, University Hospital Schleswig-Holstein, Kiel, Germany

The use of small molecules became one key cornerstone of targeted anti-cancer therapy. Among them, tyrosine kinase inhibitors (TKIs) are especially important, as they were the first molecules to proof the concept of targeted anti-cancer treatment. Since 2001, TKIs can be successfully used to treat chronic myelogenous leukemia (CML). CML is a hematologic neoplasm, predominantly caused by reciprocal translocation t(9;22)(q34;q11) leading to formation of the so-called BCR-ABL1 fusion gene. By binding to the BCR-ABL1 kinase and inhibition of downstream target phosphorylation, TKIs, such as imatinib or nilotinib, can be used as single agents to treat CML patients resulting in 80 % 10-year survival rates. However, treatment failure can be observed in 20-25 % of CML patients occurring either dependent or independent from the BCR-ABL1 kinase. Here, we review approved TKIs that are indicated for the treatment of CML, their side effects and limitations. We point out mechanisms of TKI resistance focusing either on BCR-ABL1-dependent mechanisms by summarizing the clinically observed BCR-ABL1-mutations and their implications on TKI binding, as well as on BCR-ABL1-independent mechanisms of resistances. For the latter, we discuss potential mechanisms, among them cytochrome P450 implications, drug efflux transporter variants and expression, microRNA deregulation, as well as the role of alternative signaling pathways. Further, we give insights on how TKI resistance could be analyzed and what could be learned from studying TKI resistance in CML *in vitro*.

Keywords: drug resistance, CML, BCR-ABL, CYP3A4, ABCB1, ABCG2, OCT1, miRNA

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Meyling Cheok, Institut National de la Santé et de la Recherche Médicale (INSERM), France
Diego Maria Michele Fornasari, University of Milan, Italy

****Correspondence:***
Ingolf Cascorbi, cascorbi@pharmakologie.uni-kiel.de

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INTRODUCTION

The development of tyrosine kinase inhibitors tremendously changed anti-cancer drug therapy and opened new treatment options and strategies. Successfully enabling new therapy regimen by specific blockade of the ATP-binding domain of a tyrosine kinase led to increased patient survival rates, less side effects and improved outcome for the patients. Initially established for the use in chronic myelogenous leukemia (CML) by targeting the BCR-ABL1 fusion protein, the outstanding therapeutic success made tyrosine kinase inhibitors a prominent example of the concept of targeted therapy. Meanwhile, there are multiple therapeutic options in which tyrosine kinase inhibitors (TKIs) are first-line choice in therapy or co-therapy, i.e. targeting epidermal growth factor receptor (EGFR) subtypes

using erlotinib or gefitinib in HER1-overexpressing tumors, as well as lapatinib to inhibit HER2 in HER2-positive breast cancer, targeting angiogenesis *via* vascular endothelial growth factor receptor VEGF(R) inhibition or blockade of kinases, such as c-kit (CD117), platelet derived growth factor receptor (PDGFR), or anaplastic lymphoma kinase (ALK), just to name a few (Jiao et al., 2018). Nevertheless, acquired therapy resistances occur during the treatment with TKIs. Here, we review the TKIs used in CML regarding their side effects and limitations. Moreover, we discuss potential mechanisms of impaired TKI response in CML, in particular genomics of *BCR-ABL1*, the impact of variants in cytochrome P450 enzymes and drug transporters, as well as alternative mechanisms of resistance. In addition, we summarize what can be learned from CML for the treatment other neoplasms.

Role Model of Successful TKI-Based Anti-cancer Therapy: Chronic Myelogenous Leukemia

The hematopoietic neoplasm chronic myelogenous leukemia (CML) is a rare disorder predominantly caused by reciprocal translocation t (9; 22) (q34; q11) resulting in formation of the so-called Philadelphia chromosome (Ph) and the *BCR-ABL1* fusion gene (Nowell and Hungerford, 1960; Rowley, 1973; Heisterkamp et al., 1983). This fusion gene makes up for 95% of all CML and 20% of Ph + acute lymphatic leukemia (ALL) cases and is the main driver of malignant cell progression in these leukemias (Radich, 2001; Soverini et al., 2019). For several decades, CML has been a fatal disease with hardly any effective treatment using arsenic substances, radiotherapy, cytostatic drugs, i.e., busulfan and hydroxyurea, or interferon-α, with the latter compounds at least resulting in normalization of the blood visible as hematological remission or even cytogenetic response (Kennedy, 1972; Morstyn et al., 1981; Hukku et al., 1983; Talpaz et al., 1987). Nevertheless, since the development of a tyrosine kinase inhibitor targeting BCR-ABL1 in the 90s century, CML can be effectively treated using the 2-phenylaminopyrimidine imatinib resulting in more than 80% 10-years survival rates in a life-long treatment regimen (Druker et al., 1996; Hochhaus et al., 2017). Since then, tyrosine kinase inhibitors, in particular imatinib, became first-line therapy in CML superseding previous treatment strategies (Hochhaus et al., 2020). This showed for the first time that kinases can be used as druggable targets for anti-cancer treatment. Nevertheless, CML requires a life-long treatment with the respective TKI, as discontinuation might provoke relapses of remaining CML cells. Although several markers are considered to identify suitable patients for therapy termination, e.g. duration of therapy or response rate before discontinuation, *BCR-ABL1/ABL1* ratio, or Sokal score, median relapse rate of patients is approximately 51% (Campiotti et al., 2017; Etienne et al., 2017). Therefore, further studies are needed to identify eligible patients to safely discontinue the treatment.

Tyrosine Kinase Inhibitors in CML: Indications, Side Effects and Treatment Limitations

The fusion gene *BCR-ABL1* arises from the breakpoint cluster region (*BCR*) and the Abelson tyrosine kinase 1 (*ABL1*). While the physiological function of the phosphoprotein BCR is relatively unclear, ABL1 encodes for a cytosolic tyrosine kinase involved in the regulation of proliferation (McCubrey et al., 2008; Bixby and Talpaz, 2011). In *Ph* + cells, BCR-ABL1 is constitutively active, which results in malignant progression. Imatinib binds to the type II conformation of BCR-ABL1 and inhibits binding of ATP to the ATP binding domain preventing phosphorylation of downstream target proteins (Druker et al., 1996; Nagar et al., 2002). This results in proliferation stop and apoptotic cell death. Besides, BCR-ABL1, imatinib also binds to other tyrosine kinases: ABL1 and ABL2 (also named Abelson-related gene ARG), the membrane kinase c-kit (CD117), platelet-derived growth factor receptor beta (PDGFRβ) and colony stimulating factor 1 (M-CSF) (Buchdunger et al., 1995; Buchdunger et al., 1996; Heinrich et al., 2000; Dewar et al., 2005). While inhibition of both ABL paralogs might contribute to the observed side effects of imatinib treatment (Buchdunger et al., 1996), imatinib is used to target c-kit-mutated gastrointestinal stroma tumors (GIST) or PDGFRβ-mutated chronic myelomonocytic leukemia (CMML, **Table 1**) (Poveda et al., 2017; Valent et al., 2019). For inhibition of M-CSF, the influence on therapeutic outcome or side effects remains unclear (Dewar et al., 2005). The occurring side effects of imatinib treatment (but also of later generation TKIs) are gastrointestinal disorders, i.e. nausea or emesis, dermatitis, and in severe cases leukocytopenia, heart failure or liver disorders (Hahn et al., 2003; Kalmanti et al., 2015; Steegmann et al., 2016). Although the side effects are much less severe compared to classical chemotherapy using cytostatic drugs and no absolute contraindications or life-threatening complications have been observed yet, in approximately 10% of patients, distinctive side effects lead to interruption or termination of the therapy with the majority occurring over time or after a drug holiday (O'Brien et al., 2003a; Hochhaus et al., 2020).

While the use of tyrosine kinase inhibitors in CML is tremendously successful, approximately 20–25% of all treated CML patients suffer from loss of previously achieved cytogenetic or major molecular response within 5 years of treatment (Milojkovic and Apperley, 2009; Hochhaus et al., 2017). This stresses the utter need for treatment alternatives. For this purpose, the second and third generation TKIs were developed. Besides imatinib, there are four clinically approved tyrosine kinase inhibitors namely second-generation inhibitors nilotinib, dasatinib and bosutinib and third-generation ponatinib, which differ in their potency, side effects, targets and efficacy against BCR-ABL mutations. Nilotinib, which also binds to the inactive conformation of BCR-ABL1, is 20-fold more potent than imatinib, but also binds to mitogen activated protein (MAP)-kinases and might provoke cardiovascular events in 20% of patients more frequently than imatinib (5%) (Manley et al., 2010; Hughes et al., 2019a). In addition, cerebrovascular

TABLE 1 | Therapeutic targets, impact of metabolic pathways and drug transporters of tyrosine kinase inhibitors, used for the treatment of CML.

Tyrosine kinase inhibitor	Therapeutic target	CYP3A4/5	OCT1	ABCB1	ABCG2
Imatinib	BCR-ABL1 PDGFRβ c-KIT	+	?	+	+
Nilotinib	BCR-ABL1 PDGFRβ c-KIT CSF-1R DDR	+	?	+	+
Dasatinib	Multi kinase inhibitor BCR-ABL1 src family PDGFRβ c-KIT	+	?	+	+
Bosutinib	Dual BCR-ABL1/Src inhibitor	+	?	–	-
Ponatinib	BCR-ABL1 T315I	+	?	+	+

Adapted from Deng et al., 2014. +: strong evidence, substrate or inhibitor; -: no evidence; ?: evidence unclear.

events, hypertension, hypercholesterolemia, diabetes as well as pancreatitis are contradictory (Rosti et al., 2009). Besides similar adverse effects compared to imatinib, the second generation TKI dasatinib, which binds to the active BCR-ABL1 conformation, is likely to cause pleuro-pulmonary toxicity or pleural effusion in approximately 37% of the patients, while being less specific (Kitagawa et al., 2013; Cortes et al., 2016). The broad specificity SRC/ABL inhibitor bosutinib, which was initially designed to inhibit SRC in SRC-overexpressing tumors, but also shows high activity against ABL (and BCR-ABL) (Keller et al., 2009), binds to the BCR-ABL1 kinase independent from the kinase conformation, while provoking transient diarrhea in about 30% of patients (Remsing Rix et al., 2009). In addition, increased levels of transaminases might be a temporary side effect (Hochhaus et al., 2020).

Ponatinib is considered to be a second line TKI used in case of T315I mutation (see below) and resistance to first or second generation TKIs (Cortes et al., 2013). Compared to the other TKIs, the highest number of adverse events occurs during treatment with 30% cardiovascular toxicity and cardiovascular risk factors being contraindicated. Further, the risk of arterial occlusion events should be considered by monitoring hypertension, hyperlipidemia, diabetes and smoking cessation (Hochhaus et al., 2020). Ponatinib binds to the inactive state, precisely the DFG (Asp-Phe-Gly)-out motif, of BCR-ABL1. It should be added that treatment with TKIs is especially effective in chronic phase CML, while the treatment of advanced phases or terminal blast crises, which became rare due to excellent response rates, includes classical chemotherapy or allogenic stem cell transplantation (comprehensively summarized in (Hochhaus et al., 2020).

Regarding genomics of adverse events, little is known about the relevance of SNVs during TKI treatment of CML. Overall, it seems that drug-drug interactions or variants in drug transporters play a more important role in drug resistance than in the occurrence of adverse events (see below).

Genomics of Therapy Resistances: *BCR-ABL1*-Mutations

About approximately 50% of all TKI resistances in CML occur due to mutations or overexpression/amplification of the BCR-ABL1 kinase leading to loss of TKI binding and re-activation of the downstream phosphorylation cascade (Gorre et al., 2001; Jabbour et al., 2011; Baccarani et al., 2013; Rosti et al., 2017). BCR-ABL1 consists of the breakpoint cluster region protein and the tyrosine kinase ABL. The latter is structured by the *N*-terminal lobe and *C*-terminal lobe fused by a hinge region. In the *N*-lobe, *ß*-sheets and an *a*-helix, as well as an SRC-homology domain regulating the tyrosine kinase activity are located. The two *ß*-sheets are fused by a P-loop, which contributes to binding of ATP. In the *C*-lobe, the ATP binding site and the activation loop with conserved DFG required for kinase activation (aspartate, phenylalanine, glycine 381–383) are situated (Reddy and Aggarwal, 2012). Imatinib binds to the inactive conformation of the BCR-ABL1 ATP binding pocket and requires six hydrogen bonds and the conformation switch of activation domain and P-loop into the active conformation (Reddy and Aggarwal, 2012). Therefore, mutations altering the necessary amino acids can tremendously limit the function of the drug (Eiring and Deininger, 2014). Binding of imatinib is entirely abolished by the so-called gatekeeper mutation T315I, in which one hydrogen bond is removed inside the ATP binding pocket. This mutation also leads to loss of action of the second generation TKIs. The only remaining treatment option to this date is ponatinib, which is a pan-BCR-ABL1 inhibitor and binds to the ATP binding domain independent from the T315 hydrogen bond, although this mutation requires increase of the ponatinib dose (O'Hare et al., 2012; de Lavallade and Kizilors, 2016; Braun et al., 2020; Luciano et al., 2020). Nevertheless, a second step mutation on the same residue from isoleucine to methionine results in failure of ponatinib as well (Zabriskie et al., 2014). Besides these TKIs, the allosteric inhibitor of ABL1 asciminib, as a mimic of the *N*-terminal myristoyl group of ABL1 (and therefore named specifically targeting the ABL myristoyl pocket-(STAMP)-inhibitor), might be an alternative to overcome resistances due to BCR-ABL1 mutations, which are located in the ATP binding domain. As the myristoyl group is lost in the BCR-ABL1 fusion protein, autoregulation of ABL1 is prevented resulting in malignant activation of the signaling transduction cascade, which might be overcome by asciminib (Schoepfer et al., 2018; Hughes et al., 2019b; Eide et al., 2019).

Moreover, mutations in the P-loop, i.e. G250E or Y253H, destabilizing binding of imatinib or in the activation loop, i.e. H396R, prevent the activation loop to maintain the closed position lead to imatinib failure (Reddy and Aggarwal, 2012). However, nilotinib is known to fail as well in the two depicted mutations in the P-loop, while bosutinib is partially resistant to G250E, but a therapeutic option in Y253H (Soverini et al., 2014). This shows the utter need for stratification by the BCR-ABL1 mutation pattern to determine to best TKI for the therapy

TABLE 2 | Examples of mutations in the BCR-ABL1 protein and their influence on TKI response in CML.

Protein mutation	Localisation in the protein	Consequence on structure, TKI binding	Clinical options
BCR-ABL1			
T315I	ATP binding pocket	Loss of binding of imatinib, nilotinib, dasatinib, bosutinib	Switch to ponatinib
T315M	ATP binding pocket	Second step mutation, loss of function of ponatinib	No treatment option, asciminib?
G250E	P-loop	Failure of imatinib, nilotinib, bosutinib	Use of dasatinib or ponatinib
Y253H	P-loop	Failure of imatinib, nilotinib	Switch to dasatinib, bosutinib or ponatinib
H396R	Activation loop	Failure of imatinib	Switch to second generation TKIs

Adapted from Hochhaus et al., 2020; de Lavallade et al., 2016; Soverini et al., 2014; Zabriskie et al., 2014.

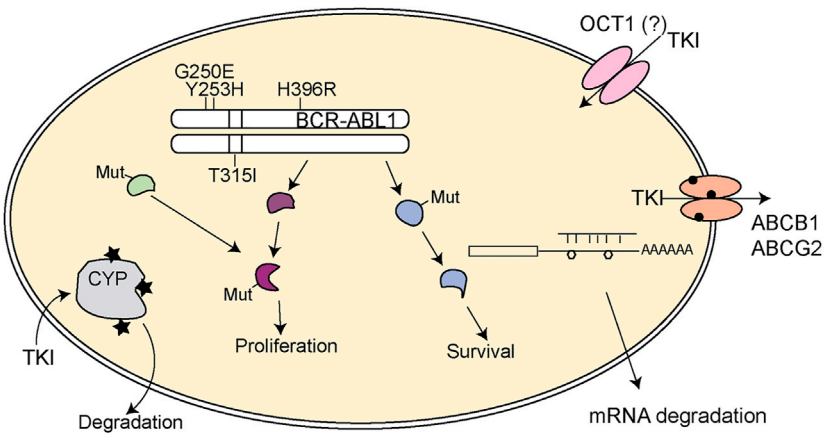

FIGURE 1 | Schematic representation of pharmacogenetic variants leading to TKI resistance in CML. Mutations in BCR-ABL1 (depicted by the protein loci of the mutation) can lead to TKI loss of function. Further, mutations in downstream signaling pathways (Mut) might provoke constitutive activation of the pathway or may lead to activation of alternative signaling pathways that undertake the signaling transduction to sustain proliferation and survival of the tumor cell. Variants in cytochrome 450 enzymes (indicated by stars) could facilitate loss of metabolism of the respective TKI and thereby impaired turnover. In addition, variants in ABCB1 or ABCB2 (circles) might lead to altered TKI efflux and TKI response. The general role of the drug importer OCT1 is still controversially discussed. SNVs in mRNAs (polygons) can also lead to impaired binding of microRNAs, which itself results in altered gene expression potentially contributing to TKI resistance. TKI: tyrosine kinase inhibitor.

(**Table 2**; **Figure 1**). This is also the case for patients with intolerance to one distinct TKIs.

Genomics of Therapy Resistances: BCR-ABL1-independent Mechanisms

Besides mutations in BCR-ABL1, resistances can occur independently from the kinase. These include multiple aspects, which will be reviewed thereafter (**Figure 1**).

Impact of Drug Metabolism: *CYP3A4/CYP3A5*

TKIs are substrates for cytochrome P450, mainly for CYP3A4 and CYP3A5 (Haouala et al., 2011). Therefore, it is not surprising that drug-drug interactions may occur with a large number of co-medications causing induction or inhibition of this metabolic pathway. These include rifampicin, anticonvulsants, i.e. carbamazepine, or herbal products, e.g. St. John's wort, that are confirmed PXR ligands inducing certain cytochrome P450 enzymes including CYP3A4 and 3A5. As a consequence, enhanced metabolism of TKIs diminishes the striven TKI plasma concentration contributing to chemoresistance (Peng et al., 2005; Tian et al., 2018). Imatinib itself is considered to be a moderate CYP3A4 inhibitor, while being a substrate (O'Brien et al., 2003b; Filppula et al., 2012). CYP3A4 metabolizes imatinib to the active, but less cytotoxic metabolite N-desmethyl-imatinib (CPG74588) (Mlejnek et al., 2011). Interestingly, the autoinhibition of CYP3A4 reveals a second pathway, namely CYP2C8, to be involved in hepatic elimination after imatinib exposure (Filppula et al., 2013). Moreover, it was observed that a higher activity of CYP3A4 and CYP3A5 was present in CML patients achieving complete molecular remission compared to poor responders (Green et al., 2010). It was discussed whether pharmacological long-acting metabolites would have contributed to this observation.

Similar to the role of the enzyme activity, the presence of pharmacogenetic variants might limit enzyme activity and thereby affecting the metabolism of imatinib. The main clinically relevant polymorphisms are *CYP3A4**20 (rs67666821) expressed as a truncated protein with loss in enzymatic activity and *CYP3A4**22 (rs35599367) resulting in

TABLE 3 | Pharmacogenetic variants in cytochrome P450 enzymes and in drug transporters and their relevance to TKI response in CML.

Pharmacogenetic variant	Rs-number	Consequence	Evidence
Cytochrome P450 enzymes			
*CYP3A4*20*	rs67666821	Truncated protein	—
*CYP3A4*22*	rs35599367	Intronic SNP, C > T	—
*CYP3A5*3*	rs776746	Cryptic splice site with premature stop codon, A > G	Unclear, contradictory data
*CYP3A5*6*	rs10264272	Synonymous, G > A	—
*CYP3A5*7*	rs41303343	Insertion, frameshift mutation	Decrease in imatinib trough concentration?
*CYP2C8*2*	rs11572103	Missense, T > A	Increase in imatinib trough level?
Drug transporters*OCT1*			
181C > T	rs1208357	R61C	—
480C > G	rs683369	L160F	—
1022C > T	rs2282143	P341L	—
1222A > G	rs628031	M408V	—
1260-1262delGAT	rs72552763	M420del	—
ABCB1			
1199G > A/T	rs2229109	S400 N/L	Relevance unclear
1236C > T	rs1128503	Synonymous	Increased imatinib response?, no association to nilotinib, dasatinib, ponatinib
2677G > T/A	rs2032582	A893 S/T	Increased imatinib response?, no association to nilotinib, dasatinib, ponatinib
3435C > T	rs1045642	Synonymous	Increased imatinib response?, no association to nilotinib, dasatinib, ponatinib
ABCG2			
34G > A	rs2231137	V12M	Improved response to imatinib?
421C > A	rs2231142	Q141K	Conflicting data
−15,622C > T	rs7699188	Low expression of BCRP	Unclear?

Adapted from Werk and Cascorbi, 2014; White et al., 2006; Watkins et al., 2015; Bruckmueller and Cascorbi, 2021; -: lack of evidence.

loss of about 20% enzyme activity, while evidence for variants with increased enzyme activity is lacking (Werk and Cascorbi, 2014; Saiz-Rodriguez et al., 2020). *CYP3A4* and *CYP3A5* share a high sequence homology and overlap in their substrate spectra (Williams et al., 2002). For *CYP3A5*, the main variants are non-functional *CYP3A5*3* (rs776746), *CYP3A5*6* (rs10264272), *CYP3A5*7* (rs41303343) that differ in their expression patterns between the ethnicities (Kuehl et al., 2001; Werk and Cascorbi, 2014). CML patients with known *CYP3A4* polymorphisms might suffer from impaired TKI metabolism resulting in increased adverse effects, but presumably also the response to the TKI might be improved. However, there is conflicting data on the role of *CYP450* variants on the response to TKIs. Interestingly, for *CYP3A5*3*, inferior imatinib response of the variant compared to wild-type carriers was observed in several studies contradicting the presumption of improved imatinib response in the presence of a non-functional CYP3A5 protein (Liu et al., 2002; Kim et al., 2009; Bedewy and El-Maghraby, 2013; Harivenkatesh et al., 2017). In contrast, a meta-analysis for *CYP3A5*3* revealed an association of higher complete cytogenetic response rates under imatinib treatment at least in the Asian population (Cargnin et al., 2018). However, future conformational studies are necessary to confirm these findings in other cohorts. An association of the TKI response to the other variants mentioned has not been fully elucidated, yet some studies point to a contribution of *CYP2C8*2* and *CYP3A4*7* to alterations in imatinib trough levels in homozygous carriers resulting either in an increase or decrease in the concentration (Adehin et al., 2019). The main genetic variants are summarized in **Table 3**. Regarding adverse events, it seems that CYP3A4 interactions play a larger role in adverse events or lack of TKI response than genetic variants in CYP3A4, as observed for e.g. phenytoin, cyclosporin A or ketoconazole (Dutreix et al., 2004; Atiq et al., 2016; Osorio et al., 2019). Therefore, assessment of cytochrome P450 genotypes or function is not performed in the clinical routine to this date. Further studies are necessary to analyze the relevance of these enzymes in relation to drug resistance and adverse events.

Impact of Drug Transporters

Besides hepatic metabolism, drug transporters are known to be involved in drug resistance impairing the intracellular drug concentration or limiting the bioavailability of a drug in certain tissues. For CML, several drug transporters are discussed being either drug importers or efflux transporters (see **Table 3**).

OCT1

The organic cation transporter 1 *OCT1/SLC22A1* is considered to be involved in the import of some TKIs into the tumor cells. However, data regarding its relevance in CML is controversial, as an upregulation of *OCT1* in imatinib resistance was shown (White et al., 2006; Engler et al., 2010), while others clearly demonstrated the absence of a *OCT1* regulation (Davies et al., 2009; Nies et al., 2014). Interestingly, it was shown that OCT1 expression and activity might be used as a prognostic marker for long-term imatinib response of CML patients (Watkins et al.,

2015). Regarding pharmacogenetics, the main variants in *OCT1* are 181C > T (R61C, rs12208357), 480C > G (L160F, rs683369), both located in exon 1; exon 6 1022C > T (P341L, rs2282143), 1222A > G (M408V, rs628031) and 1260-1262delGAT (M420del, rs72552763), both located in exon 7. Nevertheless, several studies did not confirm an influence of any *OCT1* variant on imatinib response (Watkins et al., 2015).

ABC Transporters

Regarding drug efflux transporters, the CML TKIs are discussed to be dose-dependent substrates or inhibitors of P-glycoprotein (P-gp, ABCB1) and breast cancer resistance protein (BCRP, ABCG2) being drug efflux transporters of the ATP binding cassette (ABC) family that limit the intracellular concentration of the respective TKI (Hegedus et al., 2009; Anreddy et al., 2014; Beretta et al., 2017). In particular imatinib, nilotinib, dasatinib and ponatinib were shown to be substrates of both, ABCB1 and ABCG2, whereas bosutinib shows only little affinity and cannot be considered as substrate of one of the mentioned ABC transporters (Deng et al., 2014). Being overexpressed, these transporters are known to contribute to drug resistance in several tumors (Li et al., 2016; Mohammad et al., 2018). Besides questions on drug competition and varying expression of these ABC transporters, pharmacogenetic variants in *ABCB1* or *ABCG2* might have an impact on the development of drug resistance (Bruhn and Cascorbi, 2014; Kaehler and Cascorbi, 2019).

ABCB1

ABCB1 is one of the most extensively investigated drug transporters and broadly analyzed in terms of pharmacogenetic variants. It could be expected that loss of function variants or those with impaired protein function resulting in reduction in efflux capability may lead to improved response to TKIs. However, so far there is no clear evidence that *ABCB1* variants could be applied as predictive biomarkers in any drug therapy (Bruckmueller and Cascorbi, 2021). Most pharmacogenetic studies on TKIs focused on the common variants are 1236C > T (synonymous, exon 12, rs1128503), 2677G > T/A (A893 S/T, exon 21, rs2032582) and 3435C > T (synonymous, exon 26, rs1045642). Regarding response to imatinib, there is conflicting data. Whereas in vitro-experiments using *ABCB1*-overexpressing cells demonstrated a moderately increased imatinib response in triple variants carriers compared to wild-type (Dessilly et al., 2016b), a comprehensive meta-analysis of clinical studies revealed lack of significance on molecular response in relation to any of the above mentioned *ABCB1* variants (Wang et al., 2015). In addition, the role of these variants during treatment with nilotinib, dasatinib and ponatinib also lacked a clear association (Dessilly et al., 2016b; Galimberti et al., 2017). Regarding less common variants, the influence of 1199G > A/T is also controversially discussed, as for the A variant allele increased efflux of imatinib, nilotinib and dasatinib was observed *in vitro*, while this finding was not detected in other studies (Skoglund et al., 2013; Dessilly et al., 2016a). Overall, the role of *ABCB1* polymorphisms in TKI resistance remains controversial. At least, variants do not seem to be suitable as predictive biomarkers of drug response.

ABCG2

Besides its function as drug efflux transporter, ABCG2 is also regarded as stem cell factor being highly expressed in hematopoietic precursor and stem cells (Scharenberg et al., 2002; Jordanides et al., 2006). Similar to *ABCB1*, *ABCG2* polymorphisms are discussed to alter the transport capability of this protein. The most important variants are 34G > A (V12M, exon 2, rs2231137) and 421C > A (Q141K, rs2231142). Some evidence pointed to homozygous 34G > A resulting in amino acid exchange from valine to methionine to be associated with an improved response to imatinib potentially due to reduction in *ABCG2* expression (Kim et al., 2009). For 421C > A, which presumably affects the conformation of the ATP binding domain, data is conflicting as it was shown that expression of the variant limited imatinib bioavailability, while others demonstrated no effects on the pharmacokinetics of imatinib *in vivo* (Gardner et al., 2006; Takahashi et al., 2010; Skoglund et al., 2014). Nevertheless, Jiang and colleagues suggested a potential use of this variant to predict imatinib response in CML (Jiang et al., 2017). In addition to these polymorphisms, the -15,622C > T promoter SNP (rs7699188) was associated with low expression of BCRP in multiple tissues, including the liver, likely to decrease imatinib clearance from the cell (Poonkuzhali et al., 2008). Additional variants in *ABCG2* were also analyzed, but revealed hardly any effects on TKI clearance or response (Bruckmueller and Cascorbi, 2021). To conclude, for both, ABCB1 and ABCG2, a clear association of pharmacogenetic variants to imatinib response is lacking and future studies are necessary to provide insights into their relevance in drug resistance.

Adding to the complexity, expression of ABCB1 and ABCG2 in drug resistance seems to be dose-dependent, as in several studies controversial findings were observed pointing to a dynamic expression of these proteins (Gromicho et al., 2011; Eadie et al., 2013; Kaehler et al., 2017). Interestingly, it was shown that ABCG2 expression in peripheral blood leukocytes could be used to predict treatment-free remission during imatinib discontinuation (Rinaldetti et al., 2018). Nevertheless, future studies are needed to analyze the influence of ABC transporter variants in neoplasms, such as CML.

Epigenetics and microRNAs

Besides activation or repression by transcription factors, gene expression is regulated by epigenetic factors. These imply DNA methylation or histone modifications as acetylation or ubiquitinoylation, as well as post-transcriptional regulation. For CML, there is some evidence on the influence of methylation during the progression of the CML phases, as it was shown that the *ABL1* promoter is hypermethylated in early stages of CML, as well as a global hypermethylation in CML blast crisis occurs (Machova Polakova et al., 2013; Heller et al., 2016). In TKI drug resistance, an increase in overall methylation was also observed in patients resistant or intolerant to imatinib (Jelinek et al., 2011). However, these findings are limited on distinct genes.

Besides epigenetic regulation, expression of microRNAs might be involved in the pathogenesis of CML and drug resistance. microRNAs are 19–21 nt short ribonucleotides involved in post-transcriptional regulation of gene expression by binding specifically to the 3' UTR of their target mRNAs and provoking either their degradation or translational stop (Kim, 2005; Krutzfeldt et al., 2006). As microRNAs regulate expression of tumor suppressor or oncogenes, aberrant microRNA-expression was demonstrated in several malignancies, as well as in combination with anti-cancer drugs (Zheng et al., 2010). In CML, it was shown that the presence of TKIs alters the microRNA expression pattern in blood samples of CML patients (Flamant et al., 2010). In addition, the global microRNA expression pattern seems to differ between either drug sensitivity and resistance *in vitro*, in CML patients, as well as in responding and non-responding CML patients or CML phases (San Jose-Eneriz et al., 2009; Machova Polakova et al., 2011; Turrini et al., 2012; Klumper et al., 2020). Moreover, distinct microRNAs, as shown e.g. for miR-203 or -30a/e, target the *BCR-ABL1* gene and their deregulation might contribute to altered response to TKIs (Liu et al., 2013; Shibuta et al., 2013; Hershkovitz-Rokah et al., 2014). Even beyond BCR-ABL1, fine-tuning of gene expression by microRNAs as e.g. *MYC* by miR-144/451 or miR-212/*ABCG2* might be involved to regulate the relevant target genes in the downstream signaling cascade and contribute to drug resistance (Liu et al., 2012; Kaehler et al., 2017). Therefore, it is discussed if microRNA expression could be used as biomarker for response to TKI treatment (Litwinska and Machalinski, 2017).

It has to be added that SNVs in the 3' UTRs of microRNA target genes, as well as expression of alternate 3′ UTR lengths might tremendously affect microRNA binding resulting in tumor cell escape from therapy (Kasinski and Slack, 2011). This was shown e.g., for *ABCB1* and *ABCG2* in various cancer cell lines (To et al., 2008; Bruhn et al., 2016), as well as for three members of the ABCC family (Bruhn et al., 2020). Moreover, binding of let-7 was impaired by mutated *KRAS* 3' UTR (Chin et al., 2008).

Alternative Mechanisms of Resistance

The constitutive activation of the BCR-ABL1 fusion protein leads to pleiotropic stimulation of various signaling pathways involving JAK/STAT, MAP-kinases and PI3K/Akt signaling pathways. These result in increased cell proliferation, anti-apoptotic signaling, as well as altered cell motility and adhesion to stroma cells (Cilloni and Saglio, 2012). As the majority of these signaling pathways are oncogene addicted to BCR-ABL1 activity, treatment with BCR-ABL1 inhibitors is highly successful. However, these pathways can be captured by alternate stimuli, as shown e.g. for WNT/β-catenin signaling in leukemic stem cells or JAK2 activation by external stimuli (Braun et al., 2020), which makes the tumor cell at least partially autonomous from BCR-ABL1 potentially facilitating therapy failure or unsatisfactory response rates. Moreover, adaptions of the signaling pathways cannot only occur due to differential gene expression, but also due to mutations downstream of BCR-ABL1 or in alternative signaling pathways. These include re-activation of proliferative pathways, e.g. hedgehog or PI3K/Akt signaling, or activation of autophagy (comprehensively reviewed in (Minciacchi et al., 2021). The main difficulty with this is the detection of the responsible signaling pathways to find a suitable target (and drug) combination to circumvent resistance and trigger synthetic lethality, especially of leukemic stem cells (Cilloni and Saglio, 2012). As TKIs–at least to date–require a life-long therapy, they promote the development of mutations, clonal evolution and selection, which facilitates CML progression, but also TKI resistance and thereby adaption of the therapeutic strategy. Luckily, in cases of imatinib failure, a switch to newer generation TKIs according to the guidelines leads to good responses in most patients (Baccarani et al., 2013; Hochhaus et al., 2020).

How to Analyze Genomics of Drug Resistance: In Vitro-Models

As drug resistant cell lines can hardly be established from primary material, these cell lines are utterly important to investigate drug responses. Although these tools are necessary to understand the biology and the mechanisms of drug resistance, some cancer cell lines potentially differ from the tumor they derived from and the transfer to the clinical situation might be limited (Sandberg and Ernberg, 2005; Ertel et al., 2006). Nevertheless, studies on drug efficacy using cell lines were successfully transferred to cancer patients, as shown e.g. for prediction of drug efficacy using gene expression data of cell lines by artificial intelligence and machine-learning (Borisov et al., 2018). In addition, cell lines have been used to develop treatment protocols, as shown for CML using K-562, but also NB4 cells for acute promyelocytic leukemia (Mirabelli et al., 2019). The application of drug resistant cell lines appears still to be the best model to analyze drug resistance (Rumjanek et al., 2013). These can either be generated by pulse treatment or continuous administration of increasing drug concentrations to a given cell line (McDermott et al., 2014). Regarding CML drug resistance models, the majority of studies have been performed on the K-562 cell line (e.g., Turrini et al., 2012; Kaehler et al., 2017), but other cell lines, e.g. LAMA-84 or KCL-22, have been tested as well. The major drawback with these cell lines is their origin in blast crisis of CML patients, which might not reflect the clinical situation of treatment of chronic phases, where initial therapy failure is observed. Therefore, the use of cell lines always implies future studies for the transfer of the observed resistance mechanisms to the clinical situation.

DISCUSSION

Targeted treatment of CML using specific tyrosine kinase inhibitors of the causal BCR-ABL1 fusion protein is tremendously successful. With this it was shown that targeting a single protein in the tumor cell can lead to therapeutic remission. Since CML cells are highly oncogene addicted to BCR-ABL1, inhibition of this protein and its downstream signaling pathways is sufficient to promote the demise of the tumor cells. This strategy was transferred to other tumors and is especially successful whenever the tumor cells have a high

dependency on druggable kinases and has a rather simple complexity. Additional examples are HER2 inhibition in breast cancer using lapatinib and/or HER2-specific monoclonal antibodies trastuzumab and pertuzumab or BRAF mutated malignant melanoma using the tyrosine kinase inhibitors vemurafenib or dabrafenib (Swain et al., 2015; Jiao et al., 2018). While therapy using HER2-inhibition is genuinely successful in primary or advanced HER2-positive breast cancer, BRAF inhibition is often undertaken by downstream mutations leading to time-dependent relapses (Finn et al., 2012; Pernas and Tolaney, 2019). Therefore, BRAF inhibition is often combined with immune checkpoint inhibitors, i.e., ipilimumab, nivolumab or pembrolizumab, while drastically improved the outcome (Furue et al., 2018). CML still is one of the few neoplasms, in which a single agent can be used to successfully treat the disease, as for others, the combination of different agents often exceeds the response rates of a monotherapy and reduces the likelihood of drug resistance (Palmer and Sorger, 2017; Jardim et al., 2020). To this day, a variety of TKIs can be used for several tumors and are mainly administered in a co-treatment strategy (Jiao et al., 2018). Nevertheless, identification of the right (sub) population of tumors is often the key for successful therapy.

Regarding mechanisms of drug resistance, the findings in TKI resistant CML are likely to be transferrable to other drug-tumor combinations. This is the case as e.g. the majority of TKIs are metabolized by CYP3A4 and transported by ABC efflux transporters (Di Gion et al., 2011; Scheffler et al., 2011). Studying various combinations of anti-cancer drugs and tumor entities, it can be concluded that drug-drug interactions and pharmacogenetic variants might play a role in the development of drug resistance in other drug-tumor combinations. However, a predictive role for these variants at least for ABC transporters is not possible yet (Bruckmueller and Cascorbi, 2021).

As shown for BCR-ABL1, mutations in the binding domain of a respective kinase inhibitor or its overexpression/gene amplification have been observed in multiple tumors leading to drug resistance, as shown for acquired EGFR T790M mutations and c-MET receptor tyrosine kinase amplification promoting gefitinib resistance in lung cancer or KIT exon 14 or 17 and PDGFRA exon 14 mutations providing resistance against imatinib and reduced efficacy of sunitinib in GIST (Lynch et al., 2004; Gao et al., 2013; Kobayashi et al., 2013; Zhang et al., 2019). In addition, observations derived from CML regarding activation of alternative signaling pathways can also be observed in other tumor entities. This shows that processes of drug resistance observable in CML are highly similar to other drug-tumor combinations.

Overall, the genomics of impaired response against tyrosine kinase inhibitors observed in CML (**Figure 1**) might be observed during the treatment of other tumors using alternate TKIs as well. Mechanisms of resistance against TKIs often consist of a variety of layers, on mutations of the TKI target gene, in metabolic enzymes, drug transporters or in proteins of downstream or alternative signaling pathways. Adaption of the therapeutic regimen and development of new compounds overcoming these obstacles are necessary to further improve therapy response to TKIs.

AUTHOR CONTRIBUTIONS

MK reviewed literature and wrote the manuscript. IC wrote and edited the manuscript.

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BRIEF RESEARCH REPORT
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CYP3A5 Genotype-Dependent Drug-Drug Interaction Between Tacrolimus and Nifedipine in Chinese Renal Transplant Patients

Yilei Yang[1], Xin Huang[1], Yinping Shi[1], Rui Yang[1], Haiyan Shi[1], Xinmei Yang[1], Guoxiang Hao[2], Yi Zheng[2], Jianning Wang[3], Lequn Su[1], Yan Li[1]† and Wei Zhao[1,2]*†*

[1]Department of Clinical Pharmacy, The First Affiliated Hospital of Shandong First Medical University and Shandong Provincial Qianfoshan Hospital, Shandong Engineering and Technology Research Center for Pediatric Drug Development, Shandong Medicine and Health Key Laboratory of Clinical Pharmacy, Jinan, China, [2]Department of Clinical Pharmacy, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Cheeloo College of Medicine, Shandong University, Jinan, China, [3]Department of Urology, The First Affiliated Hospital of Shandong First Medical University and Shandong Provincial Qianfoshan Hospital, Shandong Medicine and Health Key Laboratory of Organ Transplantation and Nephrosis, Shandong Institute of Nephrology, Jinan, China

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***Correspondence:**
Yan Li
li_xyan@126.com
Wei Zhao
zhao4wei2@hotmail.com

†These authors have contributed equally to this work

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Purpose: The drug-drug interactions (DDIs) of tacrolimus greatly contributed to pharmacokinetic variability. Nifedipine, frequently prescribed for hypertension, is a competitive CYP3A5 inhibitor which can inhibit tacrolimus metabolism. The objective of this study was to investigate whether CYP3A5 genotype could influence tacrolimus-nifedipine DDI in Chinese renal transplant patients.

Method: All renal transplant patients were divided into *CYP3A5*3/*3* homozygotes (group I) and *CYP3A5*1* allele carriers (*CYP3A5*1/*1* + *CYP3A5*1/*3*) (group II). Each group was subdivided into patients taking tacrolimus co-administered with nifedipine (CONF) and that administrated with tacrolimus alone (Controls). Tacrolimus trough concentrations (C_0) were measured using high performance liquid chromatography. A retrospective analysis compared tacrolimus dose (D)-corrected trough concentrations (C_0) (C_0/D) between CONF and Controls in group I and II, respectively. At the same time, a multivariate line regression analysis was made to evaluate the effect of variates on C_0/D.

Results: In this study, a significant DDI between tacrolimus and nifedipine with respect to the *CYP3A5*3* polymorphism was confirmed. In group I (n = 43), the C_0/D of CONF was significantly higher than in Controls [225.2 ± 66.3 vs. 155.1 ± 34.6 ng/ml/(mg/kg); p = 0.002]. However, this difference was not detected in group II (n = 27) (p = 0.216). The co-administrated nifedipine and *CYP3A5*3/*3* homozygotes significantly increased tacrolimus concentrations in multivariate line regression analysis.

Discussion: A CYP3A5 genotype-dependent DDI was found between tacrolimus and nifedipine. Therefore, personalized therapy accounting for CYP3A5 genotype detection as

Abbreviations: CCBs, calcium channel blockers; DDIs, drug-drug interactions; RT-PCR, real-time polymerase chain reaction; SDs, standard deviations.

well as therapeutic drug monitoring are necessary for renal transplant patients when treating with tacrolimus and nifedipine.

Keywords: tacrolimus, nifedipine, drug-drug interaction, CYP3A5, renal transplantation

INTRODUCTION

In recent years, modern transplant surgery techniques, immunosuppressants, and donor organ preservation technologies have greatly promoted the development of renal transplantation. Renal transplantation has become a standard treatment for end-stage renal failure, as it significantly improves patients' quality of life (Viklicky et al., 2020).

Tacrolimus, a calcineurin inhibitor, is one of the most widely used immunosuppressants for solid organ transplants (Staatz and Tett, 2018). However, its clinical application is limited by significant differences in treatment response among patients and a narrow therapeutic window (Rong et al., 2019). In humans, tacrolimus is metabolized by the CYP3A subfamily, which mainly includes CYP3A4 and CYP3A5 (Zhu et al., 2015; Yu et al., 2018). Therefore, drugs that affect CYP3A4/5 enzyme activity can affect tacrolimus metabolism and concentration.

Post-transplant hypertension is a common adverse reaction following renal transplantation, and it leads to the concurrent use of oral antihypertensive drugs with anti-rejection treatments. Proton pump inhibitors (PPIs) are commonly used drugs in clinic. Omeprazole increased tacrolimus concentration through inhibiting CYP3A5 of patients with variant CYP2C19 alleles in one drug interaction study (Bosó et al., 2013). The most commonly used antihypertensive drugs in renal transplant recipients are dihydropyridine calcium channel blockers (CCBs) (Moes et al., 2017; Rao and Coates, 2018; Sen et al., 2019). The interaction between tacrolimus and CCBs varies widely in clinical practice. CCBs can inhibit tacrolimus metabolism and affect tacrolimus level (Jasiak and Park, 2016). For example, an *in vitro* study showed that nifedipine inhibited tacrolimus metabolism by 60–70% (Iwasaki, 2007). Moreover, a retrospective study of liver transplant recipients showed that tacrolimus concentrations were significantly higher in those also receiving nifedipine compared to those did not (Seifeldin et al., 1997).

The importance of genotypic variations, especially in CYP3A5, have been reported in studies where tacrolimus was co-administrated with amlodipine or nicardipine (Hooper et al., 2012; Zuo et al., 2013). As one of the CYP3A5 allelic variants, CYP3A5*1 encodes functional metabolic enzyme (Hooper et al., 2012; Zuo et al., 2013). *CYP3A5*3*, an important function-reduced mutant alleles of CYP3A5, has a distinct racial distribution frequency. It is present in more than 90% of Caucasians, and decreases to about 70% in Asians and less than 50% in Africans (Chakkera et al., 2013; Tang et al., 2020). CYP3A5*3/*3 homozygotes are considered to be CYP3A5 nonexpressors even though few enzyme still has functional activity (Lamba et al., 2012). Nifedipine is mainly metabolized by CYP3A including CYP3A4 and CYP3A5. A study on the pharmacokinetics of nifedipine in healthy Chinese volunteers has demonstrated that CYP3A5*3 is associated with the decrease of nifedipine metabolism (Wang et al., 2015). Currently, the effect of CYP3A5 on the interaction between tacrolimus and nifedipine is unclear. This study aimed to assess whether the drug-drug interaction (DDI) between tacrolimus and nifedipine is associated with CYP3A5 genotype in renal transplant recipients.

METHOD

Study Population and Data Collection

Kidney transplant patients from the Department of Urology of The First Affiliated Hospital of Shandong First Medical University and Shandong Provincial Qianfoshan Hospital from January 2017 to May 2020 were included in this observational study. This process was approved by Ethics Committee of the same hospital. The informed consent was also obtained from the patients or relatives.

The inclusion criterion was that patients receiving tacrolimus as part of a standard immunosuppressive therapy in the immediate post-transplant period (≤2 weeks). The exclusion criterion were patients who had received: a previous heart or liver transplantation; a second kidney transplantation; CYP3A enzyme inducers (e.g., rifampin, phenytoin sodium, or carbamazepine) or inhibitors (e.g., fluconazole, ketoconazole, voriconazole, caspofungin, or macrolide antibiotics); other CCBs besides nifedipine; proton pump inhibitors including omeprazole and esomeprazole; herbal medication such as Wuzhi capsules or hemodialysis following renal transplantation.

Clinical characteristics including age, weight, post-operative day, glucocorticoid dose, creatinine, creatinine clearance rate, tacrolimus dose (D), and co-administration of other drugs were recorded. The post-operative day was calculated from the day of renal transplantation. The chosen beginning measurement day for glucocorticoid dose, creatinine, creatine clearance rate, tacrolimus dose was the same with post-operative day.

Immunosuppressant Therapy

Patients were treated with a post-transplant immunosuppression protocol according to the Kidney disease: Improving Global Outcomes clinical practice guideline (Kidney Disease: Improving, 2009). More specifically, intravenous methylprednisolone was administered the day after transplantation with an initial dose of 500 mg/day that was evenly tapered to 40 mg/day during the first week. During the second week, methylprednisolone tablets were given sequentially at 40 mg/day which was gradually reduced to 16 mg/day as the maintenance dose. Immunosuppression was maintained with oral mycophenolate mofetil tablets given twice daily (1.0–2.0 g/day); tacrolimus was administered twice daily with a starting dose

of 0.05–0.25 mg/kg/day. Dosages were adjusted based on the patients' tacrolimus C_0 and clinical situation.

Tacrolimus concentration generally reached to a steady state three days after the first dose. After having reached to a steady state, therapeutic drug monitoring was routinely performed in the morning before tacrolimus administration. In the first two weeks post-transplantation, tacrolimus concentrations were monitored to maintain a C_0 in the recommended therapeutic range of 10–15 ng/ml. The subsequent measurements of tacrolimus concentration were mostly finished every other day so that tacrolimus dosage can be adjusted in time. The treatment of post-transplant hypertension with nifedipine was at the discretion of the supervising physician.

Tacrolimus Analysis

Tacrolimus C_0 was quantified using high performance liquid chromatography. The linear calibration curve ranged from 0.5 to 30 ng/ml, while assay accuracy ranged from 101.3 to 103.4% with an error of 5%. The intra- and inter-assay coefficients of variation were 5 and 10%, respectively. The lower limit of quantification of the assay was 0.5 ng/ml (Bergmann et al., 2014; Nair et al., 2015).

Genotyping

The presence of *CYP3A5*3* was detected using a TaqMan real-time polymerase chain reaction (RT-PCR) assay (Applied Biosystems, Foster City, CA, United States) as previously described (Allegri et al., 2019; Cheung et al., 2019). Genomic deoxyribonucleic acid was extracted from the blood samples using the TIANamp Blood DNA Kit (DP348; TIANGEN Biotech, Beijing, China) according to the manufacturer's instructions. The primers and sequences for *CYP3A5*3* are as follows: forward primers (5'-CCTGCCTTCAATTTTCACT-3'); reverse primers (5'-GGTCCAAACAGGGAAGAGGT-3'). To validate the RT-PCR results, *CYP3A5*3* (rs776746) was confirmed *via* Sanger sequencing using a 3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, United States) (Saifullah and Tsukahara, 2018; Allegri et al., 2019).

Statistical Analysis

All data were reported as means ± standard deviations (SDs) except where otherwise specified. Nonparametric tests were applied when appropriate. Distributed data were compared using two-tailed Mann-Whitney *U* test in SPSS v16.0. The multivariate linear regression analysis were also finished by SPSS v16.0 in which C_0/D was dependent variable and other factors were independent variables. The $p< 0.05$ represents significant difference. The grouped column scatter plot was created using GraphPad Prism 5.

RESULTS

CYP3A5 Genotype Distribution

We firstly analyzed the allele distribution frequency of CYP3A5. Seventy post-renal transplantation patients were included in this study. The CYP3A5 genotypes among the patients included *CYP3A5*1/*1* (n = 5), *CYP3A5*1/*3* (n = 22) and *CYP3A5*3/*3* (n = 43). The allele frequencies of *CYP3A5*1* and *CYP3A5*3* were 22.9 and 77.1%, respectively (**Table 1**). The allele distribution of CYP3A5 was consistent with the Hardy-Weinberg equilibrium ($\chi^2 = 0.83$; $p = 0.36$).

TABLE 1 | The CYP3A5 genotype distribution of renal transplant patients.

n	Genotype (n/%)			Allele frequency (%)	
	*1/*1	*1/*3	*3/*3	*1	*3
70	5/7.2	22/31.4	43/61.4	22.9	77.1

Tacrolimus-Nifedipine DDIs in Groups I and II

The relationships between effect of DDI with CYP3A5 genotype and nifedipine co-administration were evaluated. Clinical characteristics including age, weight, post-operative day, glucocorticoid dose, creatinine, endogenous creatinine clearance rate and tacrolimus dose did not significantly differ between Controls and CONF in either group I or II, respectively ($p > 0.05$; **Tables 2**, **3**). In group I, the tacrolimus dose-corrected trough concentration (C_0/D) of CONF was significantly higher than in Controls ($p = 0.002$; **Table 2** and **Figure 1**). However, the C_0/D of CONF did not differ from Controls in group II ($p = 0.216$; **Table 3** and **Figure 1**).

For patients of Controls with no nifedipine co-administration, the C_0/D in groups I was higher than that in group II [155.12 ± 34.59 vs. 99.56 ± 22.94 ng/ml/(mg/kg); $p = 0.013$]. At the same time, the C_0/D of CONF with nifedipine co-administration showed significant differences between groups I and group II [225.18 ± 66.25 vs. 116.81 ± 28.46 ng/ml/(mg/kg); $p < 0.001$].

The Effect of Influencing Variates on C_0/D

The results of multivariate line regression analysis showed that the influencing variates including weight, post-operative day, CONF and CYP3A5*3 homozygous mutation had significant effect on C_0/D. The patients with larger weight or post-operative day had higher C_0/D ($p < 0.05$; **Table 4**). The C_0/D of CONF was above that of Controls and showed significant difference (B = 32.042, $p < 0.05$; **Table 4**). The same result of CYP3A5*3 homozygous mutation on C_0/D comparing with CYP3A5*1 allele carriers was present (B = 86.598, $p < 0.05$; **Table 4**). The other variates had no significant effect on C_0/D ($p > 0.05$; **Table 4**).

DISCUSSION

CYP3A5 genetic polymorphisms are widely accepted to play an important role in tacrolimus metabolism. The relatively balanced distribution frequency of CYP3A5 in this study indicate that the *CYP3A5*3* allele mutation is less common in Chinese than Caucasians, which facilitates to compare the effects of DDI between tacrolimus and nifedipine (Chakkera et al., 2013; Qu et al., 2017).

TABLE 2 | The clinical characteristics of the 43 patients in group I.

	Group I (mean ± SDs)		
	Controls (n = 10)	**CONF (n = 33)**	***p* value**
Age (year)	28.00 ± 11.33	37.00 ± 11.28	0.059
Weight (kg)	61.00 ± 7.20	67.00 ± 11.57	0.087
Post-operative day (day)	9.17 ± 2.67	11.00 ± 2.11	0.194
Glucocorticoid dose (mg)	33.34 ± 24.49	24.00 ± 21.87	0.226
Creatinine (μmol/L)	125.34 ± 71.78	142.33 ± 46.26	0.854
Creatinine clearance rate (ml/min)	56.26 ± 21.99	58.87 ± 17.78	0.745
Tacrolimus dose (μg/kg)	56.36 ± 7.52	50.72 ± 10.77	0.118
C_0/D [ng/ml/(mg/kg)]	155.12 ± 34.59	225.18 ± 66.25	0.002

TABLE 3 | The clinical characteristics of the 27 patients in group II.

	Group II (mean ± SDs)		
	Controls (n = 6)	**CONF (n = 21)**	***p* value**
Age (year)	31.50 ± 16.23	45.00 ± 8.99	0.345
Weight (kg)	62.00 ± 8.66	67.00 ± 9.79	0.263
Post-operative day (day)	7.70 ± 2.40	8.00 ± 1.90	0.712
Glucocorticoid dose (mg)	56.65 ± 35.00	56.00 ± 33.42	0.755
Creatinine (μmol/L)	119.84 ± 48.61	110.03 ± 39.13	0.441
Creatinine clearance rate (ml/min)	59.69 ± 18.49	76.67 ± 17.75	0.110
Tacrolimus dose (μg/kg)	54.97 ± 15.02	57.14 ± 10.18	0.798
C_0/D [ng/ml/(mg/kg)]	99.56 ± 22.94	116.81 ± 28.46	0.216

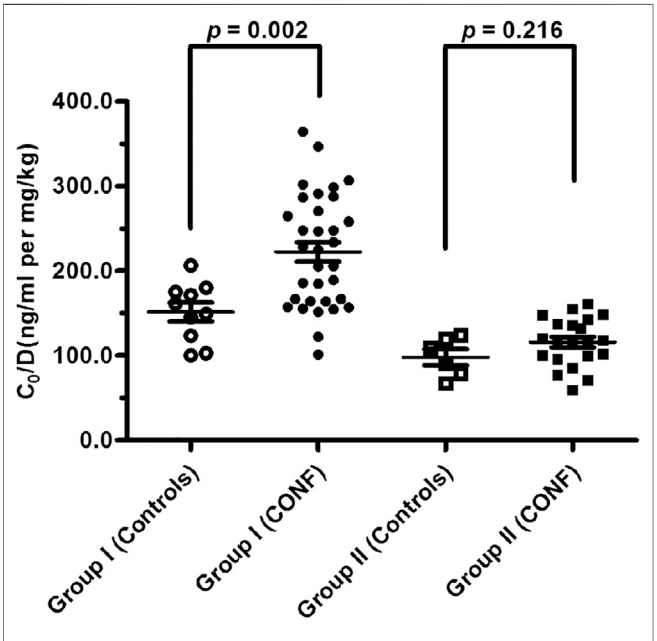

FIGURE 1 | Tacrolimus dose-corrected trough concentrations between subgroups CONF and Controls in group I and II, respectively ($p < 0.05$ denotes a significant difference between corresponding data).

TABLE 4 | The results of stepwise multivariate linear regression analysis.

Influencing variates	**B[a]**	**SE[b]**	**T**	***p***
Weight	2.226	0.542	4.107	<0.001
Post-operative day	5.503	2.590	2.124	0.037
CONF vs controls	32.042	13.591	2.357	0.020
CYP3A5*3/*3 vs CYP3A5*1 allele carriers	86.598	12.187	7.106	<0.001

[a]*B represents the coefficient of linear regression.*
[b]*SE represents the standard error of linear regression.*

As potent inhibitors of CYP3A enzyme, dihydropyridine calcium channel blockers can reduce and increase tacrolimus metabolism and concentration, respectively. The studies of DDI between tacrolimus and amlodipine or nicardipine also proved that CYP3A5 expressers had lower tacrolimus concentration than CYP3A5 nonexpressors, which was consistent with the result of this study (Hooper et al., 2012; Zuo et al., 2013).

For DDI between tacrolimus and nifedipine, nifedipine significantly increased tacrolimus concentrations in *CYP3A5*3/*3* homozygotes but not in *CYP3A5*1* allele carriers. In *CYP3A5*3/*3* homozygotes, the enzyme just retains little activity. At the same time, co-administrated nifedipine almost completely inhibited enzyme activity of CYP3A5, thereby significantly reduced tacrolimus metabolism and consequently increased C_0 levels of tacrolimus. However, nifedipine did not affect tacrolimus concentrations in *CYP3A5*1* allele carriers, who still express some CYP3A5 enzymes and therefore can counteract the tacrolimus-nifedipine DDI effect.

The results of C_0/D in Controls between group I and group II validated the importance of CYP3A5 on the metabolism of tacrolimus. The more higher difference of C_0/D in CONF between group I and group II may reveals a superimposed effect of co-administrated nifedipine and *CYP3A5*3/*3* homozygotes. Through the multivariate line regression

analysis, the post-operative day has direct relationship with C_0/D in the first two weeks after transplantation, which also was found in other DDI (Hooper et al., 2012; Zuo et al., 2013). In addition, the positive effect of co-administrated nifedipine and *CYP3A5*3/*3* homozygotes on C_0/D are further strengthened.

The main limitation of this observational study is that only the effect of CYP3A5 gene polymorphism was investigated. In fact, *CYP3A5*3/*3* homozygotes have minimal CYP3A5 activity and metabolize tacrolimus through another metabolic enzyme, CYP3A4. Although nifedipine inhibits CYP3A4 and CYP3A5, the mechanism underlying tacrolimus-nifedipine DDI needs to be further explored. Due to the low proportion of CYP3A4 mutations in the study population, the synergistic effect of CYP3A4 and CYP3A5 was not easily evaluated. In addition, the small size of including patients, especially for Control subgroup of group II is present. Therefore, more big-sample studies will be necessary to validate forcefully the DDI between tacrolimus and nifedipine.

CONCLUSION

A CYP3A5 genotype-dependent DDI between tacrolimus and nifedipine was confirmed in this study. The significant difference in *CYP3A5*3/*3* homozygotes highlights the importance of CYP3A5 genotype in tacrolimus-nifedipine DDI. *CYP3A5*3/*3* homozygotes that are administered with nifedipine require dose adjustments as part of an individualized treatment.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation, to any qualified researcher.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of The First Affiliated Hospital of Shandong First Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

XH, HS, LS, YL, and WZ designed this study. YY, XY, and JW organized to recruit renal transplant patients. YS and RY undertook genotype analysis. YY, GH, and YZ performed tacrolimus analysis. YY, YS, RY, and WZ jointly drafted this manuscript. All authors have read and agreed with the final version of the manuscript. None of the authors have any other relationships or activities that could appear to have influenced the submitted work.

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The Association Between *STX1B* Polymorphisms and Treatment Response in Patients With Epilepsy

*Shitao Wang[1], Liang Zhou[1], Chenglu He[2], Dan Wang[1], Xuemei Cai[2], Yanying Yu[1], Liling Chen[1], Di Lu[3], Ligong Bian[3], Sunbing Du[1], Qian Wu[1] and Yanbing Han[1]**

[1]Department of Neurology, The First Affiliated Hospital of Kunming Medical University, Kunming, China, [2]Department of Clinical Laboratory, The First Affiliated Hospital of Kunming Medical University, Kunming, China, [3]Biomedicine Engineering Research Center, Kunming Medical University, Kunming, China

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Youssef Daali, Geneva University Hospitals (HUG), Switzerland

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***Correspondence:**
Yanbing Han
ynhyb@163.com

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Background: Epilepsy is a debilitating brain disease with complex inheritance and frequent treatment resistance. However, the role of *STX1B* single nucleotide polymorphisms (SNPs) in epilepsy treatment remains unknown.

Objective: This study aimed to explore the genetic association of *STX1B* SNPs with treatment response in patients with epilepsy in a Han Chinese population.

Methods: We first examined the associations between *STX1B* SNPs and epilepsy in 1000 Han Chinese and the associations between *STX1B* SNPs and drug-resistant epilepsy in 450 subjects. Expression quantitative trait loci analysis was then conducted using 16 drug-resistant epileptic brain tissue samples and results from the BrainCloud database (http://eqtl.brainseq.org).

Results: The allelic frequencies of rs140820592 were different between the epilepsy and control groups ($p = 0.002$) after Bonferroni correction. The rs140820592 was associated with significantly lower epilepsy risk among 1,000 subjects in the dominant model after adjusting for gender and age and Bonferroni correction (OR = 0.542, 95%CI = 0.358–0.819, $p = 0.004$). The rs140820592 also conferred significantly lower risk of drug-resistant epilepsy among 450 subjects using the same dominant model after adjusting for gender and age and Bonferroni correction (OR = 0.260, 95%CI = 0.103–0.653, $p = 0.004$). Expression quantitative trait loci analysis revealed that rs140820592 was associated with *STX1B* expression level in drug-resistant epileptic brain tissues ($p = 0.012$), and this result was further verified in the BrainCloud database (http://eqtl.brainseq.org) ($p = 2.3214 \times 10^{-5}$).

Conclusion: The *STX1B* rs140820592 may influence the risks of epilepsy and drug-resistant epilepsy by regulating *STX1B* expression in brain tissues.

Keywords: STX1B, epilepsy, polymorphism, association, treatment

INTRODUCTION

Epilepsy is one of the most prevalent chronic neurological disorders worldwide, with an estimated globe prevalence > 0.5% (Shorvon, 1990; Fiest et al., 2017). The disease is characterized by episodes of hyper-synchronized neuronal activity leading to recurrent seizures. It is estimated that more than half of all epilepsy cases are associated with genetic factors (Pal et al., 2010). Mutations in many genes have been reported to cause epilepsy (Helbig et al., 2008; Poduri and Lowenstein, 2011; Ponnala et al., 2012; Abou El Ella et al., 2018; Butilă et al., 2018), and epilepsies associated with different mutations exhibit substantial heterogeneity in disease course, clinical manifestations, and treatment response (Wang et al., 2017), presenting significant challenges for diagnosis and management.

The *STX1B* gene (16p11) encodes Syntaxin 1B, a protein of the SNARE complex mediating calcium-dependent synaptic vesicle release (Südhof, 2013). Synaptic dysfunctions are associated with a myriad of neurological disorders, including epilepsy (Steinlein, 2004; Luscher and Isaac, 2009; van Spronsen and Hoogenraad, 2010; Lepeta et al., 2016; Torres et al., 2017). Recent studies suggest that *STX1B* is involved in epilepsy (Schubert et al., 2014; Wolking et al., 2019). However, the role of *STX1B* SNPs in epilepsy treatment remains unknown, so it is necessary to explore the genetic association of *STX1B* SNPs with treatment response in patients with epilepsy in a Han Chinese population.

In this study, we investigated the associations between seven *STX1B* tagging SNPs and treatment response in patients with epilepsy in Han Chinese, and then conducted brain expression quantitative trait loci (eQTL) analysis. The *STX1B* rs140820592 was associated with reduced risks for epilepsy and drug-resistant epilepsy, likely by regulating *STX1B* expression in brain tissues.

MATERIALS AND METHODS

Subjects

A case–control study was performed to investigate the associations between *STX1B* tagging SNPs and drug-resistant epilepsy. Clinical and demographic characteristics of the study cohort are summarized in **Table 1**. All blood samples were collected at the First Affiliated Hospital of Kunming Medical University, and stored at −80°C in the biobank of the First Affiliated Hospital of Kunming Medical University. All brain tissue samples were collected at the First Affiliated Hospital of Kunming Medical University and Xinqiao Hospital and stored at −80°C in the biobank of Kunming Medical University. All subjects included in this study were of Han Chinese ancestry. The epilepsy patients were diagnosed according to 2017 International League Against Epilepsy (ILAE) criteria, and drug-resistant epilepsy patients were diagnosed according to 2010 ILAE criteria. Carbamazepine, valproic acid, levetiracetam and Lamotrigine were prescribed to the epilepsy patients. In order to improve epilepsy care and research, the ILAE defined drug-resistant epilepsy as failure of adequate trials of two tolerated, appropriately chosen and used antiepileptic drug schedules (whether as monotherapies or in combination) to achieve sustained seizure freedom in 2010 (Kwan et al., 2010), and developed the new classification of seizures and epilepsy relevant to clinical practice in 2017 (Fisher et al., 2017; Scheffer et al., 2017). Symptomatic epilepsy was excluded through auxiliary examination and disease history review. The healthy controls had no individual or family history of epilepsy and were neurologically normal. All participates or legal representatives provided written informed consent in accordance with the tenets of the Declaration of Helsinki. All study protocols were approved by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University (No.2020-L-40).

Selection and Genotyping of SNPs

We first identified all *STX1B* SNPs in the Chinese Han South (CHS) population recorded in the 1000 Genomes database (http://www.internationalgenome.org/), and then used Haploview software (Barrett et al., 2005) to pick seven tagging SNPs (r^2 > 0.8) with minor allele frequency > 5%. Basic information for seven *STX1B* SNPs is summarized in **Table 2**. Genomic DNA was extracted from human brain tissues using the Tissue DNA Kit (OMEGA, United States) and from peripheral blood using the Blood DNA Mini Kit (OMEGA, United States). SNP genotyping was conducted using the Bio-Rad CFX96 (BioRad, United States) platform. Primers for PCR were designed using Primer Premier V6.0 (Premier Biosoft Inc., United States). Details on PCR primers are provided in Supplementary Table 1.

Association Analysis of SNPs

SPSS V23.0 (IBM Corp, Armonk, NY) was used for all analyses. Haplotypes were constructed and analyzed using SHEsis software (http://analysis.bio-x.cn/myAnalysis. php). Hardy–Weinberg equilibrium and allele frequencies of all SNPs were analyzed using the Chi-square (χ2) test or Fisher's exact test. Difference in genotype frequencies of all SNPs between cases and controls was analyzed using binary logistic regression. In order to calculate the statistical power, type I error rate of 0.05, dominant mode, and 0.

TABLE 1 | Demographic characteristics of the patients and controls.

Characteristics	Epilepsy	Control	P[a]	DT	DE	p[b]
Males/Females	214/236	244/306	0.314	57/74	157/162	0.271
Age (Mean ± SD)	24.63 ± 16.221	25.77 ± 15.664	0.260	28.74 ± 17.162	22.95 ± 15.535	0.001

SD: standard deviation, DT: drug-resistant epilepsy, DE: drug-responsive epilepsy.
Difference in gender between cases and controls was analyzed using the χ2 test.
Difference in mean age was analyzed using the independent sample t-test.
[a]*P-values were calculated between epilepsy and control.*
[b]*P-values were calculated between drug-resistant epilepsy and drug-responsive epilepsy.*

TABLE 2 | Basic information for seven *STX1B* SNPs.

SNP ID	Position	Location	Minor allele	MAF[a]	MAF[b]
rs4889606	Intron	31,011,183	A	0.1095	0.0995
rs8060857	3' UTR	31,002,720	G	0.0667	0.079
rs12445568	Intron	31,004,812	C	0.0857	0.088
rs74474326	Intron	31,009,343	T	0.0857	0.1105
rs79086360	Intron	31,009,866	C	0.0571	0.0505
rs140820592	5' UTR	3,1,021,880	T	0.081	0.0715
rs186050757	Intron	31,021,024	T	0.0571	0.034

SNP: Single nucleotide polymorphism, MAF: Minor allele frequency.
[a]MAF: Minor allele frequency in Han Chinese in 1000 Genomes project.
[b]MAF: Minor allele frequency in Han Chinese in the study population.

007 of baseline risk were assumed with QUANTO V1.2.4 (Written by John Morrison and W.James Gauderman at the University of Southern California), so the dominant model was also used to evaluate the associations between the genotypes of all SNPs and the risks of epilepsy and drug-resistant epilepsy.

eQTL Analysis

To examine if the tagging SNPs associated with epilepsy and drug-resistant epilepsy were also associated with *STX1B* expression level in drug-resistant epileptic brain tissues, we conducted eQTL analysis using temporal lobe samples from 16 patients (nine males and seven females) with drug-resistant epilepsy. Genomic RNA was extracted using the Tissue RNA Kit (OMEGA, United States), and reverse transcribed into cDNA using the FastQuant cDNA kit (Tiangen, China). Quantitative PCR was conducted using SYBR® Green I (Vazyme, China) and the ABI QuantStudio six Flex™ (ABI, United States) analyzer. Primers were designed using Primer Premier V6.0 (Premier Biosoft Inc., United States) Relative expression levels were determined using the $2^{-\Delta\Delta CT}$ method, and differences in *STX1B* mRNA expression between genotypes were analyzed by independent sample *t*-test using Graphpad Prism9.0 (www.graphpad- prism.cn). We also conducted eQTL analysis using results from the BrainCloud database (http://eqtl.brainseq.org) to validate eQTL findings from epileptic brain tissues. Details on qPCR primers are provided in Supplementary Table 2.

Statistical Analysis

Data are expressed as number, frequency, or mean ± standard deviation (SD) as appropriate. Continuous variables were compared between groups by independent samples *t*-test and categorical variables by χ2 test, Fisher's exact test and logistic regression analysis using SPSS V23.0 (IBM Corp, Armonk, NY) and Graphpad Prism9.0 (www.graphpad-prism.cn) A $p < 0.05$ (two-tailed) was considered significant. For Bonferroni correction, A $p < 0.007$ (0.05/7) was considered significant.

RESULTS

Subject Characteristics

Neither average age nor gender ratio differed significantly between the epilepsy group and control group. However, average age is significantly older in the drug-resistant epilepsy group than drug-responsive epilepsy group. Subject information is summarized in **Table 1**.

TABLE 3 | Comparison of allele frequency distributions for seven tagging SNPs between cases and controls.

SNP ID	Alleles	P[a]	P[b]	P[c]	p[d]
rs4889606	G > A	0.199	0.359	0.769	0.948
rs8060857	A > G	0.881	0.423	0.799	1.000
rs12445568	T > C	0.409	0.139	0.998	0.947
rs74474326	C > T	0.523	0.576	1.000	1.000
rs79086360	T > C	0.479	0.097	1.000	0.882
rs140820592	G > T	0.002	0.028	0.712	1.000
rs186050757	C > T	0.552	0.035	1.000	0.825

SNP Single nucleotide polymorphism.
[a]p values were calculated using χ2 test for comparison of the allele distribution frequencies between epilepsy patients and healthy controls.
[b]P values were calculated using χ2 test for comparison of the allele distribution frequencies between drug-resistant epilepsy patients and drug-responsive epilepsy patients.
[c]P values were calculated using χ2 test or Fisher's exact test for Hardy-Weinberg equilibrium in healthy control.
[d]P values were calculated using χ2 test or Fisher's exact test for Hardy-Weinberg equilibrium in drug-responsive epilepsy patients.

STX1B SNPs Associated Epilepsy Treatment Response in Han Chinese

To comprehensively evaluate the relationships between the seven selected SNPs and epilepsy treatment response in Han Chinese, we performed a case–control study involving 1,000 individuals (450 cases, 550 healthy controls). All seven tagging SNPs were in Hardy–Weinberg equilibrium among control participants ($p > 0.05$) (**Table 3**).

In the Stage I study, we found a significant difference in rs140820592 allele distribution between the epilepsy group and the control group ($p = 0.002$) (**Table 3**), and the association remained significant after adjusting for age and gender and Bonferroni correction (OR = 0.542, 95%CI = 0.358–0.819, $p = 0.004$) in the dominant model (GT + TT vs. GG) (**Table 4**).

In Stage II, we further explored the relationships between *STX1B* SNPs and drug-resistant epilepsy among 450 Han Chinese individuals (131 drug-resistant epilepsy patients, and 319 drug-responsive epilepsy patients), and again found this association remained after adjusting for age and gender and Bonferroni correction (OR = 0.260, 95%CI = 0.103–0.653, $p = 0.004$) in the dominant model (GT + TT vs. GG) (**Table 5**).

The rs8060857, rs12445568, and rs74474326 polymorphisms have strong linkage disequilibrium for both epilepsy vs. control groups and drug-resistant epilepsy vs. drug-responsive epilepsy groups (**Figure 1**). There were no significant difference in the distributions of haplotypes A-T-C, A-T-T, and G-C-C between epilepsy and control groups ($p = 0.812$, $p = 0.726$, and $p = 0.938$, respectively) (**Table 6**) or between drug-resistant epilepsy vs. drug-responsive epilepsy groups ($p = 0.936$, $p = 0.636$, and $p = 0.478$, respectively) (**Table 7**).

eQTL Analysis

We then conducted eQTL analysis based on brain tissue samples from 16 epilepsy patients with drug-resistant epilepsy and found

TABLE 4 | Associations between *STX1B* SNP genotypes and epilepsy.

SNP ID	Genotype	Epilepsy N (%)	Control N (%)	OR (95% CI)	P
rs4889606 G > A	GG	372 (82.7)	436 (79.3)	1.000	–
	GA + AA	78 (17.3)	114 (20.7)	0.855 (0.595–1.227)	0.394
rs8060857A > G	AA	382 (84.9)	469 (85.3)	1.000	–
	AG + GG	68 (15.1)	81 (14.7)	1.178 (0.800–1.735)	0.406
rs12445568 T > C	TT	379 (84.2)	453 (82.4)	1.000	–
	TC + CC	71 (15.8)	97 (17.6)	0.964 (0.662–1.404)	0.849
rs74474326 C > T	CC	359 (79.8)	431 (78.4)	1.000	–
	CT + TT	91 (20.2)	119 (21.6)	1.014 (0.713–1.442)	0.939
rs79086360 T > C	TT	408 (90.7)	492 (89.5)	1.000	–
	TC + CC	42 (9.3)	58 (10.5)	0.970 (0.620–1.518)	0.894
rs140820592 G > T	GG	405 (90.0)	456 (82.9)	1.000	–
	GT + TT	45 (10.0)	94 (17.1)	0.542 (0.358–0.819)	0.004
rs186050757 C > T	CC	417 (92.7)	515 (93.6)	1.000	–
	CT + TT	33 (7.3)	35 (6.4)	1.354 (0.804–2.281)	0.254

SNP: Single nucleotide polymorphism, OR: Odds ratio, 95% CI: 95% confidence interval.
p-values calculated by logistic regression analysis with adjustment for gender and age.

TABLE 5 | Associations between *STX1B* SNP genotypes and drug-resistant epilepsy.

SNP ID	Genotype	Drug-resistant patients N (%)	Drug-responsive patients N (%)	OR (95% CI)	P
rs4889606 G > A	GG	112 (85.5)	260 (81.5)	1.000	–
	GA + AA	19 (14.5)	59 (18.5)	0.565 (0.303–1.055)	0.073
rs8060857A > G	AA	114 (87.0)	268 (84.0)	1.000	–
	AG + GG	17 (13.0)	51 (16.0)	0.577 (0.301–1.107)	0.098
rs12445568 T > C	TT	116 (88.5)	263 (82.4)	1.000	–
	TC + CC	15 (11.5)	56 (17.6)	0.452 (0.232–0.880)	0.019
rs74474326 C > T	CC	102 (77.9)	257 (80.6)	1.000	–
	CT + TT	29 (22.1)	62 (19.4)	0.963 (0.541–1.713)	0.897
rs79086360 T > C	TT	114 (87.0)	294 (92.2)	1.000	–
	TC + CC	17 (13.0)	25 (7.8)	1.408 (0.696–2.849)	0.341
rs140820592 G > T	GG	125 (95.4)	280 (87.8)	1.000	–
	GT + TT	6 (4.6)	39 (12.2)	0.260 (0.103–0.653)	0.004
rs186050757 C > T	CC	116 (88.5)	301 (94.4)	1.000	–
	CT + TT	15 (11.5)	18 (5.6)	1.718 (0.799–3.694)	0.166

SNP: Single nucleotide polymorphism, OR: Odds ratio, 95% CI: 95% confidence interval.
p-values calculated by logistic regression analysis with adjustment for gender and age.

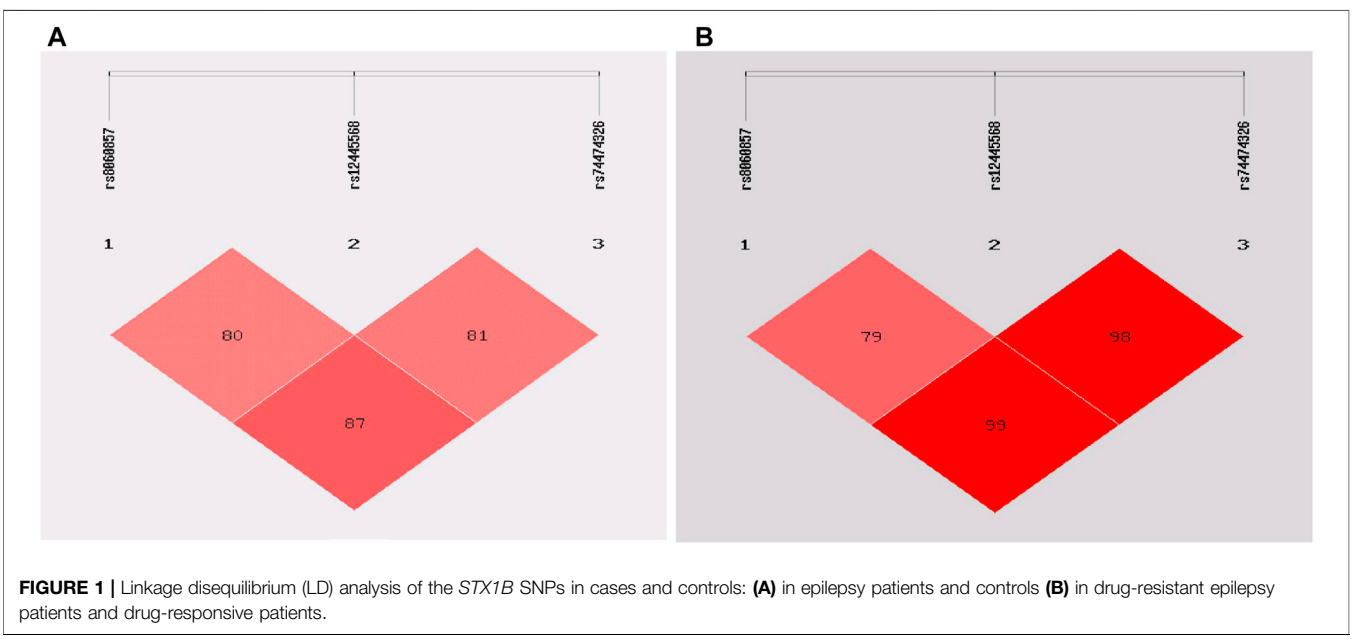

FIGURE 1 | Linkage disequilibrium (LD) analysis of the *STX1B* SNPs in cases and controls: **(A)** in epilepsy patients and controls **(B)** in drug-resistant epilepsy patients and drug-responsive patients.

TABLE 6 | Association between Haplotypes of *STX1B* SNPs and epilepsy risk.

Haplotype	Epilepsy	Control	X^2	p	OR (95% CI)
A-T-C	718.04 (0.798)	863.76 (0.785)	0.057	0.812	1.029 (0.814–1.301)
A-T-T	94.72 (0.105)	119.85 (0.109)	0.123	0.726	0.950 (0.714–1.264)
G-C-C	58.73 (0.065)	70.07 (0.064)	0.006	0.938	1.014 (0.708–1.452)

OR Odds ratio, 95% CI 95% confidence interval.
The haplotypes are combined with STX1B *rs8060857-rs12445568-rs74474326. Haplotypes (frequency < 3%) in both groups have been ignored.*

TABLE 7 | Association between Haplotypes of *STX1B* SNPs and drug-resistant epilepsy risk.

Haplotype	Drug-resistant epilepsy	Drug-responsive epilepsy	X^2	p	OR (95% CI)
A-T-C	213.00 (0.813)	505.13 (0.792)	0.007	0.936	1.016 (0.693–1.490)
A-T-T	29.99 (0.114)	64.62 (0.101)	0.224	0.636	1.117 (0.706–1.769)
G-C-C	14.98 (0.057)	43.72 (0.069)	0.504	0.478	0.803 (0.438–1.472)

OR Odds ratio, 95% CI 95% confidence interval.
The haplotypes are combined with STX1B *rs8060857-rs12445568-rs74474326. Haplotypes (frequency < 3%) in both groups have been ignored.*

that T allele carriers of rs140820592 exhibited higher *STX1B* expression than GG carriers (p = 0.012) (**Figure 2**), a finding consistent with records from the BrainCloud database (http://eqtl.brainseq.org) (**Figure 3**).

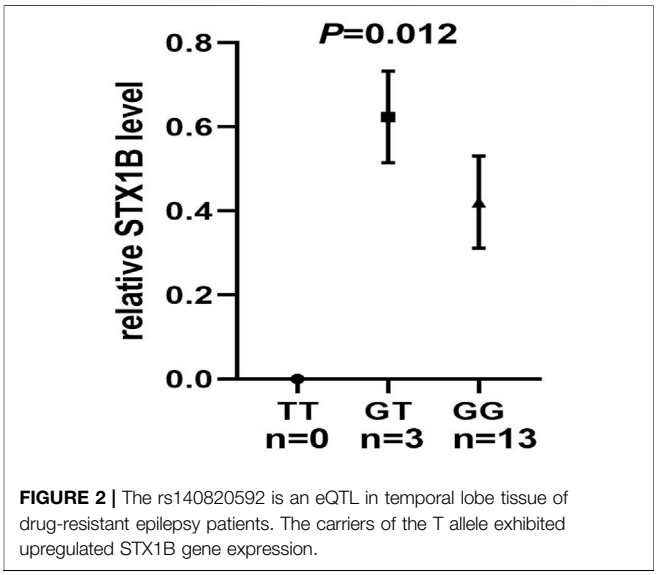

FIGURE 2 | The rs140820592 is an eQTL in temporal lobe tissue of drug-resistant epilepsy patients. The carriers of the T allele exhibited upregulated STX1B gene expression.

DISCUSSION

Syntaxin 1B together with its binding partner Syntaxin binding protein 1 (Hata et al., 1993; Ogawa et al., 1998) is a critical component of the minimal presynaptic transmitter release machinery (Jahn and Sheller, 2006; Rizo and Sudhof, 2012). Here we showed that rs140820592 in the Syntaxin 1B-coding gene *STX1B* was significantly associated with the risks of epilepsy (drug-resistant epilepsy + drug-responsive epilepsy) and drug-resistant epilepsy. This is a study combining genetic association and eQTL analyses in brain tissues and blood samples to comprehensively evaluate the relationship between the *STX1B* gene and epilepsy treatment.

Among the seven tagging SNPs, the SNP rs140820592 was significantly associated with epilepsy. Specifically, risk of epilepsy was lower in rs140820592 GT + TT genotype carriers in the dominant model after adjusting for gender and age, and significance was maintained after Bonferroni correction (**Table 4**). Furthermore, eQTL analysis showed that the T allele carriers of rs140820592 was associated with increased *STX1B* expression in drug-resistant epileptic brain tissues (**Figure 1**), suggesting rs140820592 is a functional SNP.

In addition to epilepsy (drug-resistant epilepsy + drug-responsive epilepsy), the rs140820592 GT + TT genotype decreased the risk of drug-resistant epilepsy in the dominant model after adjusting for gender and age, and significance remained after Bonferroni correction (**Table 5**). To our knowledge, there are no other reports on the relationship between *STX1B* SNPs and drug-resistant epilepsy. In summary, rs140820592 may decrease the risks of epilepsy and drug-resistant epilepsy by regulating *STX1B* gene expression in human brain tissues. However, further larger sample studies are required for confirmation.

Haplotype analysis showed a strong LD for rs8060857, rs12445568, and rs74474326 (D' = 0.79–0.99) in this study. However, the frequencies of these haplotypes, including A-T-C, A-T-T and G-C-C, did not differ between case and control groups for either epilepsy or drug-resistant epilepsy (**Table 6** and **Table 7**), at least in part because these SNPs were not associated with epilepsy or drug-resistant epilepsy in this study cohort. We did not find a strong LD between rs140820592 and other tagging SNPs, suggesting that rs140820592 may be an independent factor reducing the risks of epilepsy and drug-resistant epilepsy in Han Chinese.

Syntaxin 1B is mainly expressed in neurons, and has similar physiological functions to STX1A (Inoue et al., 1992; Kushima et al., 1997). The *STX1B* gene is highly expressed in temporal lobe according to BioGPS (http://biogps.org), so this region was chosen for eQTL analysis. Indeed, this analysis showed that rs140820592 is an eQTL in drug-resistant epileptic brain tissues. As reported in previous studies, disease-associated SNPs often influence the risk of disease by regulating gene expression (Nicolae et al., 2010; Maurano et al., 2012; Ward

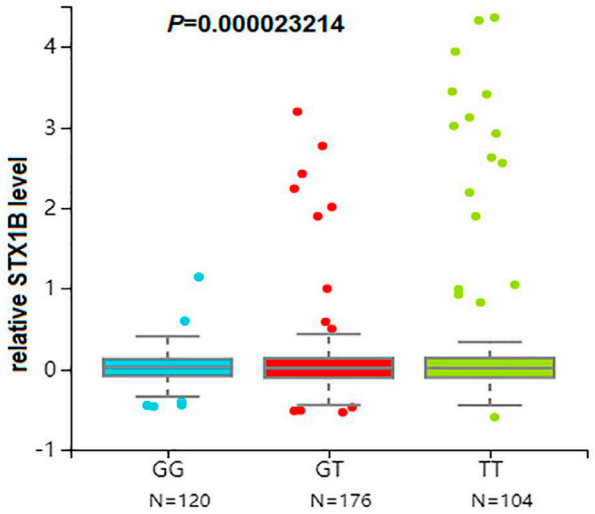

FIGURE 3 | The rs140820592 is an eQTL in the dorsolateral prefrontal cortex. Data were retrieved from the brain tissue database Braincloud (http://eqtl.brainseq.org).

and Kellis, 2012; Bi et al., 2018; Guo et al., 2018; Li et al., 2018; Wang et al., 2021), and this result further verified the association between rs140820592 and epilepsy treatment response. These findings may aid in the development of new diagnostic methods and therapeutic targets for epilepsy.

Genetic factors have been found to affect drug response in many studies (Franco and Perucca, 2015; Balestrini and Sisodiya. 2018). Understanding the associations between *STX1B* SNPs and drug response will aid in translation of genetic findings to clinical treatment. We found that the *STX1B* rs140820592 was significantly associated with the risk of drug-resistant epilepsy at the genetic and expressional levels, suggesting a role of *STX1B* in epilepsy treatment. STX1B plays an important role in the regulation of synaptic vesicle exocytosis, including release of glutamatergic and GABAergic synaptic transmission (Mishima et al., 2014). Furthermore, some important neurotransmitters, such as glutamate and GABA have been found to be involved in epilepsy and drug treatment (Teichgräber et al., 2009; Ngomba and van Luijtelaar, 2018; Chun et al., 2019; Alcoreza et al., 2021). Therefore, STX1B may be involved in epilepsy and drug responsiveness by regulating synaptic vesicle exocytosis.

However, our study in Han Chinese failed to replicate rs140820592 minor allele (T) as a increased risk for epilepsy in the United Kingdom biobank database. The opposite direction of the effect can be explained as follows: First, the difference in the distribution of alleles among different populations should be considered. Second, study subjects for different types of epilepsy may also draw inconsistent conclusions.

Although we have provided important evidence that STX1B gene is involved in the development of epilepsy and drug-resistant epilepsy at the genetic and expressional levels in Han Chinese, some limitations of this study should also be noted: First, our study only included the Han population, and the sample size was relatively small (We estimated that our study had 80% power to detect significant SNPs of MAF = 0.034 with relative risk >1.75.), so a larger sample study including different ethnic groups should be conducted to further verify our findings. Second, we performed functional analysis only in human brain tissues, and further experiments are necessary. Third, in this study, only STX1B gene was included, and other important epilepsy genes were not included in our study.

CONCLUSION

We provide multiple lines of evidence that the *STX1B* rs140820592 may decrease the risks of epilepsy (drug-resistant epilepsy + drug-responsive epilepsy) and drug-resistant epilepsy by regulating *STX1B* expression. STX1B gene may be a new therapeutic target for epilepsy. However, It is necessary to further verify our conclusions through studies with large samples in the future.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of the First Affiliated Hospital of Kunming Medical University. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YH and SW conceived and designed this research; DW, XC, YY, CH, and LC collected blood and brain tissue samples; DL, LB, and QW analyzed the data; YH, SW, and LZ wrote the manuscript; YY, LC, and SD performed the experiments. All authors read and approved the final manuscript.

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frontiers in Pharmacology

ORIGINAL RESEARCH
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CYP450 Genotype—Phenotype Concordance Using the Geneva Micrococktail in a Clinical Setting

Kuntheavy Ing Lorenzini, Jules Desmeules, Victoria Rollason, Stéphane Bertin, Marie Besson, Youssef Daali and Caroline F. Samer*

Division of Clinical Pharmacology and Toxicology, Department of Anaesthesiology, Pharmacology, Intensive Care and Emergency Medicine, University Hospitals of Geneva, Geneva, Switzerland

Pharmacokinetic variability is a major source of differences in drug response and can be due to genetic variants and/or drug-drug interactions. Cytochromes P450 are among the most studied enzymes from a pharmacokinetic point of view. Their activity can be measured by phenotyping, and/or predicted by genotyping. Depending on the presence of drugs and/or diseases that can affect their *in vivo* activity, both approaches can be complementary. In 2014, the Geneva cocktail using dried blood spots was validated in healthy volunteers for CYP450 phenotyping. Since its clinical implementation, it has been used in approximately 500 patients in various clinical situations. Our study aims to report the concordance between CYP450 genotype and phenotype in real-life patients. The prospectively collected data from patients who were genotyped and/or phenotyped between January 2014 and December 2020 were reviewed. A total of 537 patients were genotyped and/or phenotyped for CYP450 during this period, and 241 underwent simultaneous genotyping and phenotyping allowing for genotype/phenotype concordance assessment. Genotyping correctly predicted poor metabolizer phenotypes for most CYPs isoenzymes studied, whereas agreement was more variable for intermediate, normal, and ultrarapid metabolizers. Discrepancies between the phenotype predicted on the basis of genotyping and the measured phenotype were not always explained by concurrent medication (phenotypic switch). Therefore genotyping and phenotyping tests are complementary approaches when aiming to individualize drug therapy. In the 537 patients, the majority of clinical situations were observed with analgesic/anesthetic drugs (n = 187), followed by antidepressants (n = 153), antineoplastics (n = 97), and immunosuppressants (n = 93). Inefficacy (or low drug levels) and adverse drug reaction (or high drug levels) were the main reasons for testing. Genotype and/or phenotype results explained or at least contributed to the clinical event in 44% of cases.

Keywords: cytochromes P450, phenotype, genotype, phenoconversion, drug-drug interaction, inefficacy, adverse drug reaction

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Amit V. Pandey,
University of Bern, Switzerland

Reviewed by:
Volker Martin Lauschke,
Karolinska Institutet (KI), Sweden
Julio Benitez,
University of Extremadura, Spain

****Correspondence:***
Kuntheavy Ing Lorenzini
Kuntheavy-roseline.ing@hcuge.ch

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INTRODUCTION

Patients vary in their response to drugs. A dose that is effective in a given patient may cause an adverse drug reaction (ADR) in another patient or conversely be ineffective. Several causes of variability can be cited, genetic- or disease-related changes in drug concentrations or responsiveness, poor compliance, drug-drug interactions (DDI). Variability in drug response can affect pharmacokinetics, pharmacodynamics, or both (Roden et al., 2019). Pharmacokinetic variability is a major source of differences in drug response and can be due to genetic variants, diseases themselves and/or DDI. Cytochromes P450 (CYP450) are among the most studied enzymes from a pharmacokinetic point of view. Their activity can be predicted by genotyping and/or measured by phenotyping.

Genotyping consists of determining the patient DNA sequence and analyzing functional genetic variants coding for specific enzymes. It allows predicting the phenotype based on the identified alleles (Samer et al., 2013). The genotype offers the advantage of being immutable in a given patient. However, predicting metabolic phenotype from genotype may be challenging for CYP450 enzymes, especially given the continuously increasing number of novel alleles being discovered (Shah et al., 2016). In practice, the genotype does not necessarily correlate well with the phenotype (Waring, 2020). Another major issue is that genotyping does not account for any of the many environmental factors (diseases, drug interactions, dietary) which may impact phenotype (McGraw et al., 2018).

On the other hand, phenotyping can be considered a more useful tool for patient metabolism evaluation to anticipate possible inefficacy or ADR at conventional doses (Keller et al., 2017). However, it implies the oral administration of probe specific xenobiotics, followed by blood or urine sampling. This may represent a limitation in vulnerable populations such as children and pregnant women. Therefore, we believe that genotyping and phenotyping are complementary in clinical settings, depending on the presence of drugs and/or diseases that may affect the *in vivo* activity of CYP450.

At Geneva University Hospitals, we have been using, CYP450 genotyping and phenotyping methods in patients for almost two decades. *In vivo* phenotyping can be performed by administering a single probe drug metabolized by an individual CYP enzyme, or by a "cocktail" approach, consisting in administering several probe drugs. The cocktail approach allows for the simultaneous determination of several CYP enzymes activity (Keller et al., 2017). The first Geneva phenotyping cocktail for CYP450 phenotyping was developed in 2004. It contained 5 probe substrates used at therapeutic doses (100 mg caffeine, 50 mg flurbiprofen, 40 omeprazole, 25 mg dextromethorphan, and 7.5 mg midazolam), thus associated with a risk of therapeutic unwanted effects (Jerdi et al., 2004). Our clinical experience in patients using the "full dose" phenotyping cocktail and its subsequent variants has been published previously with psychotropic drugs (Lloret-Linares et al., 2016) and analgesic drugs (Rollason et al., 2020a). In 2014, a new mixed-probe of the Geneva cocktail, which is called the Geneva microcoktail, using dried blood spots, was validated in healthy volunteers. The Geneva micrococktail contains smaller doses of probe substrates: 50 mg caffeine, 20 mg bupropion, 10 mg flurbiprofen, 10 omeprazole, 10 mg dextromethorphan, 1 mg midazolam, and 25 mg fexofenadine (Bosilkovska et al., 2014), facilitates sample collection, requiring only 10 µL blood samples, and allows phenotyping for additional CYPs and P-glycoprotein. This new formulation of the Geneva cocktail recently showed an excellent safety profile in 265 healthy volunteers from different geographic regions (Rollason et al., 2020b). It was also used to characterize the variation in CYP450 activity in patients undergoing elective orthopedic surgery (Lenoir et al., 2020).

The clinical use of the Geneva micrococktail in real-life polymorbid and/or polymedicated patients has not been previously reported. Since its implementation, we have used it in approximately 500 patients. Our study aims to report the concordance between CYP450 genotype and phenotype using the Geneva micrococktail in real-life patients. Second, we aim to determine if genotyping and/or phenotyping help explaining unexpected clinical responses to drug administration (ADR, or inefficacy).

METHODS

Patients and Setting

The protocol of this retrospective study was approved by the Ethics Commission of the Canton of Geneva, Geneva (Switzerland) (study number: 15-225). We evaluated in- and outpatients with CYP450 genotyping and/or phenotyping tests since 2014, the year of implementation of the Geneva micrococktail as used in its current formulation. As described previously (Lloret-Linares et al., 2016; Rollason et al., 2020a), we retrospectively collected results of the genetic and/or phenotypic investigations made because of non-response to drugs or excessive response to drugs performed between April 2014 and December 2020. Our previously published worked included patients who underwent phenotyping until November 2014. Since we included patients from April 2014, there is an overlap of 18 patients included in the previous as well as in the present article. In the present paper, we will focus on genotype-phenotype concordance. Therefore, patients with genotype-only testing will not be discussed in detail unlike our previously published articles. Patients with only non-CYP450 genotyping (e.g. ABCB1, COMT) were excluded from the analysis.

Genotyping

Genotyping was performed by our institutional laboratory of molecular oncology and pharmacogenomics. Genotyping techniques have considerably evolved over the last few years. From 2017, the used method was based on Next Generation Sequencing (NGS) technologies with the pharmacogenomics panel from ThermoFisher. CYP2D6 gene copy number was determined by qPCR on LC480 (Roche) using CNV Exon 9 Hs00010001_cn and CNV Intron 6 Hs04502391_cn probes for CYP2D6 (Life Technologies, with RNAse P gene used as reference gene). AlleleTyper Software (Thermo Fisher

TABLE 1 | Considered alleles.

CYP	Alleles
CYP1A2	*1K, *1F, *15, *11, *3, *16, *4, *5, *7, *6, *8
CYP2B6	*22, *10, *11, c.516G > T g.15631G > T, *4A, *16/*18, *28, *5
CYP2C9	*7, *13, *2, *27, *8, *15, *9, *10, *6, *16, *11, *3, *4, *5
CYP2C19	*17, *4, *2B, *8, *6, *9, *3, *10, *2, *7, *5
CYP2D6	*2, *29, *41, *7, *2, *9, *3, *20, *4, *14, *8, *6, *29, *17, *11, *15, *12, *10, *35, *2A
CYP3A4	*20, *3, *13, *12, *6, *2, *17, *22, *15, *1B
CYP3A5	*3/*10, *10, *2, *7, *9, *6, *3B, *8

Scientific) was used to translate genetic pattern information from genotyping (Single-nucleotide polymorphisms—SNP) and copy number assay to their standardized allele name or star (*) allele nomenclature. For the NGS, the considered alleles are detailed in **Table 1**.

The predicted phenotypes were based on enzyme activities of the identified alleles, as listed in the Pharmacogene Variation (PharmVar) Consortium database (Gaedigk et al., 2018; Gaedigk et al., 2019) or the PharmGKB database (Whirl-Carrillo et al., 2012). Patients were classified into poor metabolizer (PM), intermediate metabolizer (IM), normal metabolizer (NM), and ultra-rapid metabolizer (UM). For the predicted phenotype of the combined CYP3A4 and CYP3A5 genotypes, we used the classification as described by Andreu et al. PMs were defined as CYP3A4*22 carriers with the CYP3A5*3/*3 genotype, IMs were CYP3A4*22 non-carriers with the CYP3A5*3/*3 genotype or CYP3A4*22 carriers with the CYP3A5*1/*1 genotype, and NMs were CYP3A4*22 non-carriers and CYP3A5*1 carriers (Andreu et al., 2017).

Phenotyping

Phenotyping was performed using the Geneva micrococktail which contained 50 mg caffeine, 20 mg bupropion, 10 mg flurbiprofen, 10 mg omeprazole, 10 mg dextromethorphan, 1 mg midazolam, and 25 mg fexofenadine. These probe substrates allow *in vivo* phenotyping of CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP2D6, CYP3A4/5 and P-glycoprotein, respectively (Bosilkovska et al., 2014). As previously described, the micrococktail was given orally on an empty stomach. Two hours after administration, capillary blood samples or venous blood samples were collected. In cases venous blood samples were taken, blood spots of 10 µL each were spotted on a dedicated filter card. Dried blood spots were stored at −20°C in a sealable plastic bag until analysis by a validated method using liquid chromatography tandem mass spectrometry. Phenotype determination was based on the metabolite to parent drug metabolic ratio (MR). As for genotyping, patients were classified as PM, IM, NM, and UM according to their MR (Lenoir et al., 2020).

Clinical Data

For each patient, we collected demographic data, as well as relevant medical history and current treatments, including complementary and alternative medicine therapies. When applicable, current concomitant drugs were classified as CYP inhibitors or inducers based on our table "Interactions médicamenteuses, Cytochromes P450 et P-glycoprotéine P" (Service de pharmacologie et toxicologie cliniques and HUG, 2020), and the summary of product characteristics.

We also collected the main therapeutic classes according to ATC classification and the main reason for genotyping/phenotyping, which could be one of the following: ADR/high drug levels, inefficacy/low drug levels, DDI, International Normalized Ratio (INR) variation, prescription (preemptive testing), and other (cases not concerned by any of the other categories). Finally, based on the conclusions of the interpretive report written by a senior clinical pharmacologist, we determined whether the genotype/phenotype explained the clinical event.

Statistical Analysis

Descriptive statistics were used. Categorical and continuous variables were described using frequency tables (n, %) and median (range), respectively. Genotype-phenotype concordance was considered adequate when the phenotype was equal to the predicted genotype based on the identified alleles. All analyses were performed using the SPSS® software package, version 25 (IBM corporation, Armonk, NY, United States).

RESULTS

Population Characteristics

Between January 2014 and December 2020, a total of 551 patients underwent genotyping and/or phenotyping tests. Among them, 13 patients had only non-CYP450 genotyping (ABCB1, COMT, SLCO1B1, and OPRM1) and one had missing data; these patients were therefore excluded from the analysis (**Figure 1**). The mean age of the 537 remaining patients was 48.6 years old (range: one month-90 years) and 52.3% (n = 281) were women. Among the 537 patients, 241 (45%) underwent simultaneous genotyping and phenotyping, 235 (44%) underwent phenotyping only, and 61 (11%) underwent genotyping only (**Figure 1**).

Genotype and Phenotype

A total of 241 patients underwent simultaneous genotyping and phenotyping, allowing for genotype-phenotype concordance assessment. The majority of patients received the Geneva microcktail (n = 212) while the other patients (n = 29) had targeted phenotyping, i.e. focusing on one, two, or eventually three CYP enzymes, usually in children to minimize the exposure to non-authorized substances in this population, or because

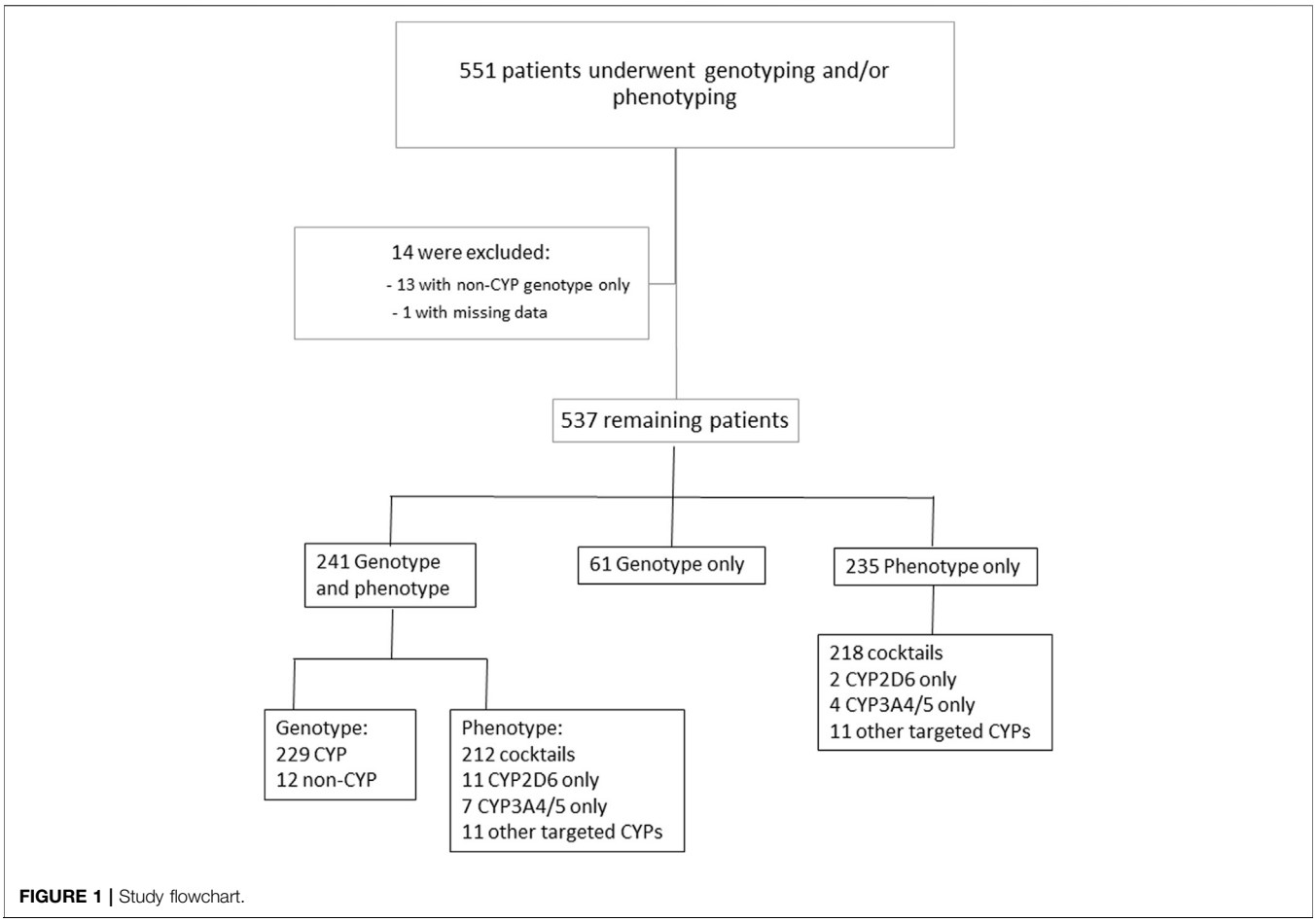

FIGURE 1 | Study flowchart.

TABLE 2 | In patients with simultaneous genotyping and phenotyping, CYP predicted phenotype based on the identified alleles.

	—	PM	IM	NM	UM	Unknown
CYP1A2	Number of individuals (%)	0	0	2 (17%)	9 (75%)	1 (8%)
	Genotypes	—	—	*1B/*1B	*1F/*1F, *1/*1F, *1A/*1F, *1B/*1F	*1N/*1N
CYP2B6	Number of individuals (%)	6 (16%)	10 (26%)	17 (45%)	4 (11%)	1 (2%)
	Genotypes	*6/*6, *9/*9	*1/*6, *1/*9, *4/*6, *5/*6	*1/*1, *1/*2, *1/*5, *2/*2B	*1/*22, *2/*22, *5/*22	*5/*10
CYP2C9	Number of individuals (%)	2 (3%)	20 (32%)	40 (65%)	NA	0
	Genotypes	*2/*3	*1/*2, *1/*3	*1/*1, *1/*9	NA	—
CYP2C19	Number of individuals (%)	7 (6.7%)	23 (21.9%)	48 (45.7%)	27 (25.7%)	0
	Genotypes	*2/*2	*1/*2, *1/*3, *2/*17	*1/*1	*17/*17, *1/*17	—
CYP2D6	Number of individuals (%)	11 (8.1%)	52 (38.5%)	66 (48.9%)	5 (3.7%)	1 (0.8%)
	Genotypes	*3/*4, *4/*4, *5/*5, *6/*6	*1/*3, *1/*4, *1/*5, *2A/*4, *4/*35, *5/*41	*1/*1, *1/*2, *1/*2A, *1/*9, *1/*10, *1/*41	Functional allele xN	*41/*119
CYP3A4-CYP3A5	Number of individuals (%)	8 (11%)	52 (73%)	10 (14%)	NA	1 (2%)
	Genotypes	*1/*22–*3/*3, *22/*22–*3/*3	*1/*1–*3/*3	*1/*1–*1/*1, *1/*1-*1/*3, *1/*1B–*1/*3	NA	*1/*1–*4/*4

PM: poor metabolizer; IM: intermediate metabolizer; NM: normal metabolizer; UM: ultra-rapid metabolizer. CYP: cytochrome P450. NA: not applicable. For CYP2D6 and CYP3A4-CYP3A5, only the principal identified genotypes are given.

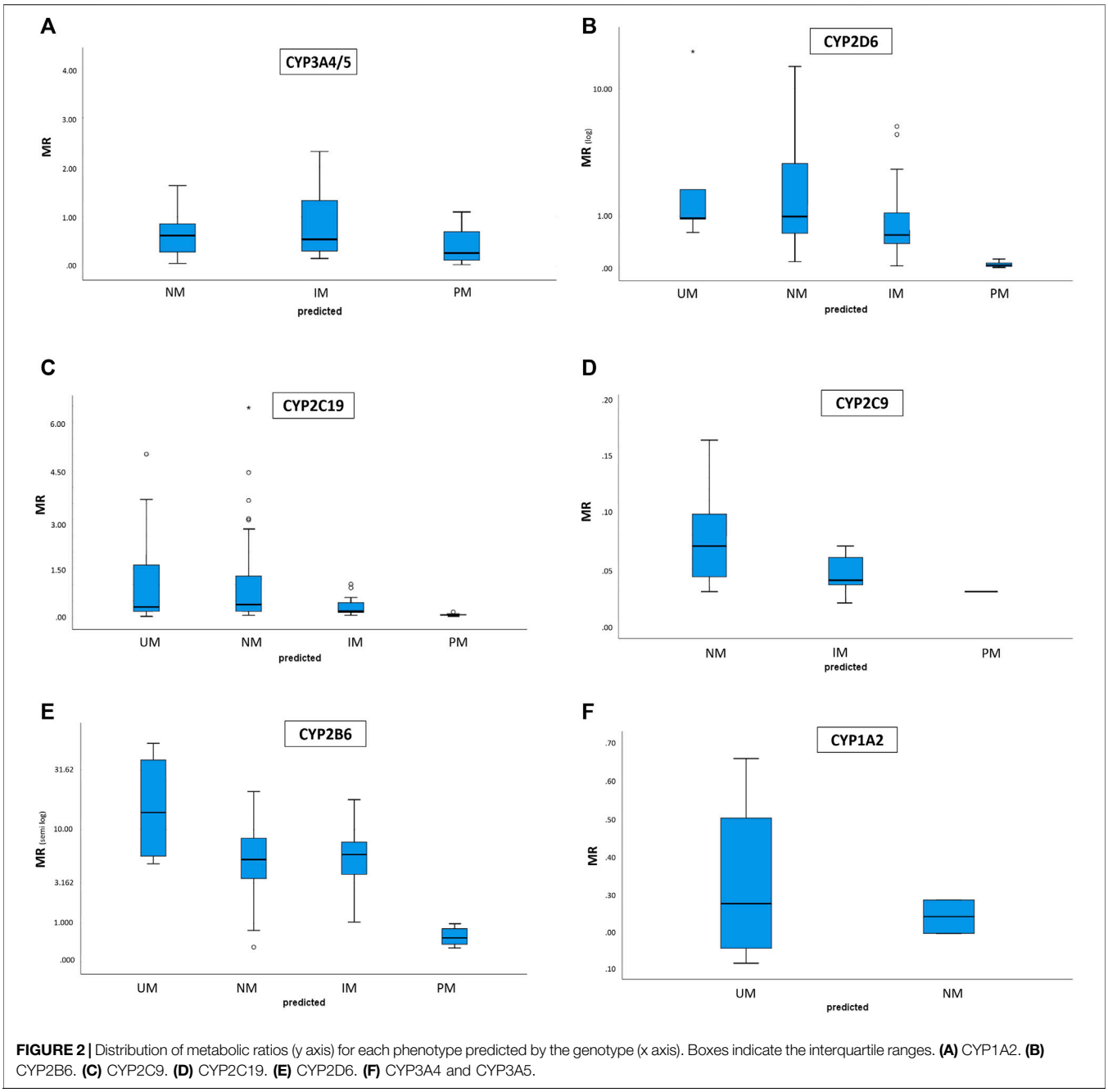

FIGURE 2 | Distribution of metabolic ratios (y axis) for each phenotype predicted by the genotype (x axis). Boxes indicate the interquartile ranges. **(A)** CYP1A2. **(B)** CYP2B6. **(C)** CYP2C9. **(D)** CYP2C19. **(E)** CYP2D6. **(F)** CYP3A4 and CYP3A5.

targeted phenotyping was justified by the clinical context. The predicted phenotypes according to the identified alleles are presented in **Table 2**. **Figure 2** shows the distribution of metabolic ratios for each phenotype predicted by the genotype. The concordance between the predicted and measured phenotypes is presented in **Table 3**. As illustrated in **Figure 2** and **Table 3**, genotyping allowed correct prediction of PM phenotypes for most of the studied CYPs isoenzymes, with a 100% concordance between the predicted and measured phenotype for CYP2C9, CYP2C19, and CYP2D6 PM. On the other hand, concordance rates between the predicted and measured phenotype for IM, NM, and UM, whatever the considered isoenzyme, varied widely with concordance rates ranging from 19 to 100%. CYP2C9 phenotypic IM and NM were correctly predicted by the genotype in approximately 60% of the cases. CYP2C19 and CYP2D6 phenotypic IM were frequently well predicted by the genotype (concordance in 91 and 73% of the cases respectively) whereas the opposite was true for CYP2C19, CYP2D6, and CYP3A4/5 NM, in which a concordance between the predicted and measured phenotype was observed in 30–38% of cases only. Finally, except for CYP2B6, individuals with a UM genotype frequently displayed a discordant phenotype (63% of cases for CYP1A2, and 80% of cases for CYP2C19 and CYP2D6). In

TABLE 3 | Concordance between the predicted and measured and phenotypes, given as n (%).

	—	PM	IM	NM	UM	Phenotypic switch[a]
CYP1A2	Concordant	—	—	2 (100%)	3 (37%)	—
	Non concordant	—	—	0	5 (63%)	2 (40%) (estradiol, paroxetine)
CYP2B6	Concordant	4 (37%)	0	6 (38%)	4 (100%)	—
	Non concordant	2 (33%)	10 (100%)	10 (62%)	0	6 (27%) (isavuconazole, cyclophosphamide)
CYP2C9	Concordant	2 (100%)	10 (59%)	25 (66%)	NA	—
	Non concordant	0	7 (41%)	13 (34%)	NA	1 (5%) (sulfamethoxazole)
CYP2C19	Concordant	7 (100%)	20 (91%)	14 (33%)	5 (19%)	—
	Non concordant	0	2 (9%)	28 (67%)	21 (81%)	24 (47%) (es)omeprazole, fluconazole, voriconazole
CYP2D6	Concordant	11 (100%)	36 (73%)	25 (38%)	1 (20%)	—
	Non concordant	0	13 (27%)	40 (62%)	4 (80%)	18 (32%) [paroxetine, venlafaxine, (es)citalopram, duloxetine, sertraline, quetiapine, risperidone]
CYP3A4/5	Concordant	5 (63%)	24 (48%)	3 (30%)	NA	—
	Non concordant	3 (37%)	26 (52%)	7 (70%)	NA	5 (14%) (azole antifungal)

PM: poor metabolizer; IM: intermediate metabolizer; NM: normal metabolizer; UM: ultra-rapid metabolizer. CYP: cytochrome P450. NA: not applicable
[a]Number of cases with a phenotypic switch explaining the non-concordance (with examples of involved comedications)

TABLE 4 | Distribution of measured phenotypes.

	—	PM	IM	NM	UM
CYP1A2	Number of individuals (%)	23 (10.5%)	0	145 (65.9%)	52 (23.6%)
	Mean metabolic ratio (SD)	0.15 (0.41)	NA	0.34 (0.1)	0.8 (0.29)
CYP2B6	Number of individuals (%)	1 (0.5%)	2 (0.9%)	181 (83.8%)	32 (14.8%)
	Mean metabolic ratio (SD)	0.39 (NA)	0.56 (0)	2.21 (1.1)	9.11 (7.1)
CYP2C9	Number of individuals (%)	26 (12.0%)	14 (6.5%)	161 (74.5%)	15 (6.9%)
	Mean metabolic ratio (SD)	0.021 (0.01)	0.039 (0.001)	0.066 (0.018)	0.14 (0.021)
CYP2C19	Number of individuals (%)	43 (20.3%)	23 (10.8%)	127 (59.9%)	19 (9.0%)
	Mean metabolic ratio (SD)	0.15 (0.07)	0.34 (0.069)	1.12 (0.59)	3.45 (1.3)
CYP2D6	Number of individuals (%)	18 (8.0%)	62 (27.7%)	128 (57.1%)	16 (7.1%)
	Mean metabolic ratio (SD)	0.08 (0.07)	0.49 (0.19)	1.78 (0.97)	8.78 (3.54)
CYP3A4-CYP3A5	Number of individuals (%)	33 (14.5%)	5 (2.2%)	164 (71.9%)	26 (11.4%)
	Mean metabolic ratio (SD)	0.17 (0.08)	0.3 (0.022)	0.74 (0.38)	3.06 (0.86)

PM: poor metabolizer; IM: intermediate metabolizer; NM: normal metabolizer; UM: ultra-rapid metabolizer. CYP: cytochrome P450. NA: not applicable. SD: standard deviation

these cases of discordance, a phenotypic switch due to the concomitant use of a CYP inhibitor explained 14–47% of cases.

As illustrated in **Figure 2**, for CYP1A2, carriers and non-carriers of the CYP1A2*1F allele, which has been associated with increased inducibility (Thorn et al., 2012), had great overlap in their MR. For CYP2B6, patients predicted as NM et IM according to their genotype also displayed great overlap in their MR. This wide distribution of MR associated with overlap between phenotype subgroups was also observed for CYP2C19 genotypic UM and NM, CYP2D6 genotypic UM and NM, and CYP3A genotypic NM and IM.

Phenotype

A total of 235 patients underwent phenotyping only. The majority of patients received the Geneva microcktail (n = 218) while the other patients (n = 17) had targeted phenotyping for the reasons explained previously. The distribution of the phenotypes, according to CYP isoenzymes is presented in **Table 4**. As expected, the majority of patients were categorized as NM, regardless of the isoenzyme examined. The proportion of patients displaying slow CYP metabolism (PM + IM) was low for CYP2B6 (approximately 2%), intermediate for CYP1A2 (10%), CYP2C9 (18%), and CYP3A (17%), and high for CYP2C19 and CYP2D6 (more than 30%). Finally, the proportion of patients categorized as UM was quite high for CYP1A2 (24%) and CYP2B6 (15%), while it was measured around 7–11% for the other isoenzymes (CYP2C9, CYP2C19, CYP2D6, and CYP3A).

Genotype

Sixty-one patients underwent genotyping only, with a total of 115 genes evaluated. The most frequently investigated enzyme was CYP2D6 (n = 33), followed by CYP3A4 and CYP3A5 (n = 23 for both). Other genes were infrequently (CYP2C19 n = 16; CYP2C9 n = 10; CYP2B6 n = 8) or very rarely (CYP1A2 n = 2) investigated.

Association Between Clinical Response and Genotype And/Or Phenotype

The majority of clinical situations were observed with analgesic/anesthetic drugs (n = 187), followed by antidepressants (n = 153), antineoplastics (n = 97), and immunosuppressants (n = 93) (**Figure 3**).

Genotypic and/or phenotypic explorations were mainly performed because of inefficacy/low drug levels (38%) followed by ADR/high drug levels (33%), and preemptively before prescribing (18%). Based on the clinical report, the genotype

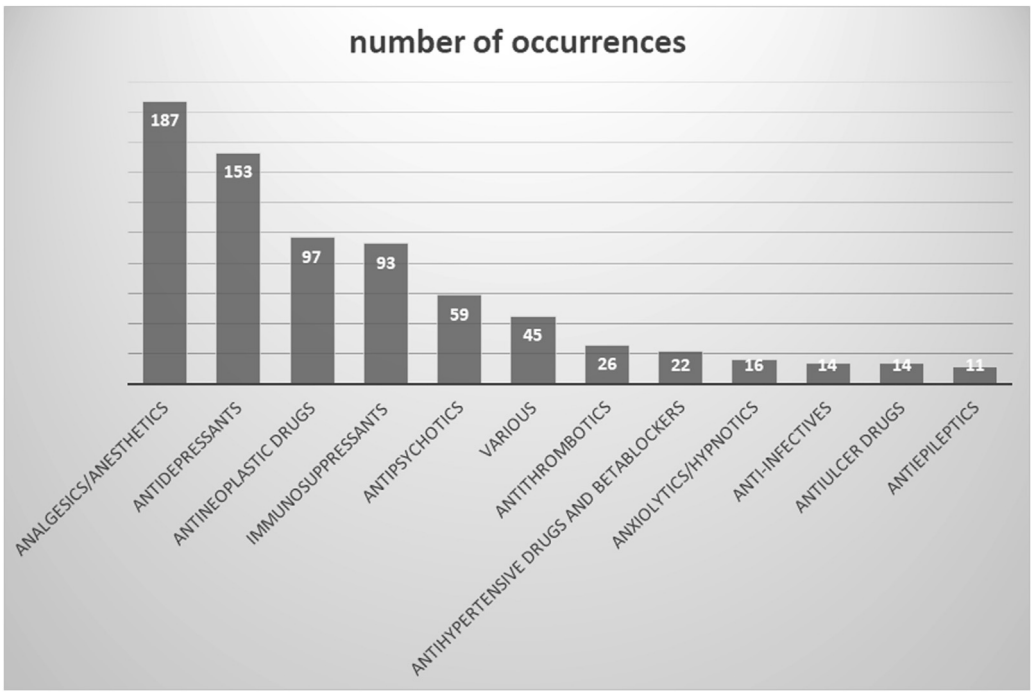

FIGURE 3 | Involved therapeutic classes.

TABLE 5 | Characteristics of patients and reasons for genotyping/phenotyping.

	Number	%
Gender (*n* total = 537)	—	—
Female	281	52.3
Male	256	47.7
Reason for genotyping/phenotyping (*n* total = 546[a])	—	—
inefficacy/low drug levels	208	38.1
ADR/high drug levels	178	32.6
prescription (preemptive testing)	100	18.3
DDI	16	2.9
INR variation	11	2.0
other	33	6.0
Clinical event explained by genotype/phenotype	—	—
Yes	237	44.1
No	161	30.0
Not applicable	139	25.9

[a]*Some patients had two reasons for genotyping/phenotyping*
ADR: adverse drug reaction, DDI: drug-drug interaction, INR: international normalized ratio

and/or phenotype results interestingly explained or at least contributed to almost half of the clinical event (44% of the cases). In 26% of the cases, the association between the genotype/phenotype and a clinical event could not be evaluated (preemptive testing, evaluation of a DDI, INR variation) (**Table 5**). In the 237 cases where the genotype/phenotype could contribute to the clinical event, the differential contribution of the different tests were as followed. In 47% of the patients who underwent genotyping only, the results explained or contributed to the clinical event. This proportion was 34% in patients who underwent phenotyping only, and 53% in patients who underwent simultaneous genotyping and phenotyping. In most of the patients in whom the genotype provided an explanation, the clinical event was ADR/high drug levels and the most frequently involved therapeutic classes were psychotropic and analgesic/anesthetic drugs. In most of the patients in whom the phenotype provided an explanation, the clinical event was of inefficacy/low drug levels, with also psychotropic and analgesic/anesthetic drugs frequently involved. Finally, combined genotype and phenotype mostly explained ADR/high drug levels with various therapeutic classes involved (psychotropic, analgesic, antineoplastic, immunosuppressant, and antithrombotic drugs).

DISCUSSION

Our retrospective study describes for the first time the clinical use of the Geneva CYP450 phenotyping micrococktail in patients suffering from various comorbidities and taking numerous comedications. The new version of the Geneva phenotyping cocktail using low doses of probe substrates was validated in 2014 in healthy volunteers (Bosilkovska et al., 2014) using CYP inhibitors and inducers to generate MR thresholds allowing to characterize the different phenotypes (e.g. PM, NM, UM). We report here concordance between the genotypes and the measured phenotypes as well as associated MR. We showed that the concordance between the predicted and measured

phenotypes was excellent for PM of all CYPs. Genotypic prediction of CYP2C9, CYP2C19, and CYP2D6 IM was satisfying to good, and prediction performance of CYPs NM was moderate. On the other hand, discrepancies were frequent for patients predicted as UM. Discrepancies between the predicted phenotype as based on genotyping and the measured phenotyping were sometimes explained by concurrent medication (phenotypic switch). A previous article from our research group showed that the overall concordance rate between the predicted and measured phenotype was around 50% for CYP2D6 and CYP2C9 using a previous version of the Geneva cocktail (Rollason et al., 2020b). The poor correlation between genotype and phenotype for UM had already been shown previously for CYP2D6 using the AmpliChip CYP450 test for genotyping and dextromethorphan/dextrorphan urine metabolic ratio for phenotyping (Rebsamen et al., 2009). More recently, Dorado et al. also showed that CYP2D6 genotype was not a good predictor of UM phenotype as measured with debrisoquin probe drug. Only 25% of phenotypic UM were explained by their genotype (carrying more than two active CYP2D6 genes) (Dorado et al., 2017). De Andrés et al. evaluated the correlation between CYPs genotype and phenotype using a cocktail approach in several populations (De Andrés et al., 2016; de Andrés et al., 2017; de Andrés et al., 2020). Their different studies showed that the drug-metabolizing capacity predicted from the genotype was frequently not concordant with the actual capacity as measured by phenotyping. For example, in their study in Mexican Amerindian, CYP2C19 genotypic UM displayed a wide range of MR, and several CYP2C19 genotypic NM had higher metabolic activity than UM. Similar to our results, they observed no association between CYP1A2*1F and enhanced CYP1A2 metabolic capacity (de Andrés et al., 2017).

Our results and those from others underscore the complementary roles of genotyping and phenotyping tests when aiming to personalize patient treatment. To minimize the risk of ADRs or therapeutic failure, the individual's drug metabolic capacities should be assessed at the time of initiation of treatment, for drugs metabolized by CYPs. Since such preemptive testing is time-consuming and might be difficult to implement in clinical practice, the simpler approach of genotyping has been suggested. Indeed, since the genome is constant throughout a lifetime, this might in theory be a useful surrogate marker for drug toxicity or inefficacy (Waring, 2020). However, prediction from the genotype can be limited by the lack of accurate results for unknown genotypes/variants (particularly with NGS technologies) and misclassification of phenotype due to errors in genetic predictions. Moreover, measured phenotype can be influenced by environmental factors such as concomitant drugs, dietary habits, or concomitant diseases (McGraw et al., 2018).

The complex interplay between genetics and drug-drug interaction has been recently reviewed by Storelli et al. Through a systematic review of case reports, they identified several mechanisms of complex gene-drug interaction such as enhancement of the magnitude of interaction due to a genetic variant directly impacting the CYP isoform of interest, increased vulnerability to phenoconversion caused by a genetic variant directly affecting the inhibited/induced metabolic pathway, increased exposure of the perpetrator drug due to genetic polymorphisms, and modification of the relative contribution of a minor pathway by a genetic variant affecting the major pathway. For example, a PM of a specific CYP isoform will not be greatly affected by an inhibitor of this isoform. In healthy CYP2D6 PM Caucasian volunteers, no increase in the AUC of metoprolol was observed when coadministered with dronedarone, a moderate CYP2D6 inhibitor (Storelli et al., 2018). In other words, CYP2D6 PMs are not sensitive to phenoconversion or phenotypic switch by CYP2D6 inhibitors. Phenoconversion corresponds to the modification of the activity of a drug-metabolizing enzyme by an inhibitor/inducer that mimics a genetic defect. For example, a CYP2D6 genotypic EM patient undergoing phenoconversion with a CYP2D6 inhibitor would behave pharmacologically as a PM. Other causes of phenoconversion include liver transplant or liver disease (Shah and Smith, 2015) as well as possibly other diseases (inflammation, surgery, etc.).

From a clinical point of view, the genotypes and/or phenotypes contributed to the observed clinical event (i.e. inefficacy, ADR) in 44% of the cases globally. When looking in more detail into the differential contribution of genotyping versus phenotyping results, we observed that simultaneous genotyping and phenotyping allowed explaining the clinical event in a greater proportion of patients then when doing genotyping or phenotyping only. This 44% proportion is quite similar to previously published results from our group focusing on analgesic (Rollason et al., 2020a) and psychotropic drugs (Lloret-Linares et al., 2017), with data collected until the end of 2014. As in the previous analysis, the main therapeutic classes in our study were analgesic/anesthetic drugs and antidepressants. A possible bias in this observation is the other clinical specialty of clinical pharmacologists in our division (experts in pain and psychopharmacology). On the other hand, many drugs from these two therapeutic classes are also subject to metabolism by CYP450, therefore potentially influenced by CYP genetic polymorphisms. This is reflected by numerous genotype-based dosing recommendations found on the PharmaGKB website (https://www.pharmgkb.org/).

When classifying patients in metabolic subgroups according to their genotypes, there were around 8% of CYP2D6 PM, 39% of IM, 49% of NM, and 4% of UM. Geneva is considered to have a population from various ethnicities. Our frequencies of extreme metabolizer (PM and UM) were similar to those reported by del Tredici et al. in their study conducted in more than 100'000 patients in the US from multiple ethnic groups (6% PM and 2% UM), while IM were more prevalent in our study (39 versus 11%) (Del Tredici et al., 2018). As reported by Gaedick et al., the distribution frequency of CYP2D6 shows considerable differences across different world populations (Gaedigk et al., 2017). Regarding CYP2C19, we found 7% of PM, 20% of IM, 45% of NM, and 25% of rapid/ultrarapid metabolizers. Our frequency of PM was higher than what is usually observed in Caucasians from different parts of the world as reported by Fricke-Galindo et al. (2–3%

approximately), but the frequency of UM was comparable (Fricke-Galindo et al., 2016). The frequency of CYP2C19 PM was also much higher in our study than reported by Fricke-Galindo et al. for Europe (20.3 versus 2.2% (Fricke-Galindo et al., 2016).

Our study has some limitations. Bias related to retrospective analysis might have led to missing information, such as follow-up data if the genotyping and/or phenotyping results led to dosage/therapeutic changes. For some isoenzymes such as CYP1A2 and CYP2B6, the limited number of patients undergoing simultaneous genotyping and phenotyping limited definitive conclusions on genotype/phenotype concordance.

CONCLUSION

Our study reported for the first time the clinical use of the Geneva micrococktail in patients as well as genotype/phenotype concordance. We showed that genotyping and/or phenotyping tests were useful in explaining or solving clinical events in almost half of the cases. We also observed that genotype/phenotype concordance was excellent for poor metabolizers but more variable for normal, and ultrarapid metabolizers. Our results highlight the complementary aspects of genotyping and phenotyping tests in helping to individualize drug therapy, and these tests should therefore be offered concomitantly more routinely in the clinic.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Commission of the Canton of Geneva. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

KI, VR, MB, JD, and CS conceived the study concept and design. YD performed the phenotyping analyses. KI and SB acquired and analyzed the data. KI wrote the first draft of the manuscript. All authors participated in refining, and reviewing the manuscript, and approved the final version.

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REVIEW
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A Review of CYP3A Drug-Drug Interaction Studies: Practical Guidelines for Patients Using Targeted Oral Anticancer Drugs

Laura Molenaar-Kuijsten[1]*, *Dorieke E. M. Van Balen*[1], *Jos H. Beijnen*[1,2], *Neeltje Steeghs*[3] and *Alwin D. R. Huitema*[1,4,5]

[1]Department of Pharmacy & Pharmacology, The Netherlands Cancer Institute—Antoni van Leeuwenhoek, Amsterdam, Netherlands, [2]Department of Pharmaceutical Sciences, Utrecht University, Utrecht, Netherlands, [3]Department of Medical Oncology and Clinical Pharmacology, The Netherlands Cancer Institute—Antoni van Leeuwenhoek, Amsterdam, Netherlands, [4]Department of Clinical Pharmacy, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands, [5]Department of Pharmacology, Princess Máxima Center for Pediatric Oncology, Utrecht, Netherlands

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***Correspondence:**
Laura Molenaar-Kuijsten
l.kuijsten@nki.nl

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Many oral anticancer drugs are metabolized by CYP3A. Clinical drug-drug interaction (DDI) studies often only examine the effect of strong CYP3A inhibitors and inducers. The effect of moderate or weak inhibitors or inducers can be examined using physiologically based pharmacokinetic simulations, but data from these simulations are not always available early after approval of a drug. In this review we provide recommendations for clinical practice on how to deal with DDIs of oral anticancer drugs if only data from strong CYP3A inhibitors or inducers is available. These recommendations were based on reviewed data of oral anticancer drugs primarily metabolized by CYP3A and approved for the treatment of solid tumors from January 1st, 2013 to December 31st, 2015. In addition, three drugs that were registered before the new EMA guideline was issued (i.e., everolimus, imatinib, and sunitinib), were reviewed. DDIs are often complex, but if no data is available from moderate CYP3A inhibitors/inducers, a change in exposure of 50% compared with strong inhibitors/inducers can be assumed. No *a priori* dose adaptations are indicated for weak inhibitors/inducers, because their interacting effect is small. In case pharmacologically active metabolites are involved, the metabolic pathway, the ratio of the parent to the metabolites, and the potency of the metabolites should be taken into account.

Keywords: drug interaction, cytochrome P450 enzyme, CYP3A inhibitor, CYP3A inducer, cancer, kinase inhibitor

INTRODUCTION

Oral targeted anticancer drugs are important drugs for the treatment of cancer. Most oral anticancer drugs are metabolized by CYP3A; therefore, patients are at risk for drug-drug interactions (DDI). Because many of these drugs show an exposure-efficacy and an exposure-toxicity relationship, a change in exposure to these drugs can be highly relevant (Verheijen et al., 2017; Groenland et al., 2019). This change in exposure as a consequence of a DDI could result in adverse events if exposure is increased, or treatment failure if exposure is decreased (in case of prodrugs vice versa).

DDI studies are performed before registration of a drug, based on the metabolism of the drug and following the recommendations of the EMA and FDA (Food and Drug Administration. Center for Drug

Evaluation and Research, 2009; Food and Drug Administration. Center for Drug Evaluation and Research, 2020a; European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2014a). These studies use strong CYP3A inhibitors (e.g., itraconazole or ketoconazole) and inducers (e.g., rifampin) since the guidelines of the EMA and FDA advise a worst-case approach. Subsequently, the effects of moderate and weak inhibitors or inducers are extrapolated from these data using physiologically based pharmacokinetic (PBPK) simulations (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2015a; Food and Drug Administration. Center for Drug Evaluation and Research, 2020a). In short, conducting a PBPK simulation consists of three steps: model development, model verification, and model application. First a physiologically based model is built for the substrate and interacting drug (for the latter also the SimCYP library can be used), including for example PK data. Secondly, the models are verified, e.g., by simulating a concentration-time profile and comparing it with the data from clinical studies. Subsequently, the two models are linked and drug-drug interactions can be simulated. Before the effects of moderate and weak inhibitors and inducers can be predicted, first the models should be verified using data from clinical DDI studies with strong inhibitors and inducers. The use of PBPK models is described in several guidelines of the FDA (Food and Drug Administration. Center for Drug Evaluation and Research, 2018; Food and Drug Administration. Center for Drug Evaluation and Research, 2020a; Food and Drug Administration. Center for Drug Evaluation and Research, 2020b). There is, however, a critical problem with the above described DDI studies performed before drug approval. Despite the fact that moderate and weak inhibitors and inducers are far more frequently used than the strong CYP3A inhibitors and inducers, clinical data on moderate and weak inhibitors and inducers is often lacking. This problem is partly overcome by the, increasingly performed, PBPK simulations. But, data from these PBPK simulations are not always available early after approval of a drug. This is for example the case for drugs that are conditionally approved, as is the case for, for instance, larotrectinib, and lorlatinib (Food and Drug Administration, 2017; Food and Drug Administration, 2018; Chen et al., 2020; Patel et al., 2020).

To determine which drugs might influence the metabolism of oral anticancer drugs, the Flockhart Table can be consulted (Flockhart, 2007). The Flockhart Table displays drugs that inhibit or induce specific CYP enzymes, for example CYP3A (Flockhart, 2007). The interacting drugs are placed in groups according to the inhibition or induction capacity, and are classified in broad ranges. Weak inhibitors increase the AUC by ≥1.25–<2-fold, moderate inhibitors by ≥2–<5-fold, and strong inhibitors by ≥5-fold (Flockhart, 2007; Food and Drug Administration. Center for Drug Evaluation and Research, 2020a). Weak inducers decrease the AUC by ≥20–<50%, moderate inducers by ≥50–<80%, and strong inducers by ≥80% (Food and Drug Administration, 2020a).

The aim of this review was to provide recommendations for clinical practice on how to deal with DDIs of oral anticancer drugs if only data from strong CYP3A inhibitors or inducers is available. To achieve this goal, we compared results from DDI studies with strong inhibitors or inducers with results with moderate or weak inhibitors or inducers, to extrapolate results to clinical practice and formulate an advice on how to deal with DDIs for which data is lacking.

METHODS

Oral anticancer drugs, used for the treatment of solid tumors, were selected based on their metabolism and year of approval. On January 1st, 2013, the EMA guideline on the investigation of DDIs came into effect (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2015b). To allow several years of follow-up after approval, in which clinical DDI studies with these drugs might be conducted, an inclusion cut-off in December 2015 was chosen. Therefore, all drugs primarily metabolized by CYP3A and approved for the treatment of solid tumors from January 1st, 2013 to December 31st, 2015 were selected. In addition, we included three drugs that were registered before the new EMA guideline was issued (i.e., everolimus, imatinib, and sunitinib), to illustrate how DDI studies were performed with the prior guideline. An overview of the drug selection is shown in **Figure 1**. Firstly, the US FDA Clinical Pharmacology and Biopharmaceutics Review and the Summary of Product Characteristics of these drugs were studied for data on DDI studies. Second, PubMed was searched using the search terms (drug name) AND [drug-drug interaction (study)] OR (drug name of most used potent inhibitor and inducer). Furthermore, citation snowballing was used to find other articles of interest. The articles, including case reports, in which no AUCs were reported, or in which the dose of the victim drug was different between the control group and group with combination treatment, and *in vitro* studies were excluded. We searched the articles for the change in AUC (preferably the $AUC_{0-\infty}$) of the victim drug in combination with the studied CYP3A inhibitor or inducer, compared with administration of the victim drug alone. We visualized this by making graphs using the ratios of adjusted means of the combination versus the victim drug alone, whereby the victim drug alone was rated as 100% exposure. The studied inhibitors and inducers were grouped according to their interaction potential, which was reported in the reviewed articles and checked with the Flockhart Table (Flockhart, 2007).

RESULTS

Table 1 gives a summary of the DDI studies of the twelve selected oral anticancer drugs. In **Table 2** a detailed overview of the results is shown. The results are described for the drugs without active metabolites first and for the drugs with active metabolites thereafter.

Drugs Without Active Metabolites

Ceritinib

When the strong CYP3A inhibitor ketoconazole was combined with a single-dose of ceritinib, the $AUC_{0-\infty}$ of ceritinib increased by 190% (n = 19) (Food and Drug Administration,

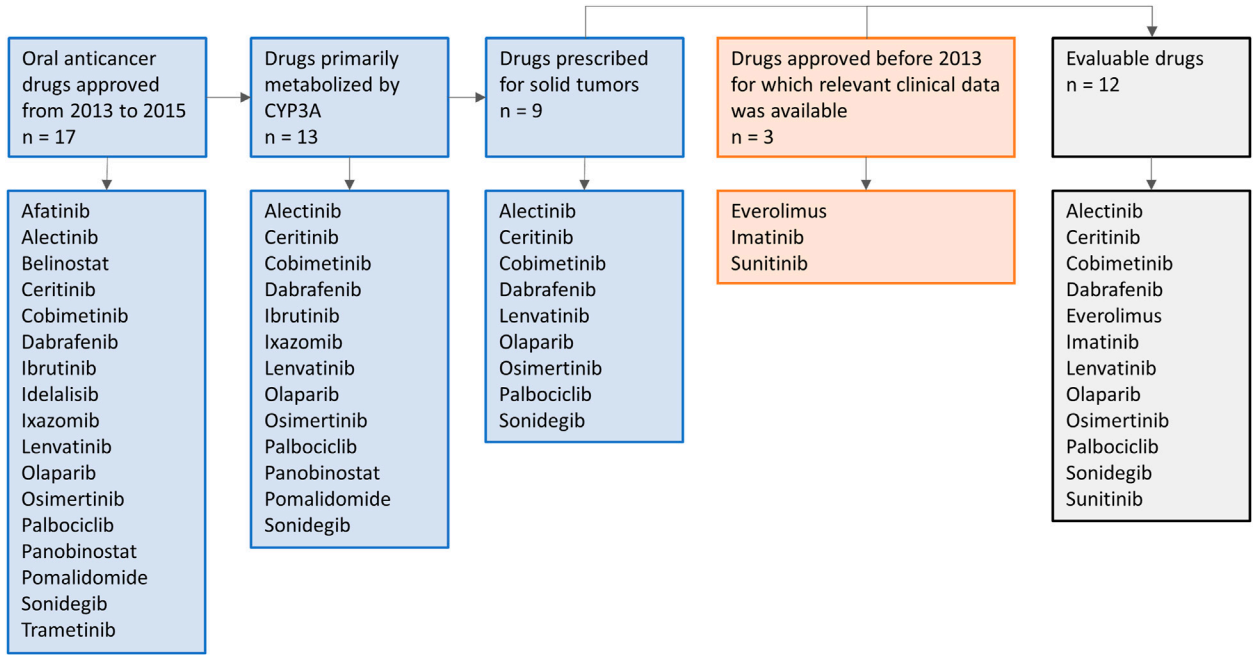

FIGURE 1 | Schematic overview of drug selection.

TABLE 1 | Summary table of the results of DDI studies performed with the reviewed oral oncolytic drugs.

Drug	Effect CYP3A inhibitors[a]			Effect CYP3A inducers[a]		
	Strong	Moderate	Weak	Strong	Moderate	Weak
Alectinib[b]	36% ↑			18.4% ↓		
Ceritinib	118.5% ↑ (51–186)	37% ↑		68.5% ↓ (67–70)	43% ↓	
Cobimetinib	572% ↑	280.5% ↑ (226–335)	3% ↑	83% ↓	72% ↓	13% ↓
Dabrafenib	71% ↑			34% ↓		
• Hydroxy-dabrafenib	82% ↑			30% ↓		
• Desmethyl-dabrafenib	68% ↑			73% ↑		
• Carboxy-dabrafenib	16% ↓					
Everolimus	1,430% ↑	220% ↑ (74–340)		63% ↓		
Imatinib	18.5% ↑ (–3.1–40.1)			73.3% ↓ (72.5–74)		37.1% ↓ (30.2–44)
• N-desmethyl-imatinib	16.75% ↑ (–5–38.5)			10.8% ↓ (9.8–11.7)		4.1% ↑
Lenvatinib	14.5% ↑			6.2% ↑ (–18.2–30.6)		
Olaparib	161% ↑ (152–170)	115% ↑ (98–126)	1.5% ↑ (1–2)	79% ↓ (71–87)	57.3% ↓ (53–60)	0%↓
Osimertinib	24.2% ↑			78.5% ↓	42% ↓	0%↓
Palbociclib	86.8% ↑	40% ↑ (38–42)	0.4% ↑ (0.3–0.4)	85.2% ↓	35% ↓ (32–38)	
Sonidegib	122.8% ↑ (42–253)	98% ↑ (36–179)		76.6% ↓ (66–88)	49% ↓ (29–65)	
Sunitinib[c]	51% ↑	11% ↑		46% ↓		

[a]*Reported as percentage of AUC change, if multiple DDI studies were performed the mean AUC change and range are reported.*
[b]*Sum of alectinib and M4.*
[c]*Sum of sunitinib and SU12662, except for the moderate inhibitor.*

2014a). In a PBPK study the effect of ketoconazole on steady-state exposure of ceritinib was simulated. Steady-state exposure increased by 51% (Food and Drug Administration, 2014a). The difference between the effect of ketoconazole on single-dose and steady-state ceritinib concentrations can be explained by the auto-inhibition of CYP3A4 by ceritinib. Hereby, the fraction of ceritinib metabolized by CYP3A4 will be decreased at steady-state concentrations, thus the effect of a strong inhibitor will be smaller. (Food and Drug Administration, 2014a). The moderate inhibitor fluconazole increased the steady-state exposure of ceritinib by 37% in a PBPK simulation (Food and Drug Administration, 2014a). The strong CYP3A inducer rifampin decreased the AUC $_{0-\infty}$ of single-dose ceritinib by 70% (n = 19) and it was predicted to decrease the AUC on steady-state by 67%. In a simulation study, the moderate inducer efavirenz decreased the AUC of ceritinib by approximately half with 43% (Food and Drug Administration, 2014a).

TABLE 2 | Detailed overview of the results of DDI studies performed with the reviewed oral oncolytic drugs.

Drug (year of market approval)	(Primary) metabolism	Target Verheijen et al. (2017)	Inter-patient variability (%CV)	Dose-linearity	DDI study with (interaction potential)	Change in AUC	Recommendations Summary of product Characteristics	Type of trial	References
Alectinib (2015)	CYP3A	ALK	46%	Dose proportional exposure	Posaconazole (strong CYP3A inhibitor)	$AUC_{0-\infty}$ 75% ↑ (90% CI 57–95) M4 $AUC_{0-\infty}$ 24.9% ↓ (90% CI 12.3–35.6) Sum alectinib and M4 $AUC_{0-\infty}$ 36% ↑ (90% CI 24–49)	Be careful when combining alectinib with strong inhibitors of CYP3A	Clinical trial	Food and Drug Administration (2015a) and Morcos et al. (2017)
					Rifampin (strong CYP3A inducer)	$AUC_{0-\infty}$ 73.2% ↓ (90% CI 69.9–76.2) M4 $AUC_{0-\infty}$ 79% ↑ (90% CI 58–102) Sum alectinib and M4 $AUC_{0-\infty}$ 18.4% ↓ (90% CI 9.9–26)	Be careful when combining alectinib with strong inducers of CYP3A	Clinical trial	Food and Drug Administration (2015a) and Morcos et al. (2017)
Ceritinib (2014)	CYP3A	ALK	74%	Nonlinear PK	Ketoconazole (strong CYP3A inhibitor)	Single dose $AUC_{0-\infty}$ 186% ↑ (90% CI 146–233) Steady–state AUC 51% ↑ (90% CI 43–59)	Avoid coadministration of strong CYP3A inhibitors or reduce the dose of ceritinib to 150 mg QD	Clinical trial PBPK simulation	Food and Drug Administration (2014a)
					Fluconazole (moderate CYP3A inhibitor)	AUC 37% ↑ (90% CI 31–42)		PBPK simulation	Food and Drug Administration (2014a)
					Rifampin (strong CYP3A inducer)	Single dose $AUC_{0-\infty}$ 70% ↓ (90% CI 61–77) Steady-state AUC 67% ↓ (90% CI 64–70)	Avoid coadministration of strong CYP3A inducers	Clinical trial PBPK simulation	Food and Drug Administration (2014a)
					Efavirenz (moderate CYP3A inducer)	AUC 43% ↓ (90% CI 38–48)		PBPK simulation	Food and Drug Administration (2014a)
Cobimetinib (2015)	CYP3A	MEK	61%	Dose proportional exposure	Itraconazole (strong CYP3A inhibitor)	$AUC_{0-\infty}$ 572% ↑ (90% CI 464–702)	Avoid coadministration of strong and moderate CYP3A inhibitors or reduce the dose of cobimetinib to 20 mg QD (short term use)	Clinical trial	Food and Drug Administration (2014b)
					Erythromycin (moderate CYP3A inhibitor)	AUC 335% ↑		PBPK simulation	Food and Drug Administration (2014b) and Budha et al. (2016)
					Diltiazem (moderate CYP3A inhibitor)	AUC 226% ↑		PBPK simulation	Food and Drug Administration (2014b) and Budha et al. (2016)
					Fluvoxamine (weak CYP3A inhibitor)	AUC 3% ↑		PBPK simulation	Food and Drug Administration (2014b) and Budha et al. (2016)
					Rifampin (strong CYP3A inducer)	AUC 83% ↓	Avoid coadministration of strong and moderate CYP3A inducers	PBPK simulation	Food and Drug Administration (2014b) and Budha et al. (2016)
					Efavirenz (moderate CYP3A inducer)	AUC 72% ↓		PBPK simulation	Food and Drug Administration (2014b) and Budha et al. (2016)
					Vemurafenib (weak CYP3A inducer)	AUC_{0-24h} 13% ↓		Clinical trial	Food and Drug Administration (2014b)

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TABLE 2 | (*Continued*) Detailed overview of the results of DDI studies performed with the reviewed oral oncolytic drugs.

Drug (year of market approval)	(Primary) metabolism	Target Verheijen et al. (2017)	Inter-patient variability (%CV)	Dose-linearity	DDI study with (interaction potential)	Change in AUC	Recommendations Summary of product Characteristics	Type of trial	References
Dabrafenib (2013)	CYP2C8/ CYP3A	BRAF	38%	Dose proportional exposure at single dose, but less than dose-proportional after repeat twice daily dosing (likely due to auto-induction)	Ketoconazole (strong CYP3A inhibitor)	AUC_{0-12h} 71% ↑ (90% CI 55–90) Hydroxy-dabrafenib AUC_{0-12h} 82% ↑ (90% CI 61–105) Desmethyl-dabrafenib AUC_{0-12h} 68% ↑ (90% CI 47–93) Carboxy-dabrafenib AUC_{0-12h} 16% ↓ (90% CI 4–27)	Be careful when combining dabrafenib with strong inhibitors of CYP3A	Clinical trial	Food and Drug Administration (2012), Suttle et al. (2015), and European Medicines Agency Committee for Medicinal Products For Human Use (CHMP) (2018)
					Rifampin (strong CYP3A inducer)	AUC 34% ↓ Desmethyl-dabrafenib AUC 30% ↓ Carboxy-dabrafenib AUC 73% ↑	Avoid coadministration of CYP3A inducers	Clinical trial	European Medicines Agency Committee for Medicinal Products For Human Use (CHMP) (2018)
Everolimus (2003)	CYP3A/P-gp	mTOR	36%	Dose proportional exposure	Ketoconazole (strong CYP3A inhibitor)	$AUC_{0-\infty}$ 1,430% ↑ (90% CI 1020–2,150)	Avoid coadministration of strong CYP3A inhibitors. Avoid coadministration of moderate CYP3A inhibitors or reduce the dose of everolimus to 2.5 or 5 mg QD	Clinical trial	Kovarik et al. (2005b) and Food and Drug Administration (2008)
					Erythromycin (moderate CYP3A inhibitor)	$AUC_{0-\infty}$ 340% ↑ (90% CI 250–440)		Clinical trial	(European Medicines Agency Committee for Medicinal Products For Human Use (CHMP); Kovarik et al. (2005c) and Food and Drug Administration (2008)
					Verapamil (moderate CYP3A inhibitor)	$AUC_{0-\infty}$ 250% ↑ (90% CI 210–290)		Clinical trial	(European Medicines Agency Committee for Medicinal Products For Human Use (CHMP); Kovarik et al., (2005a) and Food and Drug Administration (2008)
Everolimus (2003)	CYP3A/P-gp	mTOR	36%	Dose proportional exposure	Imatinib (moderate CYP3A inhibitor)	AUC 270% ↑	Avoid coadministration of strong CYP3A inhibitors. Avoid coadministration of moderate CYP3A inhibitors or reduce the dose of everolimus to 2.5 or 5 mg QD	Clinical trial	(European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2006)
					Cyclosporine (moderate CYP3A inhibitor)	Neoral® $AUC_{0-\infty}$ 168% ↑ (90% CI 122–224) Sandimmune® $AUC_{0-\infty}$ 74% ↑ (90% CI 49–104)		Clinical trial	Kovarik et al. (2002b) and Kovarik et al. 2006)

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TABLE 2 | (*Continued*) Detailed overview of the results of DDI studies performed with the reviewed oral oncolytic drugs.

Drug (year of market approval)	(Primary) metabolism	Target Verheijen et al. (2017)	Inter-patient variability (%CV)	Dose-linearity	DDI study with (interaction potential)	Change in AUC	Recommendations Summary of product Characteristics	Type of trial	References
					Rifampin (strong CYP3A inducer)	AUC 63% ↓ (90% CI 54–70)	Avoid coadministration of strong CYP3A inducers or increase the dose of everolimus to 10 or 20 mg QD	Clinical trial	(European Medicines Agency Committee for Medicinal Products For Human Use (CHMP); Kovarik et al. (2002a) and Food and Drug Administration (2008)
Imatinib (2001)	CYP3A	KIT, PDGFR, Bcr-Abl	40–60%	Dose proportional exposure	Ketoconazole (strong CYP3A inhibitor)	Single dose $AUC_{0-\infty}$ 40.1% ↑ (90% CI 31–49.9) N-desmethylimatinib $AUC_{0-\infty}$ 5% ↓ (90% CI −3–12.5)	Be careful when combining imatinib with inhibitors of CYP3A	Clinical trial	Food and Drug Administration, (2008); European Medicines Agency Committee for Medicinal Products For Human Use (CHMP) (2006)
					Ritonavir (strong CYP3A inhibitor)	Steady-state AUC_{0-24h} 3.1% ↓ (90% CI −12.5–16.5) N-desmethylimatinib AUC_{0-24h} 38.5% ↑ (90% CI 15.9–65.6)		Clinical trial	Van Erp et al. (2007)
					Rifampin (strong CYP3A inducer)	$AUC_{0-\infty}$ 74% ↓ (90% CI 71–76) N-desmethylimatinib $AUC_{0-\infty}$ 11.7% ↓ (90% CI 3.3–19.4)	Avoid coadministration of strong CYP3A inducers	Clinical trial	Bolton et al. (2004) and European Medicines Agency Committee for Medicinal Products For Human Use (CHMP) (2006)
Imatinib (2001)	CYP3A	KIT, PDGFR, Bcr-Abl	40–60%	Dose proportional exposure	Enzyme-inducing antiepileptic drugs (EIAEDs; e.g., carbamazepine, oxcarbazepine and phenytoin) (mixed potency; carbamazepine and phenytoin are potent inducers; oxcarbazepine is a weak inducer)	$AUC_{0-\infty}$ 72.5% ↓ N-desmethylimatinib $AUC_{0-\infty}$ 9.8% ↓	Avoid coadministration of strong CYP3A inducers	Clinical trial	Wen et al. (2006)
					St John's Wort (weak CYP3A inducer)	Study 1 $AUC_{0-\infty}$ 30.2% ↓ (90% CI 25–34.9) N-desmethylimatinib AUC_{0-72h} 4.1% ↑ (90% CI −8.4–18.3) Study 2 $AUC_{0-\infty}$ 44% ↓ (90% CI 30–54)		Clinical trial	Frye et al. (2004) Smith et al. (2004)

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TABLE 2 | (*Continued*) Detailed overview of the results of DDI studies performed with the reviewed oral oncolytic drugs.

Drug (year of market approval)	(Primary) metabolism	Target Verheijen et al. (2017)	Inter-patient variability (%CV)	Dose-linearity	DDI study with (interaction potential)	Change in AUC	Recommendations Summary of product Characteristics	Type of trial	References
Lenvatinib (2015)	CYP3A	VEGFR	36–78%	Dose proportional exposure	Itraconazole (strong CYP3A inhibitor)	$AUC_{0-\infty}$ 14.5% ↑ (90% CI 8.5–20.9)	None	Clinical trial	Food and Drug Administration (2015b) and Shumaker et al. (2015)
					Rifampin (strong CYP3A inducer)	Single dose $AUC_{0-\infty}$ 30.6% ↑ (90% CI 22.7–39) Multiple doses $AUC_{0-\infty}$ 18.2% ↓ (90% CI 8.7–26.7)		Clinical trial	Shumaker et al. (2014) and Food and Drug Administration (2015b)
Olaparib (2014)	CYP3A	PARP	38%	Dose-proportionality cannot be concluded based on available PK data	Itraconazole (strong CYP3A inhibitor)	Study 1; tablet $AUC_{0-\infty}$ 170% ↑ (90% CI 144–197) Study 2; capsule AUC 152% ↑ (95% CI 139–167)	Reduce dose of olaparib to 150 mg BID when combined with strong CYP3A inhibitors and reduce dose to 200 mg BID when combined with moderate CYP3A inhibitors (tablets)	Clinical trial PBPK simulation	Food and Drug Administration (2014c) and Dirix et al. (2016) Pilla Reddy et al. (2019)
					Fluconazole (moderate CYP3A inhibitor)	Study 1; tablet AUC 126% ↑ (95% CI 115–130) Study 2; tablet AUC 121% ↑ (95% CI 114–128) Study 2; capsule AUC 98% ↑ (95% CI 92–105)		PBPK simulation PBPK simulation	Food and Drug Administration (2014c) Pilla Reddy et al. (2019)
Olaparib (2014)	CYP3A	PARP	38%	Dose-proportionality cannot be concluded based on available PK data	Fluvoxamine (weak CYP3A inhibitor)	Tablet AUC 2% ↑ (95% CI 1–2) Capsule AUC 1% ↑ (95% CI 1–2)	Reduce dose of olaparib to 150 mg BID when combined with strong CYP3A inhibitors and reduce dose to 200 mg BID when combined with moderate CYP3A inhibitors (tablets)	PBPK simulation	Pilla Reddy et al. (2019)
					Rifampin (strong CYP3A inducer)	Study 1; tablet $AUC_{0-\infty}$ 87% ↓ (90% CI 84–89)	Avoid coadministration of strong and moderate CYP3A inducers	Clinical trial	Food and Drug Administration, (2014c); Dirix et al. (2016)
						Study 2; capsule AUC 71% ↓ (95% CI 69–73)		PBPK simulation	Pilla Reddy et al. (2019)
					Efavirenz (moderate CYP3A inducer)	Study 1; tablet AUC 59% ↓ (95% CI 58–62)		PBPK simulation	Food and Drug Administration, (2014c)
						Study 2; tablet AUC 60% ↓ (95% CI 57–62)		PBPK simulation	Pilla Reddy et al. (2019)
						Study 2; capsule AUC 53% ↓ (95% CI 50–56)			
					Dexamethasone (weak CYP3A inducer)	Tablet AUC 0 (95% CI −1–0) Capsule AUC 0 (95% CI −1–0)		PBPK simulation	Pilla Reddy et al. (2019)

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TABLE 2 | (*Continued*) Detailed overview of the results of DDI studies performed with the reviewed oral oncolytic drugs.

Drug (year of market approval)	(Primary) metabolism	Target Verheijen et al. (2017)	Inter-patient variability (%CV)	Dose-linearity	DDI study with (interaction potential)	Change in AUC	Recommendations Summary of product Characteristics	Type of trial	References
Osimertinib (2015)	CYP3A	EGFR	37%	Dose proportional exposure	Itraconazole (strong CYP3A inhibitor)	$AUC_{0-\infty}$ 24.2% ↑ (90% CI 14.6–34.5) AZ5104 $AUC_{0-\infty}$ 8.3% ↑ (90% CI −5.6–24.2) AZ7550 AUC 51% ↓ (90% CI 45–56.3)	None	Clinical trial	European Medicines Agency Committee for Medicinal Products For Human Use (CHMP) (2016a) and Vishwanathan et al. (2018)
					Rifampin (strong CYP3A inducer)	AUC_{0-24h} 78.5% ↓ (90% CI 76.2–80.5) AZ5104 AUC_{0-24h} 81.2% ↓ (90% CI 78.8–83.4) AZ7550 AUC_{0-24h} 29.8% ↑ (19.1–41.4)	Avoid coadministration of strong and moderate CYP3A inducers	Clinical trial	European Medicines Agency Committee for Medicinal Products For Human Use (CHMP) (2016a) and Vishwanathan et al. (2018)
					Efavirenz (moderate CYP3A inducer)	AUC 42% ↓ (95% CI 40–44)		PBPK simulation	Reddy et al. (2018)
					Dexamethasone (weak CYP3A inducer)	AUC 0.001% ↓ (95% CI 0.001–0.001)		PBPK simulation	Reddy et al. (2018)
Palbociclib (2015)	CYP3A	CDK4/6	29%	Dose proportional exposure	Itraconazole (strong CYP3A inhibitor)	$AUC_{0-\infty}$ 86.8% ↑ (90% CI 72.9–101.9)	Avoid coadministration of strong CYP3A inhibitors or reduce dose of palbociclib to 75 mg QD	Clinical trial	Food and Drug Administration (2014d) and European Medicines Agency Committee for Medicinal Products For Human Use (CHMP) (2016b)
					Diltiazem (moderate CYP3A inhibitor)	AUC_{0-216h} 42% ↑		PBPK simulation	Yu et al. (2017)
					Verapamil (moderate CYP3A inhibitor)	AUC_{0-216h} 38% ↑		PBPK simulation	Yu et al. (2017)
					Fluvoxamine (weak inhibitor)	AUC_{0-216h} 0.4% ↑		PBPK simulation	Yu et al. (2017)
					Fluoxetine (weak CYP3A inhibitor)	AUC_{0-216h} 0.3% ↑		PBPK simulation	Yu et al. (2017)
					Rifampin (strong CYP3A inducer)	$AUC_{0-\infty}$ 85.2% ↓ (90% CI 81.4–88.2)	Avoid coadministration of strong CYP3A inducers	Clinical trial	Food and Drug Administration (2014d)
					Efavirenz (moderate CYP3A inducer)	AUC_{0-168h} 38% ↓		PBPK simulation	Yu et al. (2017)
Palbociclib (2015)	CYP3A	CDK4/6	29%	Dose proportional exposure	Modafinil (moderate CYP3A inducer)	$AUC_{0-\infty}$ 32% ↓	Avoid coadministration of strong CYP3A inducers	Clinical trial	European Medicines Agency Committee for Medicinal Products For Human Use (CHMP) (2016b)

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TABLE 2 | (*Continued*) Detailed overview of the results of DDI studies performed with the reviewed oral oncolytic drugs.

Drug (year of market approval)	(Primary) metabolism	Target Verheijen et al. (2017)	Inter-patient variability (%CV)	Dose-linearity	DDI study with (interaction potential)	Change in AUC	Recommendations Summary of product Characteristics	Type of trial	References
Sonidegib (2015)	CYP3A	Smoothened	CL/F 67% V/F 213%	Dose proportional exposure with doses up to 400 mg, with higher dose less than proportional (due to dose-dependent absorption)	Ketoconazole (strong CYP3A inhibitor)	Study 1; healthy subjects AUC_{0-240h} 125% ↑ (90% CI 78–186)	Reduce dose of sonidegib to 200 mg every other day when combined with strong CYP3A inhibitors	Clinical trial	European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), (2015c), and Food and Drug Administration (2015d)
						Study 2; cancer patients, sonidegib 1 day, ketoconazole 14 days AUC_{0-24h} 42% ↑ (90% CI 39–45)		PBPK simulation	Food and Drug Administration (2015d) and Einolf et al. (2017)
						Study 2; sonidegib 120 days, ketoconazole 120 days AUC_{0-24h} 253% ↑ (90% CI 231–276)			
						Study 2; sonidegib 133 days, ketoconazole 14 days AUC_{0-24h} 101% ↑ (90% CI 92–111)			
						Study 2; sonidegib QOD 133 days, ketoconazole 14 days AUC_{0-24h} 93% ↑ (90% CI 84–102)			
Sonidegib (2015)	CYP3A	Smoothened	CL/F 67% V/F 213%	Dose proportional exposure with doses up to 400 mg, with higher dose less than proportional (due to dose-dependent absorption)	Erythromycin (moderate CYP3A inhibitor)	Sonidegib 1 day, erythromycin 14 days AUC_{0-24h} 36% ↑ (90% CI 33–39)	Reduce dose of sonidegib to 200 mg every other day when combined with strong CYP3A inhibitors	PBPK simulation	Food and Drug Administration (2015d) and Einolf et al. (2017)
						Sonidegib 120 days, erythromycin 120 days AUC_{0-24h} 179% ↑ (90% CI 76–361)			
						Sonidegib 133 days, erythromycin 14 days AUC_{0-24h} 79% ↑ (90% CI 71–86)			
					Rifampin (strong CYP3A inducer)	Study 1; healthy subjects AUC_{0-240h} 72.4% ↓ (90% CI 65.1–78.1)	Avoid coadministration of strong CYP3A inducers, but if necessary, consider to increase the dose to 400–800 mg	Clinical trial	European Medicines Agency Committee for Medicinal Products For Human Use (CHMP) (2015c) and Food and Drug Administration (2015d)

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TABLE 2 | (*Continued*) Detailed overview of the results of DDI studies performed with the reviewed oral oncolytic drugs.

Drug (year of market approval)	(Primary) metabolism	Target Verheijen et al. (2017)	Inter-patient variability (%CV)	Dose-linearity	DDI study with (interaction potential)	Change in AUC	Recommendations Summary of product Characteristics	Type of trial	References
						Study 2; cancer patients, sonidegib 1 day, rifampin 14 days AUC_{0-24h} 66% ↓ (90% CI 63–68) Study 2; sonidegib 120 days, rifampin 120 days AUC_{0-24h} 88% ↓ (90% CI 87–89) Study 2; sonidegib 133 days, rifampin 14 days AUC_{0-24h} 80% ↓ (90% CI 78–82)		PBPK simulation	Food and Drug Administration (2015d) and Einolf et al. (2017)
Sonidegib (2015)	CYP3A	Smoothened	CL/F 67% V/F 213%	Dose proportional exposure with doses up to 400 mg, with higher dose less than proportional (due to dose-dependent absorption)	Efavirenz (moderate CYP3A inducer)	Sonidegib 1 day, efavirenz 14 days AUC_{0-24h} 29% ↓ (90% CI 26–31) Sonidegib 120 days, efavirenz 120 days AUC_{0-24h} 65% ↓ (90% CI 62–67) Sonidegib 133 days, efavirenz 14 days AUC_{0-24h} 53% ↓ (90% CI 50–56)	Avoid coadministration of strong CYP3A inducers, but if necessary, consider to increase the dose to 400–800 mg	PBPK simulation	Food and Drug Administration (2015d) and Einolf et al. (2017)
Sunitinib (2006)	CYP3A	VEGFR	40%	Dose proportional exposure	Ketoconazole (strong CYP3A inhibitor) Grapefruit juice (moderate CYP3A inhibitor)	Sum sunitinib and SU12662 $AUC_{0-\infty}$ 51% ↑ AUC_{0-24h} 11% ↑	Reduce dose of sunitinib to 37,5 mg QD in GIST and MRCC patients and to 25 mg QD in pancreatic/NET patients when combined with strong CYP3A inhibitors	Clinical trial Clinical trial	Food and Drug Administration (2005) Van Erp et al. (2011)
					Rifampin (strong CYP3A inducer)	Sum sunitinib and SU12662 $AUC_{0-\infty}$ 46% ↓	Increase the dose of sunitinib in steps of 12.5 mg with a maximum of 87.5 mg QD when combined with CYP3A inducers	Clinical trial	Food and Drug Administration (2005)

Cobimetinib

Figure 2 shows the results of the DDI studies conducted with cobimetinib. It can be seen that CYP3A based DDIs have a large influence on the exposure to cobimetinib. The strong inhibitor itraconazole increased the $AUC_{0-\infty}$ of cobimetinib by almost 600% (n = 15) (Food and Drug Administration, 2014b). The moderate CYP3A inhibitors erythromycin and diltiazem increased the AUC by around 300% in a PBPK simulation, which is half the effect of strong inhibitors, while weak inhibitors had no effect (Food and Drug Administration, 2014b; Budha et al., 2016). The effect of rifampin on the exposure of cobimetinib was studied in a PBPK simulation study instead of a clinical trial, which is in contrast with most DDI studies performed with rifampin. In this simulation the AUC of cobimetinib decreased by 83% when combined with rifampin (Food and Drug Administration, 2014b). Furthermore, the effect of the moderate CYP3A inducer efavirenz was studied in a PBPK simulation and a decrease in AUC of 72% was predicted (Food and Drug Administration, 2014b; Budha et al., 2016). The weak inducer vemurafenib showed a decrease in AUC_{0-24h} of only 13% in a clinical trial (n = unknown) (Food and Drug Administration, 2014b).

Everolimus

The strong inhibitor ketoconazole increased the $AUC_{0-\infty}$ of everolimus by 1,430% (n = 12) (Kovarik et al., 2005b; Food and Drug Administration, 2008). Therefore, it is not recommended to coadminister strong CYP3A4 inhibitors with everolimus (Food and Drug Administration, 2008). The effect size of moderate inhibitors was around 25% compared with ketoconazole (increase in exposure of 340% for erythromycin (n = 16), 250% for verapamil (n = 16), 270% for imatinib (n = unknown), and 121% as average for two different cyclosporin formulations (n = 12) (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP); Kovarik et al., 2002a; Kovarik et al., 2002b; Kovarik et al., 2005a; Kovarik et al., 2005c; Kovarik et al., 2006). Rifampin decreased the AUC of everolimus by 63% (n = 12) (Kovarik et al., 2002a). The effect of the moderate inhibitors was small compared with the strong inhibitor ketoconazole. An explanation for this finding is that ketoconazole also inhibits P-glycoprotein (P-gp), which influences the pharmacokinetics (PK) of everolimus in addition to CYP3A (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2014; Ravaud et al., 2014).

Lenvatinib

Lenvatinib is for more than 80% metabolized by CYP3A to different metabolites *in vitro*. Furthermore, *in vitro* data suggests that lenvatinib is a substrate for P-gp. But *in vivo*, oxidation by aldehyde oxidase and glutathione conjugation play an important role in the metabolism of lenvatinib, next to the metabolism *via* CYP3A (Food and Drug Administration, 2015b). Since the potency of lenvatinib is at least 20 times higher than of the metabolites, the metabolites were considered inactive (Shumaker et al., 2014; Food and Drug Administration, 2015b). The strong CYP3A inhibitor ketoconazole increased the $AUC_{0-\infty}$ of lenvatinib by 15% (n = 18) (Food and Drug Administration, 2015b; Shumaker et al., 2015). The strong CYP3A inducer rifampin decreased the $AUC_{0-\infty}$ of lenvatinib by 18% when multiple doses were given (n = 15) (Shumaker et al., 2014; Food and Drug Administration, 2015b). In contrast, a single dose of rifampin increased the $AUC_{0-\infty}$ of lenvatinib by 31%. Shumaker et al. explained this by a presystemic inhibition of P-gp, which is consistent with the study of Rietman et al. who described that rifampin can inhibit the efflux of drugs into the intestinal lumen (Reitman et al., 2011; Shumaker et al., 2014). The marginal effects of ketoconazole and rifampin on the lenvatinib AUC suggest that the role of CYP3A in the metabolism of lenvatinib is small. In addition, the effects of ketoconazole and rifampin on the AUC of lenvatinib could also be caused by inhibition and induction of P-gp, because both ketoconazole and rifampin have an effect on P-gp (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2014b; Food and Drug Administration, 2015b).

Olaparib

Clinical DDI studies investigated the influence of itraconazole and rifampin on the AUC of olaparib administered as tablets (Food and Drug Administration, 2014c; Dirix et al., 2016). In PBPK simulations, the effect of inhibitors and inducers on the AUC of olaparib formulated as capsules was simulated. The effect on olaparib tablets and capsules were predicted to be similar (Pilla Reddy et al., 2019).

The strong CYP3A inhibitor itraconazole increased the $AUC_{0-\infty}$ of olaparib by 170% (n = 59) (Food and Drug Administration, 2014c; Dirix et al., 2016). The moderate inhibitor fluconazole increased the AUC of olaparib with an average of 115% in three PBPK simulations (Food and Drug Administration, 2014c; Pilla Reddy et al., 2019). Furthermore, the weak inhibitor fluvoxamine, was simulated to have no effect on the AUC of olaparib (Pilla Reddy et al., 2019). Rifampin, a strong CYP3A inducer, decreased the olaparib $AUC_{0-\infty}$ by 87% (n = 22) (Food and Drug Administration, 2014c). The moderate inducer efavirenz decreased the AUC of olaparib by approximately 75%, compared with rifampin, with a decrease of 60% in a PBPK simulation (Pilla Reddy et al., 2019). The weak inducer dexamethasone, was simulated to have no effect on the AUC of olaparib (Pilla Reddy et al., 2019).

Palbociclib

Figure 3 shows the results of the DDI studies performed with palbociclib. The strong inhibitor itraconazole increased the palbociclib $AUC_{0-\infty}$ by 87% (n = 12) (Food and Drug Administration, 2014d; European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2015b). The moderate CYP3A inhibitors diltiazem and verapamil were simulated to increase the AUC_{0-216h} of palbociclib by half compared with itraconazole, with an increase of 40% (Food and Drug Administration, 2014d; Yu et al., 2017). No effect of the weak inhibitors fluvoxamine and fluoxetine on the AUC_{0-216h} of palbociclib was predicted in a simulation study (Yu et al., 2017). Moderate CYP3A inducers decreased the palbociclib AUC by approximately half compared with strong CYP3A inducers. The strong inducer rifampin decreased the $AUC_{0-\infty}$ of palbociclib by

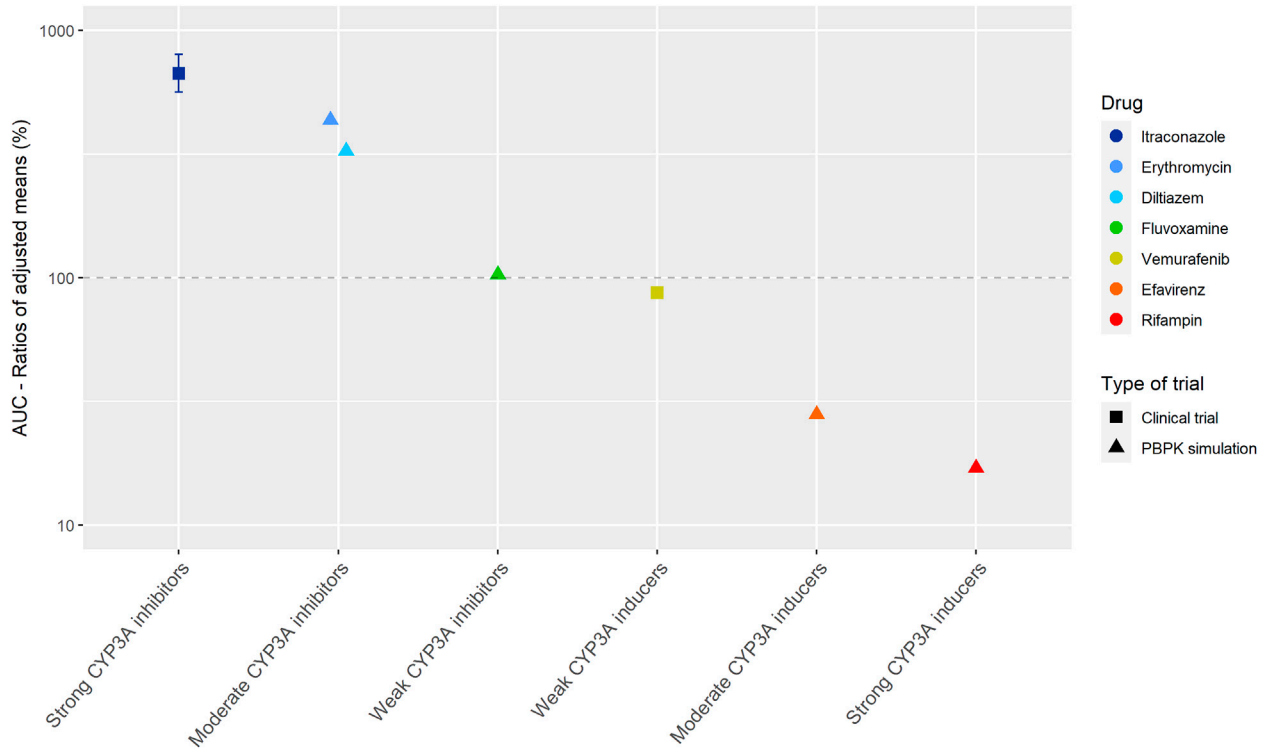

FIGURE 2 | Overview of the results from DDI studies of cobimetinib combined with CYP3A inhibitors and inducers. The coloured symbols represent the increase or decrease in AUC caused by the interacting drug, expressed as adjusted mean ±90% confidence interval (if available). The dashed line represents the baseline AUC (Food and Drug Administration, 2014b).

85% (n = 14) (Food and Drug Administration, 2014d). The moderate inducer efavirenz decreased the AUC_{0-168h} by 38% in a simulation study and modafinil decreased the $AUC_{0-\infty}$ by 32% in a clinical trial (n = 14) (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2016b; Yu et al., 2017).

Sonidegib

In a clinical trial with healthy subjects, the strong CYP3A inhibitor ketoconazole increased the AUC_{0-240h} of sonidegib 800 mg by 125% (same was simulated for sonidegib 200 mg) (parallel study; n = 16 in control group and n = 15 in combination group) (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2015c; Food and Drug Administration, 2015d; Einolf et al., 2017). Ketoconazole was simulated to increase the AUC_{0-24h} of sonidegib given as a single dose by 42% in cancer patients (Food and Drug Administration, 2015d; Einolf et al., 2017). The smaller effect of ketoconazole in cancer patients, can be explained by a decreased hepatic clearance with an elimination half-life of 28 days in cancer patients, and 10 days in healthy subjects (Food and Drug Administration, 2015d). After long-term exposure to sonidegib, ketoconazole was simulated to increase the AUC_{0-24h} by 101–253%, dependent on the duration of combined use (Food and Drug Administration, 2015d; Einolf et al., 2017).

The moderate CYP3A inhibitor erythromycin increased the AUC_{0-24h} of sonidegib given as a single dose by 36% (Food and Drug Administration, 2015d; Einolf et al., 2017). The AUC_{0-24h} of sonidegib given long-term was increased by 79–179%, dependent on the duration of combined use with erythromycin (Food and Drug Administration, 2015d; Einolf et al., 2017). Compared with the simulations for ketoconazole, according to the same treatment schedule, the increases in sonidegib AUC were more than half.

In a clinical trial with healthy subjects, the strong CYP3A inducer rifampin decreased the AUC_{0-240h} of sonidegib 800 mg by 72.4% (same was simulated for sonidegib 200 mg) (parallel study; n = 16 in control group and n = 16 in combination group) (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2015c; Food and Drug Administration, 2015d; Einolf et al., 2017). Rifampin was simulated to decrease the AUC_{0-24h} of sonidegib given as a single dose by 66% in cancer patients (Food and Drug Administration, 2015d; Einolf et al., 2017). The smaller decrease in cancer patients can be explained by a decreased hepatic clearance. The AUC_{0-24h} of sonidegib was decreased by 80–88% when sonidegib given long-term and rifampin were combined, dependent on the duration of combined use (Food and Drug Administration, 2015d; Einolf et al., 2017).

The moderate CYP3A inducer efavirenz was simulated to decrease the AUC_{0-24h} of sonidegib given as a single dose by

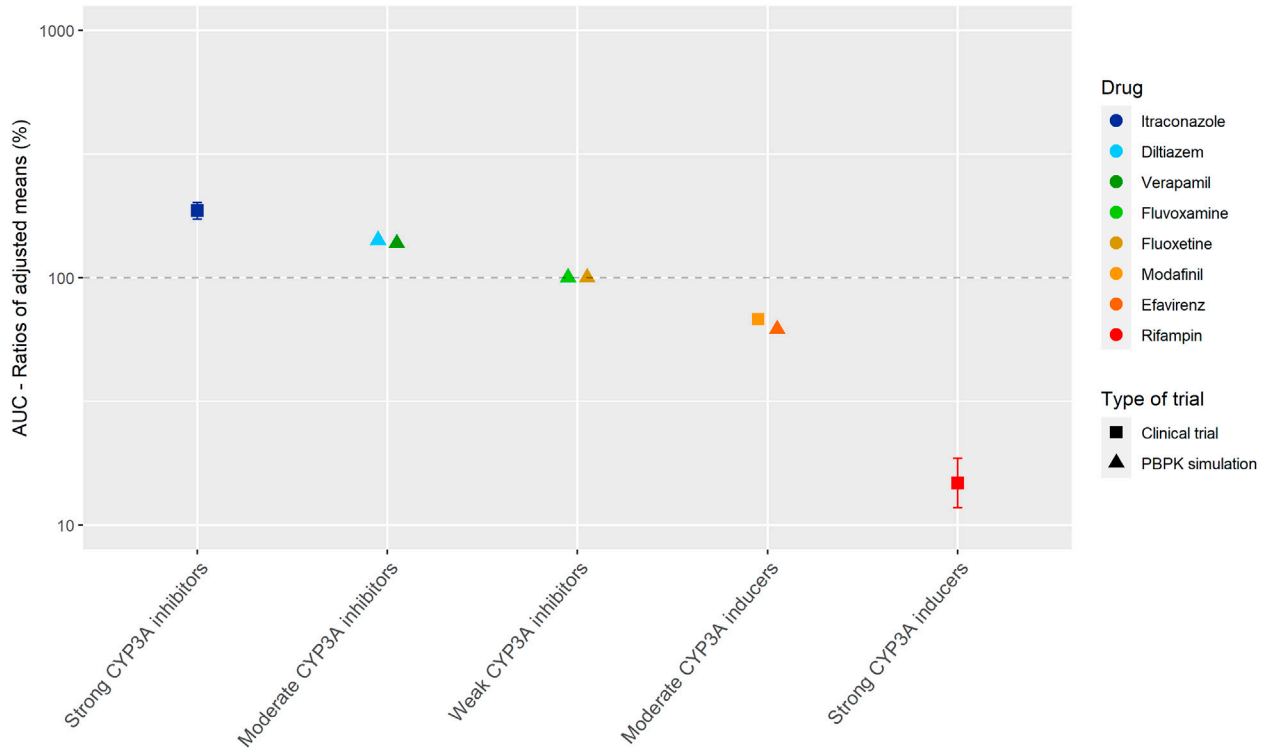

FIGURE 3 | Overview of the results from DDI studies of palbociclib combined with CYP3A inhibitors and inducers. The coloured symbols represent the increase or decrease in AUC caused by the interacting drug, expressed as adjusted mean ±90% confidence interval (if available). The dashed line represents the baseline AUC (Food and Drug Administration, 2014d; European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2016b; Yu et al., 2017).

29% (Food and Drug Administration, 2015d; Einolf et al., 2017). Efavirenz decreased the AUC_{0-24h} of sonidegib given long-term by 53–65%, dependent on the duration of combined use (Food and Drug Administration, 2015d; Einolf et al., 2017). Compared with the simulations of rifampin, according to the same treatment schedule, a decrease of approximately 70% was seen in sonidegib steady-state AUC.

To summarize, the interacting effect on sonidegib is influenced by the patient population and duration of therapy with sonidegib and the interacting agent.

Drugs With Active Metabolites

Alectinib

Alectinib is mainly metabolized by CYP3A to the active metabolite M4. Alectinib and M4 show a similar potency and plasma protein binding *in vitro* (Fowler et al., 2017; Morcos et al., 2017). Therefore, the sum of alectinib and M4 concentration was reported as the pharmacologically active exposure in the DDI studies with posaconazole and rifampin (Morcos et al., 2017).

Figure 4 shows the results of the DDI studies performed with alectinib. The strong inhibitor posaconazole increased the exposure to the sum of alectinib and M4 by 36% (n = 17) (Food and Drug Administration, 2015a; Morcos et al., 2017). The strong inducer rifampin decreased the sum of exposure by 18% (n = 24) (Food and Drug Administration, 2015a; Morcos et al., 2017). Based on the small effects of posaconazole and rifampin, the effects of other CYP3A inhibitors and inducers on the exposure of alectinib and M4 were considered clinically irrelevant.

Dabrafenib

Dabrafenib is partially metabolized to active metabolites. It is firstly oxidized by CYP enzymes to hydroxy-dabrafenib, which is further oxidized to carboxy-dabrafenib. Carboxy-dabrafenib is converted to desmethyl-dabrafenib *via* a non-enzymatic process or excreted in urine or bile. Subsequently, desmethyl-dabrafenib is oxidized to other metabolites (Bershas et al., 2013). Dabrafenib auto-induces its metabolism *via* CYP3A4 (Food and Drug Administration, 2012). Hydroxy-dabrafenib and desmethyl-dabrafenib show a similar potency and may contribute to the clinical activity of dabrafenib, on the other hand carboxy-dabrafenib does not relevantly contribute to the activity (Suttle et al., 2015).

The strong inhibitor ketoconazole increased the AUC_{0-12h} of dabrafenib and the metabolites hydroxy-dabrafenib and desmethyl-dabrafenib by 71, 82, and 68%, respectively, while the AUC_{0-12h} of carboxy-dabrafenib decreased by 16% (n = 16) (Suttle et al., 2015; European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2018). In the DDI study with the strong inducer rifampin the opposite was seen, the AUCs of dabrafenib and desmethyl-dabrafenib decreased by 34

and 30%, respectively, and the AUC of the inactive carboxy-dabrafenib increased by 73% (n = 23) (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2018). These results for both parent and metabolites when combined with a strong inhibitor versus a strong inducer were as expected because the conversion of dabrafenib, hydroxy-dabrafenib, and desmethyl-dabrafenib is mediated by CYP enzymes and thus influenced by inhibitors and inducers of CYP3A. On the contrary, the non-enzymatic conversion of carboxy-dabrafenib is not affected by CYP3A inhibitors and inducers (Bershas et al., 2013). The comparable or even higher increase in AUC for hydroxy-dabrafenib and desmethyl-dabrafenib compared to the parent, indicates higher involvement of CYP3A in elimination of the metabolites compared to their production (Suttle et al., 2015; European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2018).

Imatinib

Imatinib is mainly metabolized by CYP3A. Other CYP enzymes play a minor role. Auto-inhibition of CYP3A by imatinib was shown *in vitro*, but no *in vivo* data is available (Food and Drug Administration, 2001). The main metabolite is N-desmethylimatinib also known as CGP 74588. N-desmethylimatinib is as potent as the parent compound *in vitro*. The exposure to N-desmethylimatinib is approximately 10% compared to the exposure to imatinib, therefore the effect of the metabolite is considered clinically irrelevant (Peng et al., 2005; Whirl-Carrillo et al., 2012).

Ketoconazole in combination with a single dose of imatinib increased the imatinib $AUC_{0-\infty}$ by 40% (n = 14) (Food and Drug Administration, 2001; European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2006). Ritonavir combined with imatinib, at imatinib steady-state, decreased the imatinib AUC_{0-24h} by 3% (n = 11) (Van Erp et al., 2007). According to the Flockhart Table, ritonavir and ketoconazole share the same interaction potential (Flockhart, 2007). But ritonavir is also an inhibitor of CYP2D6 and inducer of CYP2C19 (Flockhart, 2007), which both play a minor role in the metabolism of imatinib (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2006; Whirl-Carrillo et al., 2012). Especially the induction of CYP2C19 could be an explanation for the difference seen between the effects of ketoconazole and ritonavir. Furthermore, the difference could be caused by a shift to alternative elimination routes when imatinib is administered chronically, especially because auto-inhibition of CYP3A was shown *in vitro* (Food and Drug Administration, 2001). The two described hypotheses are supported by the *in vitro* experiment of Van Erp et al. which showed that ritonavir completely inhibited the metabolism of imatinib *via* CYP3A, but in human liver microsomes by only 50% (Van Erp et al., 2007). In DDI studies with CYP3A inducers large effects of the drugs rifampin and enzyme-inducing antiepileptic drugs (EIAEDs) such as carbamazepine, oxcarbazepine and phenytoin on imatinib AUC were seen. The strong inducer rifampin decreased the $AUC_{0-\infty}$ of imatinib by 74% (n = 14) (Bolton et al., 2004; European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2006). EIAEDs (mixed potency; carbamazepine and phenytoin are potent inducers, oxcarbazepine is a weak inducer (Riva et al., 1996)) decreased the $AUC_{0-\infty}$ of imatinib by 72.5% (n = 50; n = 27 in EIAED group and n = 23 in non-EIAED group) (Wen et al., 2006). The effect of St John's Wort on imatinib exposure was smaller with an average decrease of 37% in 2 studies (n = 12 in study Frye et al.; n = 10 in study Smith et al.) (Frye et al., 2004; Smith et al., 2004). To summarize, DDI studies with mostly strong CYP3A inhibitors and inducers were performed. The effects of these drugs on imatinib were variable. This can be due to differences in study design, characteristics of the interacting drugs and also the inter-individual variability of 40–60% will have an effect (Food and Drug Administration, 2001).

Osimertinib

Osimertinib is converted into different metabolites by predominantly CYP3A, among which the active metabolites AZ5104 and AZ7550. The exposure to the active metabolites is, however, less than 10% of the total drug exposure, therefore the effects of the metabolites are considered clinically irrelevant (Vishwanathan et al., 2018). Next to the metabolism by CYP3A, in *in vitro* studies CYP1A2, CYP2A6, CYP2C9, CYP2E1 also play a minor role in the metabolism of osimertinib (Dickinson et al., 2016; Vishwanathan et al., 2018). *In vitro* studies also showed that osimertinib is an inhibitor of CYP3A, but no *in vivo* data is available (Food and Drug Administration, 2015c).

The strong inhibitor itraconazole increased the $AUC_{0-\infty}$ of osimertinib by 24% (n = 38) (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2015a; Vishwanathan et al., 2018). On the other hand, the effect of rifampin on osimertinib exposure was large, rifampin decreased the AUC_{0-24h} by 78.5% (n = 32) (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2016a; Vishwanathan et al., 2018). The moderate inducer efavirenz was simulated to decrease the exposure by approximately 50% compared with rifampin, with a decrease in AUC of 42% (Reddy et al., 2018). Dexamethasone, a weak CYP3A inducer, had no effect on the AUC of osimertinib in a PBPK simulation (Reddy et al., 2018).

The presence of a clinically relevant effect for the interaction of osimertinib with rifampin, while it was lacking for the interaction between osimertinib and itraconazole, could be explained by the fact that rifampin induces multiple enzymes and transporters, and that, next to CYP3A, other CYP enzymes play a role in the metabolism of osimertinib (Vishwanathan et al., 2018). For the drugs tivozanib and ixazomib, also a clinically relevant effect was shown for rifampin, while it was lacking for a CYP3A inhibitor (Cotreau et al., 2015; Gupta et al., 2018; Vishwanathan et al., 2018).

Sunitinib

Sunitinib is metabolized by CYP3A to the active metabolite SU12662, which is equally potent (Food and Drug

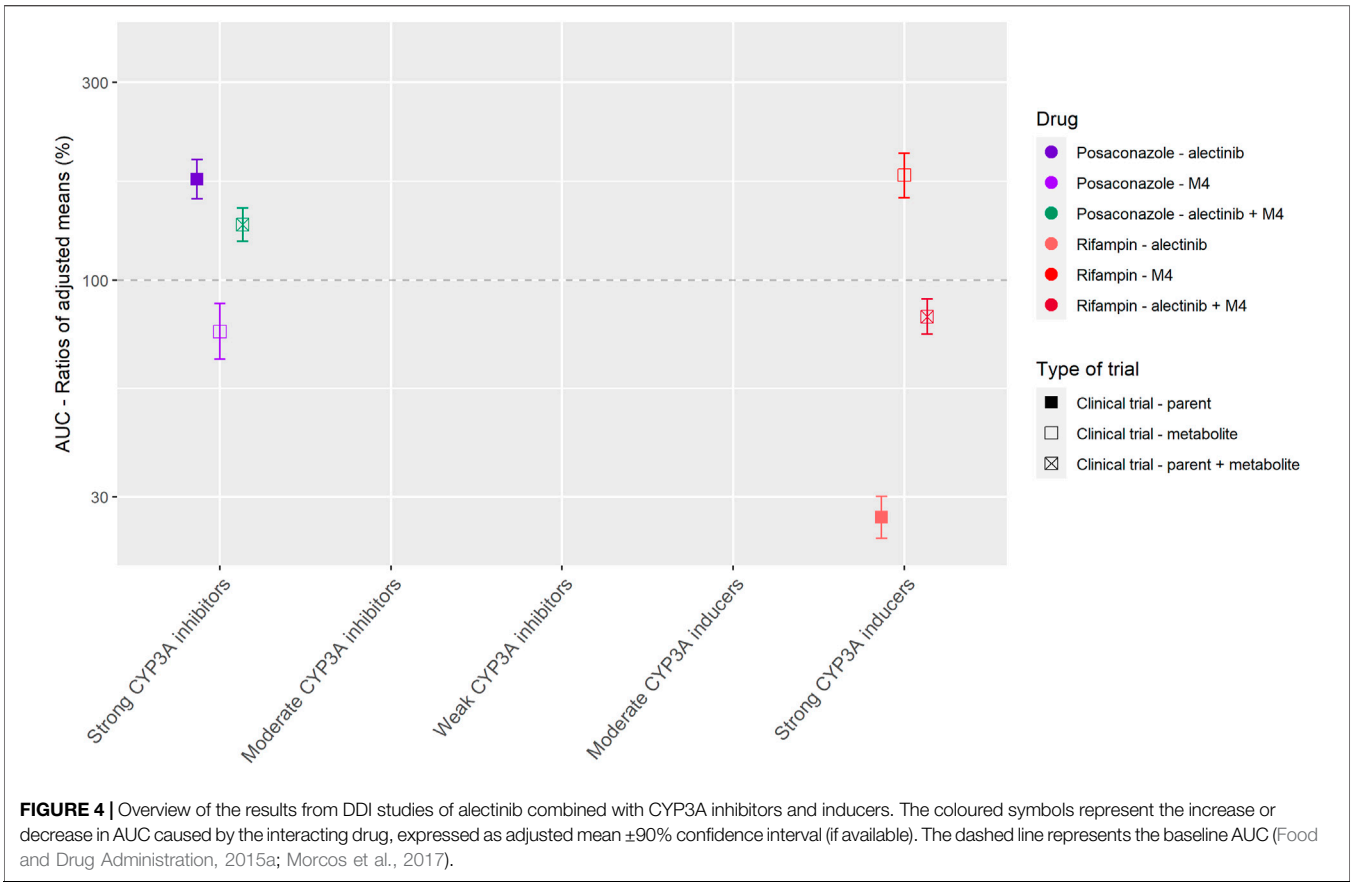

FIGURE 4 | Overview of the results from DDI studies of alectinib combined with CYP3A inhibitors and inducers. The coloured symbols represent the increase or decrease in AUC caused by the interacting drug, expressed as adjusted mean ±90% confidence interval (if available). The dashed line represents the baseline AUC (Food and Drug Administration, 2015a; Morcos et al., 2017).

Administration, 2005). SU12662 is metabolized further by CYP3A and transported by P-gp (Heath et al., 2011).

The strong inhibitor ketoconazole increased the sum of the $AUC_{0-\infty}$ of sunitinib and SU12662 only by 51% (n = 27) (Food and Drug Administration, 2005). Grapefruit juice, a moderate CYP3A inhibitor, increased the AUC $_{0-24h}$ of sunitinib by 11%, which was considered negligible (n = 8) (Van Erp et al., 2011). In this study only the AUC of sunitinib was measured and not the AUC of the metabolite SU12662. Grapefruit juice mainly inhibits intestinal CYP3A with little effect on hepatic CYP3A, while ketoconazole inhibits both (Saito et al., 2005). In addition, the small increase in AUC could be explained by the fact that in the study with ketoconazole (Food and Drug Administration, 2005), only a single dose of sunitinib was administered in contrast to the multiple dosing in the grapefruit juice study (Van Erp et al., 2011), which could lead to a shift to other metabolic pathways. The strong CYP3A inducer rifampin reduced the sum of the $AUC_{0-\infty}$ of sunitinib and SU12662 by 46% (n = 28) (Food and Drug Administration, 2005).

DISCUSSION

Most currently used oral targeted anticancer drugs have a narrow therapeutic range. Furthermore, most of these drugs are substrates of CYP3A and are, therefore, prone to DDIs with inhibitors or inducers of CYP3A. It is of crucial importance for clinical practice to have guidelines on how to deal with these DDIs in cases where data is lacking, which might be the case early after drug approval. This study reviewed the literature for DDI studies performed with twelve oral anticancer drugs. Based on this data, we formulated recommendations for clinical practice on how to deal with DDIs of oral anticancer drugs when only data from strong inducers or inhibitors is available.

In our approach, we extrapolated results from dedicated DDI studies with strong inhibitors and inducers to clinical practice. Since the extrapolation of the effects of CYP3A inhibitors and inducers is more complex in the presence of active metabolites, separate recommendations are given for the drugs metabolized to inactive and with active metabolites. The recommendations are summarized in a flowchart (**Figure 5**). When interested in a victim drug without active metabolites, start in the left of the figure in the upper blue box. Follow the flowchart depending on the characteristics (inhibitor or inducer; interaction potential) of the drug you are interested in. The last box will show you our recommendation regarding the interaction. When interested in a victim drug with active metabolites, start in the right of the figure in the upper orange box. When the metabolite contributes less than 10% to total drug exposure or less than 50% to total drug effect, the presence of an active metabolite can be neglected. Therefore, the part of the flowchart for drugs without active metabolites can be followed. If the metabolite has a relevant

contribution to total drug exposure and effect, the part of the flowchart for drugs without active metabolites can be followed, using the sum of parent and metabolite, or assessing the effect of parent and metabolite separately.

For the studied drugs without active metabolites, **Tables 1** and **2** show that the effect of moderate CYP3A inhibitors on the AUC is roughly approximate to 50% of the effect of the strong inhibitors. The same effect can be seen for moderate inducers in comparison with strong inducers. Furthermore, it can be noted that weak inhibitors and inducers had marginal effects on the exposure of the studied drugs. In **Figures 2** and **3**, these results are visualized for the drugs cobimetinib and palbociclib, which gives a good representation of the effects seen for all seven drugs without active metabolites (the **Supplementary Material** shows figures for the other drugs).

Regarding drug selection for this review we made the following decisions. Drugs which have been approved for solid tumors from January 1st, 2013 to December 31st, 2015, and three drugs (everolimus, imatinib, sunitinib) authorized before 2013 based on the availability of relevant clinical data were selected. This resulted in a selection of twelve drugs. This was decided since 1) no difference is to be expected in quality of PBPK simulations performed in early years (2013–2015) compared to later years, and 2) the results of all twelve analyzed drugs in this review roughly indicate the same direction on the extrapolation of the effects of DDI studies. For the twelve drugs selected in our analysis, only for sunitinib and palbociclib a clinical trial was performed with a moderate CYP3A inhibitor and inducer, respectively. Also for the seven drugs that were approved after 2015 and met the inclusion criteria regarding metabolism and indication (abemaciclib, brigatinib, entrectinib, larotrectinib, lorlatinib, neratinib, and ribociclib) no clinical DDI studies with moderate inhibitors/inducers, but only PBPK simulations were performed (or no DDI studies at all). Furthermore, we decided to focus on oral anticancer drugs in our review. However, our recommendations are probably also applicable to other drugs metabolized by CYP3A.

It is important to take into account the following, regarding our recommendations. First, a large variability in the PK after multiple doses occurred in the studied drugs, with a range of 23–78%. Similarly, Verheijen et al. showed that there is a high inter-individual variability in the exposure to kinase inhibitors (Verheijen et al., 2017). This is also reflected by the large variability in the effect of CYP3A inhibitors and inducers for some drugs. Possibly, this variability in exposure could partly be explained by the highly variable CYP3A4 activity among patients, which is for 60–90% genetically determined (Özdemir et al., 2000; Westlind-Johnsson et al., 2003). For example, the CYP3A4*22 polymorphism has been described, resulting in a two-fold increase of the formation of a non-functional variant of CYP3A4 (Wang et al., 2011). If the CYP3A4 activity is decreased by a genetic polymorphism, the magnitude of the effect of a CYP3A inhibitor will theoretically be decreased. Furthermore, caution should be taken while using the flowchart for drugs in which auto-induction or -inhibition plays a role and drug-drug interaction studies are not performed on steady-state, or for drugs with nonlinear dose-exposure relationships. In these cases it might not be possible to extrapolate results from DDI studies with strong inhibitors and inducers, or dose recommendations based on these results. While interpreting the results of this review it is necessary to bear in mind this large variability in PK, and the exceptions in which our recommendations might not be applicable.

Next to the results of the drugs without active metabolites, **Tables 1** and **2** show that for drugs that have active metabolites the results are less straightforward. As a visual example **Figure 4** was made, which shows the effect of interacting drugs on the AUC of the parent drug alectinib and its active metabolite (similar figures are presented in the **Supplementary Material** for the other studied drugs). There are three factors to take into account while interpreting the results of DDI studies with drugs with active metabolites. Firstly, the metabolic pathway is important. For example, in case of dabrafenib not only the parent, but also two of the active metabolites are metabolized by CYP3A, whereas the third metabolite is converted non-enzymatically. This results in an effect of CYP3A inhibitors and inducers on both parent and some of the metabolites, but not all of them. Secondly, the ratio between parent and metabolites should be taken into account. As a cut-off value a contribution of less than 10% of the metabolite to total drug exposure could be used. This is in line with the EMA recommendation to characterize metabolites structurally that contribute to more than 10% of the AUC of a drug in in vitro studies (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2015b). An example of a drug with an active metabolite which contributes to less than 10% of total drug exposure is osimertinib. Thirdly, the potency of the metabolites plays an important role. A cut-off value of 50% contribution to the total drug effect can be used when considering the relevance of the contribution of an active metabolite. This cut-off value is supported by the EMA (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2015b). Shown by the recommendation to conduct an *in vivo* DDI study not only for drugs where enzymes contribute to at least 25% of the overall elimination but also for drugs with pharmacologically active metabolites which contribute to 50% or more of the effect of the drug (and enzymes are involved in the formation or elimination of these metabolites) (European Medicines Agency Committee for Medicinal Products For Human Use (CHMP), 2015b). For example, if a metabolite is as potent as the parent drug, the effect of an interacting drug on the sum of parent and metabolite might be reported as measure of total drug activity, as was done in the case of alectinib and sunitinib.

A practical example for the drug palbociclib is given. The assumption of an effect of 50% in comparison to that of strong inhibitors and inducers can be used to extrapolate the advice of the manufacturer. In case of palbociclib, the standard dose is 125 mg once daily (QD). The manufacturer recommends to reduce the dose of palbociclib to 75 mg (QD) if combination with a strong CYP3A inhibitor cannot be avoided. In combination with a moderate CYP3A inducer it could be considered to reduce the dose with 50% compared with the

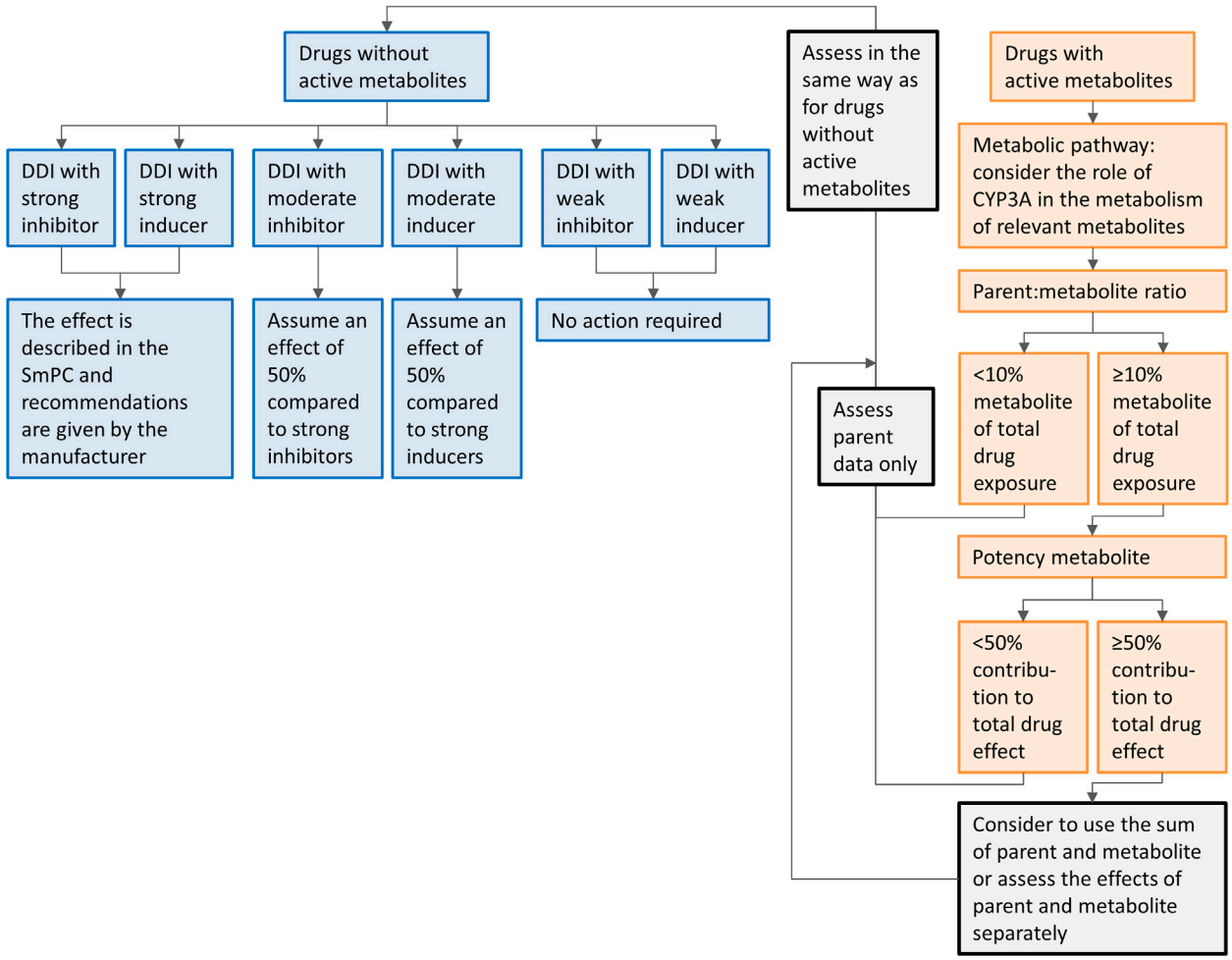

FIGURE 5 | Flowchart of the recommendations on how to handle DDIs for oral anticancer drugs metabolized by CYP3A if only clinical data from strong CYP3A inhibitors or inducers is available. Caution should be taken while using the flowchart for drugs in which auto-induction or -inhibition plays a role and drug-drug interaction studies are not performed on steady-state, or for drugs with nonlinear dose-exposure relationships.

reduction in combination with strong inhibitors. This would result in a dose of 100 mg QD (Food and Drug Administration, 2014d). A reason to reduce the dose of palbociclib is that a higher palbociclib exposure is associated with increased toxicity, specifically a larger decrease in absolute neutrophil count when compared with baseline. However, the limited data available on exposure-response and exposure-toxicity relationships could be a consideration to start with the standard starting dose and decrease the dose in case toxicity occurs (Flaherty et al., 2012; Food and Drug Administration, 2014d; Verheijen et al., 2017).

After initiation of therapy with oral anticancer drugs in a reduced or increased dose, attainment of adequate drug exposure could be monitored by means of Therapeutic Drug Monitoring. Many of the oral anticancer drugs show an exposure-efficacy and an exposure-toxicity relationship, the strength of the evidence for these relationships is and recommendations for target plasma trough levels are discussed by Verheijen et al. (Verheijen et al., 2017).

CONCLUSION

In conclusion, DDIs are often very complex and dependent on multiple factors. But, if only data from strong CYP3A inhibitors or inducers is available, in case of drugs without active metabolites, a change in exposure of 50% for moderate inhibitors/inducers compared with strong inhibitors/inducers can be assumed. We therefore recommend to start with a 50% dose reduction compared with the advised reduction in combination with strong inhibitors, and with a 50% dose increase compared to the advised increase in combination with strong and inducers.

Since an effect of weak CYP3A inhibitors on the AUC of oral anticancer drugs is small in the twelve reviewed drugs, *a priori* dose adaptations are not indicated.

In the presence of active metabolites, the response on DDIs should be based on the metabolic pathway, the exposure to the metabolites compared with the parent drug and to the potency of the metabolites. Options are to ignore the presence of a metabolite (for example when a metabolite is not

pharmacologically active or contributes minimal to the exposure of the drug) or to use the sum of the parent and metabolite (at least do this when parent and metabolite are equally potent).

AUTHOR CONTRIBUTIONS

LM-K, NS, and AH contributed to conception and design of the review. LM-K wrote the review, and designed figures and tables. All authors contributed to manuscript revision, read, and approved the submitted version.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.670862/full#supplementary-material

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Effect of Pharmacogenetics Variations on Praziquantel Plasma Concentrations and Schistosomiasis Treatment Outcomes Among Infected School-Aged Children in Tanzania

Rajabu Hussein Mnkugwe[1,2], *Omary Minzi*[3], *Safari Kinung'hi*[4], *Appolinary Kamuhabwa*[3] *and Eleni Aklillu*[2]*

[1]Department of Clinical Pharmacology, School of Medicine, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, [2]Division of Clinical Pharmacology, Department of Laboratory Medicine, Karolinska University Hospital-Huddinge, Karolinska Institutet, Stockholm, Sweden, [3]Department of Clinical Pharmacy and Pharmacology, School of Pharmacy, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania, [4]National Institute for Medical Research (NIMR), Mwanza Research Centre, Mwanza, Tanzania

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Caroline Flora Samer,
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Julio Benitez,
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***Correspondence:**
Eleni Aklillu
eleni.aklillu@ki.se

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Studies on pharmacogenetics of praziquantel (PZQ) and its relevance on plasma drug concentrations and schistosomiasis treatment outcomes are lacking. We investigated the effect of pharmacogenetics variations of PZQ on plasma drug levels and schistosomiasis treatment outcomes among infected Tanzanian school-aged children. A total of 340 *Schistosoma mansoni* infected children were enrolled and treated with single-dose PZQ. Stool samples analysis was done by thick smear Kato-Katz technique, and treatment efficacy was assessed at 3-weeks post-treatment. Safety was assessed within 4 h after PZQ intake. Plasma samples were collected at 4 h post-dose, and PZQ and *trans*-4-OH-PZQ concentrations were quantified using UPLCMS/MS. Genotyping for *CYP3A4*1B*, *CYP3A5* (*3, *6, *7), *CYP2C19* (*2, *3, *17), and *CYP2C9* (*2, *3) were done by Real-Time PCR. The median age (range) of the study participants was 12 years (7–17). There was a significant association of CYP2C19 genotypes with PZQ concentrations and its metabolic ratio (*trans*-4-OH-PZQ/PZQ). PZQ concentration was significantly higher among *CYP2C19* (*2, *3) carriers than *CYP2C19 *1/*1* and *CYP2C19 *17* carriers (ultra-rapid metabolizers) ($p = 0.04$). The metabolic ratio was significantly higher among *CYP2C19*17* carriers than *CYP2C19* (*2, *3) carriers ($p = 0.01$). No significant effect of *CYP3A4*, *CYP3A5*, *CYP2C19*, and *CYP2C9* genotypes on treatment efficacy or adverse events were observed. Baseline infection intensity and *CYP3A5* genotype were significant predictors of treatment associated-adverse events. In conclusion, *CYP2C19* genotype significantly affects plasma PZQ concentration and its metabolic ratio. For the first time, we report the importance of pharmacogenetic variation for the treatment of schistosomiasis, a neglected tropical disease.

Keywords: CYP2C19, schistosomiasis, treatment efficacy, adverse events, Tanzania, Praziquantel, CYP3A5, Africa

INTRODUCTION

Since 1984, praziquantel (PZQ) has been used in large-scale mass drug administration (MDA) programs for the treatment, control, and prevention of schistosomiasis worldwide (WHO, 2015). To date, PZQ is the only drug of choice recommended by the World Health Organization (WHO) (WHO, 2015). PZQ is reported to be safe and efficacious against all *Schistosoma* species, including *Schistosoma haematobium* (urogenital schistosomiasis) and *Schistosoma mansoni* (intestinal schistosomiasis). Globally, more than 800 million people are at risk of schistosomiasis infection, and about 250 million are infected and need treatment (Hotez et al., 2014; Mazigo, 2019). In Tanzania, schistosomiasis was first reported back in 1895 (Doumenge et al., 1984). To date, the disease is still endemic throughout the country despite ongoing interventions (Mazigo et al., 2012; Mnkugwe et al., 2020b). In 2017, approximately 99 million people of whom 81.1 million were school-aged children, received treatment worldwide (WHO, 2018). The WHO target is to control (heavy infections <5%) and eliminate (heavy infections <1%) the disease as a public health problem by the year 2025 (Gebreyesus et al., 2020).

As per WHO recommendation, preventive chemotherapy using mass PZQ treatment targeting school-going children is the main control strategy in endemic countries (WHO, 2015). PZQ MDA has played a significant role in reducing severe disease-associated morbidity and mortality in endemic settings (Andrade et al., 2017). However, the WHO recommended standard dose 40 mg/kg body weight of PZQ has been associated with varying results in both treatment efficacy, incidence, and profile of adverse events as reported in previous studies conducted in different populations (Kabuyaya et al., 2018; Mnkugwe et al., 2019; Mnkugwe et al., 2020a). In such studies, both high and low cure rates were reported, particularly with *Schistosoma mansoni* infection. The incidence and profile of treatment-associated adverse events also varied widely between populations. The causes for variability in drug response are multifactorial, including genetics, environment, and disease itself, which could potentially affect drug disposition (Wilkinson, 2005). Studies conducted in other infectious diseases such as malaria, tuberculosis, and HIV have reported variability in drug responses both treatment efficacy and adverse events in different populations due to genetic variations (Mugusi et al., 2012; Ngaimisi et al., 2013; Maganda et al., 2016).

However, studies to assess the contribution of genetic variations on PZQ plasma concentration and schistosomiasis treatment outcomes are lacking (Zdesenko et al., 2020). Although MDA poses a challenge for implementing individualized treatment, knowledge on how genetic variations affect PZQ blood levels and treatment outcomes is vital for improving treatment outcomes (Mukonzo et al., 2014; Mutagonda et al., 2017). Indeed, the utility of pharmacogenetic data to improve treatment outcomes has recently been intensified in Africa (Dandara et al., 2019). Furthermore, genetic variations can partly explain some of the reported variability on PZQ exposure, cure rates, and the incidence and profile of adverse events, as suggested previously (Bustinduy et al., 2016).

Factors such as age, pre-treatment infection intensity, and anemia are reported to affect schistosomiasis treatment outcomes among treated children (Zwang et al., 2017; Mnkugwe et al., 2019). Pharmacogenetic variations can potentially affect plasma drug levels and hence treatment efficacy and adverse events (Maganda et al., 2016; Ahmed et al., 2019). PZQ undergoes extensive phase 1 metabolism by *CYP3A4*, *CYP3A5*, *CYP2C19*, and *CYP2C9* enzymes to produce several metabolites, including 4-OH-PZQ (*Trans*- and *cis*-), which is a major metabolite of PZQ in humans (Wang et al., 2014; Nleya et al., 2019). The *trans*-4-OH-PZQ metabolite has been reported to possess antischistosomal activity (Kovač et al., 2017). *CYP3A4*, *CYP3A5*, *CYP2C19*, and *CYP2C9* are genetically polymorphic, displaying inter-individual variability in enzyme activity. The inherited defective/variant alleles may increase or decrease CYP enzyme activity resulting in variability in plasma drug levels. In pharmacokinetics -pharmacodynamics (PK-PD) studies, plasma drug concentration has been used as a surrogate marker for drug concentration at the site of action in the tissues (Bustinduy et al., 2016). High plasma drug exposure may increase the risk of adverse events, and low drug exposure results in poor therapeutic efficacy (Yimer et al., 2012). Therefore, genetic variations in CYP enzymes relevant for PZQ biotransformation can affect both PZQ plasma exposure and treatment outcomes (efficacy and safety).

To the best of our knowledge, no study has investigated pharmacogenetics variations of PZQ and its relevance on plasma concentration and schistosomiasis treatment outcomes despite reported variability in drug levels, cure rates, incidence, and profile of adverse events between treated populations (Zwang et al., 2017; Kabuyaya et al., 2018). We report the first pharmacogenetics study of PZQ and its relevance on plasma drug concentrations, treatment efficacy defined by cure rates, and adverse events among *Schistosoma mansoni* infected children treated with single-dose PZQ in Tanzania.

MATERIALS AND METHODS

Study Design and Population

This was a prospective pharmacogenetics-pharmacokinetics-pharmacodynamics study aimed at investigating the effect of pharmacogenetics variations on PZQ plasma concentration and schistosomiasis treatment outcomes among *Schistosoma mansoni* infected children. The study was conducted between February 2017 and January 2018. The study was conducted in Nyamikoma village, North-western Tanzania. The Nyamikoma village is a rural area endemic for intestinal schistosomiasis (Mnkugwe et al., 2020b). The area has received five rounds of PZQ MDA targeting school-aged children. A total of 340 *Schistosoma mansoni* infected children (aged 7–17 years) were enrolled in this study.

Data Collection Methods

Socio-demographic characteristics such as age and sex were obtained through interviews and school registries and recorded

in case record forms (CRFs). Clinical data, including pre-treatment and post-treatment infection status, treatment-associated adverse events, body weight, and height and haemoglobin concentration, were recorded in the CRFs and categorized according to the existing WHO guidelines.

Haemoglobin Concentration and Undernutrition Assessment

Pre-treatment haemoglobin concentration was determined by the HemoCue Hb 201 + analyzer (HemoCue AB Angelholm, Sweden) using finger-prick blood. Presence of anaemia was defined by haemoglobin (Hb) concentration of <11.5 g/dl (WHO, 2011). Undernutrition such as stunting and wasting were assessed by converting the height for age and body mass index (BMI) for age values into height for age Z score (HAZ) and BMI for age Z score (BAZ) using the WHO Anthro plus software version 1.0.4 (WHO, 2009). All values less than two standard deviations for both HAZ and BAZ scores were considered abnormal and classified as stunting and wasting, respectively.

Therapeutic Procedures, Follow-Up and Safety Monitoring

Treatment was given following the WHO guidelines and recommendations for assessing the efficacy of an antihelminthic drug against schistosomiasis (WHO, 2013). Following a pre-treatment meal, a standard dose 40 mg/kg body weight of PZQ (Praziquantel 600 mg/tablet, Batch BZ6043, S Kant Health Care Ltd., India) was administered to each infected child as a directly observed treatment (DOT) (Mnkugwe et al., 2019). A follow-up visit was done 3-weeks post-treatment as recommended by the WHO guideline (WHO, 2013). Treatment-associated adverse events were monitored within 4 h after drug intake.

Blood Samples Collection for DNA Extraction and Pharmacokinetics Analysis

A 2 ml pre-treatment whole blood sample was collected in EDTA tube from 340 study participants for genomic DNA extraction and stored at −80°C freezer. Another 2 ml whole blood sample was collected 4 h post-drug administration from 287 study participants in heparinized tubes and immediately centrifuged at 1,000 rpm for 10 min to obtain plasma, which was then kept at −80°C freezer until analysis. Blood and plasma samples were shipped to Karolinska Institutet (Stockholm, Sweden) for laboratory analysis.

Laboratory Analyses

Thick Smear Kato-Katz Technique for *Schistosoma mansoni* Detection

The details of methods for stool sample processing and microscopic examination were presented previously (Mnkugwe et al., 2019). All laboratory procedures were done according to the WHO guidelines (WHO, 1991). Briefly, two fresh stool samples were collected from each participating child on two consecutive days and analyzed by thick smear Kato-Katz method both at pre-treatment and follow-up visit. The slides were then double read under light microscopy by trained and experienced laboratory technicians, and egg counts were recorded (Mnkugwe et al., 2019).

Quantification of PZQ and *trans*-4-OH-PZQ Plasma Concentrations

Chemicals and Reagents

Rac-PZQ, an eleven-fold rac-deuterated-PZQ (rac-PZQ-d11) [internal standard (IS) for PZQ], *trans*-4-OH-PZQ and a five-fold *trans*-4-OH-PZQ (*trans*-4-OH-PZQ -d5) [internal standard (IS) for *trans*-4-OH-PZQ] were purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Acetonitrile, methanol, and formic acid of mass spectrometry (MS) grade were purchased from Merck (Darmstadt, Germany). Ultra-pure MilliQ water was prepared using a Milli-Q water purification system (Merck Millipore, Massachusetts, United States). Blank plasma was kindly supplied by the blood bank of the Karolinska University Hospital Huddinge (Stockholm, Sweden).

Analytical Method

The UPLC-MS/MS method for quantification of PZQ and *trans*-4-OH-PZQ was adapted from Astra Zeneca laboratories (Sweden) and was recently used by Nleya et al., 2019 (Nleya et al., 2019) with minor modifications. In brief, plasma calibration samples were freshly prepared by spiking blank plasma samples with rac- PZQ and *trans*-4-OH-PZQ and were included in each analytical run. Quality control samples were also prepared by spiking plasma blanks to obtain low, medium, and high concentrations for both PZQ and *trans*-4-OH-PZQ. The quantification range of the method was set to 3.9–2,500 ng/ml for PZQ and 31.2–50,000 ng/ml for *trans*-4-OH-PZQ.

For extraction of analytes of interest, 50 µL of plasma samples went through protein precipitation with 150 µL of internal standards solution (25 nM of rac-PZQ -d11 and 25 nM of *trans*-4-OH-PZQ -d5 in 50:50 mixture of acetonitrile: methanol) and the mixture was vortexed for 3 min followed by centrifugation for 20 min at 3,220 g at 4°C. Then, 75 µL of the supernatant was diluted with 75 µL MilliQ water and 5 µL was injected into the UPLC-MS/MS for analysis. The chromatographic system was using an Aqcuity UPLC®HSS T3 column [2.1 × 50 mm, 1.8 µm (Waters, Ireland)]. The mobile phase consisted of solvent A (0.1% formic acid and 2% acetonitrile in water) and solvent B (0.1% formic acid in acetonitrile) with a flow rate of 0.8 ml/min. The column temperature was maintained at 60°C.

The chromatographic run was 4.7 min, starting at 4% of solvent B with an increase to 70% of solvent B at 2.6 min. From 3.1 min, the column was washed with 96% of solvent B until 4.1 min, with two dips to 4% of solvent B in the middle to ensure efficient washing. Column re-equilibration was done from 4.2 to 4.7 min but was in effect longer when including the injection time. T*rans*-4-OH-PZQ eluted first at a retention time of 1.15 min, followed by PZQ at 1.89 min. PZQ was monitored by the transition m/z 313.2 > 203.1 and the IS rac-

PZQ -d11by 324.2 > 204.1 and for *trans*-4-OH-PZQ by the transition m/z 313.2 > 203.1 and the IS t*rans*-4-OH-PZQ -d5 by 324.2 > 204.1. Because of the very high concentrations of *trans*-4-OH-PZQ in the samples, a detuned (sub-optimized) MS method was used by decreasing the collision energy setting for that transition. Quantification of PZQ and *trans*-4-OH-PZQ was done using Target Lynx software (Waters). The calibration curves were constructed by linear regression of the analyte/internal standard area ratios, with a quadratic curve fit and an applied weighing of 1/x. A minimum of 12 calibration points were used, and calibrators were injected at start and end of each analysis. Three quality control samples were injected at regular intervals throughout the analyses. The PZQ and *trans*-4-OH-PZQ concentrations were estimated based on the ratio of the analyte peak area to the internal standard area.

Accuracy and recovery of the method was measured from three quality control samples each, at low (QCL), mid (QCM), and high (QCH) levels. For PZQ, recovery was 105% for QCL, 87% for QCM, and 100% for QCH at 5, 8.7, and 1% RSD, respectively. For *trans*-4-OH-PZQ the recovery was 104, 109, and 97.11 for the three QC levels, and accuracy was 2.6, 2.7, and 1.9% RSD. The precision for PZQ was measured by injection of six replicates and was 6.7% RSD at LLOQ, and 4.1% RSD at QCH. For *trans*-4-OH-PZQ, the area precision was 6.4% RSD at LLOQ and 5.3 at QCH. The calibration curves for both PZQ and *trans*-4-OH-PZQ had a coefficient of determination (r^2) of >0.98. No carry-over was detected for the compounds analyzed. The analytical method was partially validated according to the European Medicines Agency Guideline on bioanalytical method validation (EMA, 2009).

DNA Extraction and Genotyping for CYP3A4, CYP3A5, CYP2C19 and CYP2C9

Genomic DNA was extracted from the peripheral leucocytes using the QIAamp DNA Midi Kit (Qiagen GmbH, Germany) according to the manufacturer's instructions. Genotyping for common variant alleles for *CYP3A4* (*1B), *CYP3A5* (*3, *6, *7), *CYP2C19* (*2, *3, *17), and *CYP2C9* (*2, *3), which are relevant for PZQ disposition were determined as described previously (Maganda et al., 2016). In brief, genotyping was performed using TaqMan® drug metabolism genotyping assay reagents for allelic discrimination (Applied Biosystems Genotyping Assays) with the following ID numbers for each SNP: C__11711730_20 for *CYP3A4*1B* (−392A > G, rs2740574), C__26201809_30 for *CYP3A5*3* (c.6986A4G, rs776746), C__30203950_10 for *CYP3A5*6* (g.14690G4A,rs10264272), C__32287188_10 for *CYP3A5*7* (g.27131_27132insT rs41303343), C__25986767_70 for *CYP2C19*2* (rs4244285), C__2,7861809_10 for *CYP2C19*3* (rs4986893), C__469857_10 for *CYP2C19*17* (rs12248560), C__25625805_10 for *CYP2C9*2* (rs1799853), and C__27104892_10 for *CYP2C9*3* (rs1057910). Genotyping was done by 7500 Fast Real-Time PCR (Applied Biosystems, United States). The final volume for each reaction was 10 μL, consisting of 9 μL TaqMan fast advanced master mix (Applied Biosystems, Waltham, MA, United States) and 1 μL genomic DNA. The PCR profile consisted of an initial step at 60°C for 30 s, hold stage at 95°C for 10 min, and PCR stage for 40 cycles step 1 with 95°C for 15 min and step 2 with 60°C for 1 min and after reading stage with 60°C for 30 s.

Study Outcomes

The primary study outcome was the effect of *CYP3A4*, *CYP3A5*, *CYP2C19* and *CYP2C9* genotypes on PZQ, *trans*-4-OH-PZQ concentrations and metabolic ratio (*trans*-4-OH-PZQ/PZQ). The secondary outcomes were the effect of *CYP3A4*, *CYP3A5*, *CYP2C19* and *CYP2C9* genotypes on treatment efficacy (cure rate and eggs count reduction) and adverse events. The cure rate was defined as the proportion of infected children who were eggs positive for *Schistosoma mansoni* infection at baseline and turned negative at 3 weeks post-treatment (Mnkugwe et al., 2019). Eggs count reduction was defined by the mean percent change in eggs count per Gram between baseline and at 3 weeks' post-treatment. An adverse event was defined as any symptom reported by a child, which is temporally associated with PZQ intake, but not necessarily causally related (Zwang et al., 2017).

Statistical Data Analyses

Data was entered into Microsoft Excel and analyzed using the Statistical Package for Social Sciences (SPSS) version 20 (SPSS, IBM Corp, Armonk, NY, United States). Descriptive statistics were used for the analysis of both socio-demographic and clinical data. Socio-demographic characteristics were summarized into a frequency Tables as proportions for categorical data and mean ± standard deviations (SD) or median (range or Interquartile range- IQR) depending on the normality distribution of the data. Descriptive statistics were also used to analyze the treatment efficacy (i.e., cure rates) and treatment-associated adverse events as proportions in different CYP enzyme genotypes. Chi-square test was used to compare the genotype and allele frequencies between the observed and expected according to the Hardy-Weinberg equilibrium. The CYP2C19 genotype was categorized as *CYP2C19 *17* carriers (*17/*17 or *1/*17), wild type (*1/*1), and *CYP2C19* *2, *3 carriers (*1/*2 or *1/*3 or *2/*17 or *3/*17 or *2/*2 or *2/*3 and *3/*3). The means of the log-transformed PZQ, *trans*-4-OH-PZQ and t*rans*-4-OH-PZQ/PZQ concentrations were antilogged to obtain geometric means. One-way ANOVA was used to compare the geometric means of the PZQ, *trans*-4-OH-PZQ and t*rans*-4-OH-PZQ/PZQ concentrations between different CYP450 genotypes. The Pearson's Chi-square test or Fisher's exact test depending on test appropriateness was used for assessing the association between cure rates, adverse events and CYP genotypes. A univariate followed by multivariate regression analysis were used to identify the predictors of cure rate at week 3 post-treatment, and treatment-associated adverse events. Variables with $p < 0.2$ from univariate analysis were included in the multivariate regression model. One-way ANOVA was used to compare the mean percent change in eggs count (egg reduction) between different CYP genotypes. A negative binomial regression model was used to assess the predictors of eggs reduction at 3 weeks' post-treatment. A variable with p-value < 0.05 was considered as a significant predictor.

TABLE 1 | Baseline characteristics of the studied population.

Variable		N (%)
Age (years)	Mean ± SD	11.8 ± 1.7
	≤12 years	235 (69.1)
	>12 years	105 (30.9)
Sex	Male	159 (46.8)
	Female	181 (53.2)
Baseline eggs/Gram of stool	Median (IQR)	222 (96–468)
Baseline infection intensity	Light	87 (25.6)
	Moderate	152 (44.7)
	Heavy	101 (29.7)
Weight (kg)	Median (IQR)	30.2 (26.3–34.8)
Height (cm)	Median (IQR)	138.5 (130.4–144.0)
Stunting status (HAZ)	Stunted	116 (34.1)
	Not stunted	224 (65.9)
Wasting status (BAZ)	Wasted	34 (10.0)
	Not wasted	306 (90.0)
Haemoglobin concentration	Median (IQR)	12.7 (11.6–13.5)
Anaemia status	Anaemic	76 (22.4)
	Not anaemic	264 (77.6)

SD-Standard deviation; IQR-Interquartile range: BAZ-Body Mass Index (BMI) for Age Z score; HAZ: Height for Age Z score

RESULTS

Baseline Characteristics of the Study Participants

A total of 340 children were enrolled in this study. The median age (range) in years of the study population was 12 years (7–17). Females were 53.2% of the study participants. The median baseline eggs/gram of stool (IQR) was 222 epg (96–468). At enrolment, about 22.4% of the study participants had anaemia (Hb < 11.5 g/dl). The prevalence of undernutrition as defined by stunting and wasting were 34.1 and 10.0%, respectively (**Table 1**).

Genotypes and Alleles Frequencies

The overall genotype and allele frequencies for *CYP3A4*1B*, *CYP3A5* (*3, *6, *7), *CYP2C19* (*2, *3, *17) and *CYP2C9* (*2, *3) among Tanzanian children are summarized in **Table 2**. There were no significant differences in the observed and expected genotypes frequencies according to the Hardy Weinberg Equilibrium. *CYP3A4 *1B* allele occur at a highest frequency (66.7%), followed by *CYP3A5*6* at 24.4%, and the lowest allele frequency was 0.4% for *CYP2C9*2* (**Table 2**). Our previous *CYP3A* haplotype analysis in various black African population including Tanzanians indicated no linkage disequilibrium between the genotyped SNPs (Gebeyehu et al., 2011; Ngaimisi et al., 2014; Mutagonda et al., 2017). Likewise, there was no linkage disequilibrium between *2 and *3 alleles in *CYP2C9* and *CYP2C19* (Gebeyehu et al., 2011; Ahmed et al., 2019).

The defective variant alleles occur at lower frequencies and the number of participants homozygous for defective variant alleles were very few in our study population. Therefore, to investigate impact of genotype on plasma PZQ metabolic ratio (**Table 3**) or treatment outcomes (**Table 4**), genotypes were categorized as normal metabolizers (*1/*1), and carriers of any defective variant alleles (intermediate or slow metabolizers) for *CYP3A4*, *CYP3A5* and *CYP2C9* genotype. For *CYP2C19*, participants were genotyped for both the high activity allele (*2C19*17*) and the loss of function alleles (*2C19*2* and **3*). Therefore, CYP2C19 genotype was categorized as CYP2C19*17 carriers (ultrarapid or rapid metabolizers i.e., *17/*17 or *1/*17), normal metabolizers (*1/*1), and carriers of *2 or *3 defective variant alleles (intermediate or slow metabolizers) as recommended by Clinical Pharmacogenetics Implementation Consortium (CPIC) Guidelines for CYP2C19 (Hicks, et al., 2017).

The Effect of CYP Genotypes on PZQ, *trans*-4-OH-PZQ Concentrations and Metabolic Ratio

The overall geometric means ± SD of PZQ, t*rans*-4-OH-PZQ and t*rans*-4-OH-PZQ/PZQ in the study population were 257.0 ± 3.6, 9,289.7 ± 1.9 and 36.1 ± 3.0 ng/mL, respectively. Comparison of the geometric means of PZQ, t*rans*-4-OH-PZQ and t*rans*-4-OH-PZQ/PZQ between different CYP450 genotypes are summarized in **Table 3**. There was a significant association between PZQ concentration, t*rans*-4-OH-PZQ/PZQ and *CYP2C19* genotype

TABLE 2 | Genotypes and allele frequencies for *CYP3A4*, *CYP3A5*, *CYP2C9* and *CYP2C19* in the study population.

Genotype		Frequency N (%)
*CYP3A4*1B* (-392A > G)	*1/*1	42 (12.3)
	*1/*1B	143 (42.1)
	*1B/*1B	155 (45.6)
*CYP3A5*3* (c.6986A > G)	*1/*1	244 (71.8)
	*1/*3	84 (24.7)
	*3/*3	12 (3.5)
*CYP3A5*6* (c.14690G > A)	*1/*1	192 (56.5)
	*1/*6	130 (38.2)
	*6/*6	18 (5.3)
*CYP3A5*7* (27,131_27132insT)	*1/*1	279 (82.1)
	*1/*7	56 (16.4)
	*7/*7	5 (1.5)
*CYP2C19*2*	*1/*1	228 (67.1)
	*1/*2	103 (30.3)
	*2/*2	9 (2.6)
*CYP2C19*3*	*1/*1	328 (96.5)
	*1/*3	12 (3.5)
	*3/*3	0 (0.0)
*CYP2C19*17*	*1/*1	236 (69.4)
	*1/*17	92 (27.1)
	*17/*17	12 (3.5)
*CYP2C9*2*	*1/*1	337 (99.1)
	*1/*2	3 (0.9)
	*2/*2	0 (0.0)
*CYP2C9*3*	*1/*1	335 (98.5)
	*1/*3	5 (1.5)
	*3/*3	0 (0.0)
Allele	Minor allele	Percentage
*CYP3A4*1B*	*1B	66.7
*CYP3A5*3*	*3	15.9
*CYP3A5*6*	*6	24.4
*CYP3A5*7*	*7	9.7
*CYP2C19*2*	*2	17.8
*CYP2C19*3*	*3	1.7
*CYP2C19*17*	*17	17.1
*CYP2C9*2*	*2	0.4
*CYP2C9*3*	*3	0.7

TABLE 3 | Comparison of the geometric means of PZQ, *trans*-4-OH-PZQ concentrations (ng/mL) and metabolic ratio (*trans*-4-OH-PZQ/PZQ) between CYP450 genotypes using One-way ANOVA.

Genotype		N	PZQ GM ± SD	p-value	Trans-4-OH-PZQ	p-value	trans-4-OH-PZQ/PZQ	p-value
CYP3A4	*1/*1	40	249.5 ± 3.3	0.88	9,299.7 ± 2.1	0.99	37.2 ± 3.0	0.86
	*1B carriers	247	258.2 ± 3.6		9,289.7 ± 1.9		36.0 ± 3.0	
CYP3A5	*1/*1	77	261.2 ± 3.5	0.89	9,462.4 ± 1.0	0.77	36.2 ± 2.8	1.00
	*3, *6, *7 carriers	210	255.3 ± 3.6		9,225.7 ± 1.9		36.1 ± 3.1	
CYP2C19	*17 carriers	79	191.9 ± 3.3	0.04	9,311.1 ± 1.8	0.92	48.5 ± 3.0	0.01
	*1/*1	109	267.9 ± 3.3		9,440.6 ± 1.9		35.2 ± 2.6	
	*2, *3 carriers	99	310.5 ± 4.0		9,099.1 ± 2.0		29.3 ± 3.3	
CYP2C9	*1/*1	279	258.2 ± 3.5	0.68	9,246.9 ± 1.9	0.37	35.7 ± 2.9	0.32
	*2, *3 carriers	8	214.3 ± 4.6		11,350.1 ± 1.6		52.9 ± 5.1	

GM–Geometric mean.

TABLE 4 | Association of genotype with praziquantel efficacy (cure rates) and treatment-associated adverse events.

Genotype		Cure rates		*p* Value	Adverse events		*p* Value
		Cured N (%)	Not Cured N (%)		Yes N (%)	No N (%)	
CYP3A4	*1/*1	33 (12.0)	9 (14.1)	0.39	12 (13.2)	30 (12.0)	0.85
	*1B carriers	243 (88.0)	55 (85.9)		79 (86.8)	219 (88.0)	
CYP3A5	*1/*1	69 (25.0)	16 (25.0)	0.57	30 (33.0)	55 (22.1)	0.048
	*3, *6, or *7 carriers	207 (75.0)	48 (75.0)		61 (67.0)	194 (77.9)	
CYP2C19	*17 carriers	68 (24.6)	19 (29.7)	0.26	21 (23.1)	56 (26.5)	0.64
	*1/*1	104 (37.7)	28 (43.8)		39 (42.9)	93 (37.3)	
	*2, or *3 carriers	104 (37.7)	17 (26.6)		31 (34.1)	90 (36.1)	
CYP2C9	*1/*1	269 (97.5)	63 (98.4)	0.54	89 (97.8)	243 (97.6)	1.00
	*2, or *3 carriers	7 (2.5)	1 (1.6)		2 (2.2)	6 (2.4)	

TABLE 5 | Univariate and Multivariate logistic regression analysis for predictors of cure at 3 weeks' post-treatment.

Variable		Cured N (%)	Univariate analysis		Multivariate analysis	
			cOR (95%)	*p*-value	aOR (95%)	*p*-value
Age (years)	≤12	190 (80.9)	1	0.82		
	>12	86 (81.9)	1.07 (0.59–1.94)			
Sex	Male	127 (79.9)	1	0.57		
	Female	149 (82.3)	1.17 (0.68–2.02)			
Baseline infection intensity	Light	72 (82.8)	1			
	Moderate	126 (82.9)	0.71 (0.34–1.46)	0.35		
	Heavy	78 (77.2)	0.70 (0.37–1.31)	0.27		
Anaemia	Yes	67 (88.2)	0.51 (0.24–1.09)	0.08	0.51 (0.24–1.09)	0.08
	No	209 (79.2)	1		1	
Stunting (HAZ)	Yes	96 (83.6)	0.78 (0.43–1.41)	0.41		
	No	179 (79.9)	1			
Wasting (BAZ)	Yes	30 (88.2)	0.55 (0.19–1.61)	0.27		
	No	246 (80.4)	1			
CYP3A4	*1/*1	33 (78.6)	1	0.65		
	*1B carriers	243 (81.5)	0.83 (0.38–1.83)			
CYP3A5	*1/*1	69 (25.0)	1			
	*3,*6,*7 carriers	207 (75.0)	1.00 (0.53–1.87)	1.00		
CYP2C19	*17 carriers	68 (24.6)	1		1	
	*1/*1	104 (37.7)	0.59 (0.28–1.21)	0.15	0.58 (0.28–1.21)	0.15
	*2,*3 carriers	104 (37.7)	0.96 (0.49–1.86)	0.91	0.97 (0.49–1.88)	0.92
CYP2C9	*1/*1	269 (97.5)	1	0.65		
	*2,*3 carriers	7 (2.5)	0.61 (0.17–5.05)			

cOR- Crude odd ratio; aOR–Adjusted odd ratio.

TABLE 6 | Univariate and Multivariate logistic regression analysis for predictors of adverse events.

Variable		Adverse Events Yes N (%)	Univariate analysis		Multivariate analysis	
			cOR (95%)	*p*-value	aOR (95%)	*p*-value
Age (years)	≤12	68 (28.9)	1.45 (0.85–2.49)	0.18	1.59 (0.90–2.80)	0.11
	>12	23 (21.9)	1		1	
Sex	Male	38 (23.9)	1	0.26		
	Female	53 (29.3)	0.76 (0.47–1.23)			
Baseline infection intensity	Light	11 (12.6)	1		1	
	Moderate	40 (26.3)	0.22 (0.11–0.47)	≤0.001	0.20 (0.09–0.43)	≤0.001
	Heavy	40 (39.6)	0.55 (0.32–0.93)	0.03	0.50 (0.29–0.87)	0.01
Anaemia	Yes	25 (32.9)	1.47 (0.85–2.56)	0.17	1.43 (0.80–2.57)	0.23
	No	66 (25.0)	1		1	
Stunting (HAZ)	Yes	28 (24.1)	0.81 (0.49–1.36)	0.43		
	No	63 (28.1)	1			
Wasting (BAZ)	Yes	8 (23.5)	0.83 (0.36–1.89)	0.65		
	No	83 (27.1)	1			
CYP3A4	*1/*1	12 (28.6)	1			
	*1B carriers	79 (26.5)	0.90 (0.44–1.85)	0.78		
CYP3A5	*1/*1	30 (33.0)	1		1	
	*3,*6,*7 Carriers	61 (67.0)	0.58 (0.34–0.98)	0.04	0.62 (0.36–1.07)	0.09
CYP2C19	*17 carriers	21 (23.1)	1			
	*1/*1	39 (42.9)	1.08 (0.57–2.05)	0.81		
	*2,*3 carriers	31 (34.1)	1.32 (0.71–2.44)	0.38		
CYP2C9	*1/*1	89 (97.8)	1			
	*2,*3 carriers	2 (2.2)	0.91 (0.18–4.59)	0.91		

cOR- Crude odd ratio; aOR–Adjusted odd ratio.

($p < 0.05$). PZQ concentration was significantly higher among *CYP2C19 *2, *3* carriers than wild type (*CYP2C19 *1/*1*) and *CYP2C19 *17* carriers. The metabolic ratio (t*rans*-4-OH-PZQ/PZQ) was significantly higher among *CYP2C19 *17* carriers than those who are *CYP2C19 *1/*1* and *CYP2C19 *2, *3* carriers. There was no significant effect of *CYP3A4*, *CYP3A5* and *CYP2C9* genotypes on PZQ, t*rans*-4-OH-PZQ concentrations and t*rans*-4-OH-PZQ/PZQ ($p > 0.05$) (**Table 3**).

The Effect of CYP Genotypes on Treatment Efficacy

Overall, 81.2% (276/340) of the treated children were cured at 3-weeks post-treatment. There was no significant association between *CYP3A4*, *CYP3A5*, *CYP2C19*, and *CYP2C9* genotypes and cure rates ($p > 0.05$) (**Table 4**).

On multivariate logistic regression analysis, *CYP3A4*, *CYP3A5*, *CYP2C19* and *CYP2C9* genotypes were not significant predictors of cure at 3-weeks post-treatment. The model was a good fit with the Hosmer and Lemeshow test for the goodness of fit for multivariate analysis $\chi^2 = 6.40$ and $p = 0.60$ (**Table 5**).

The overall mean percent change in eggs counts (egg reduction) at 3 weeks' post-treatment was 101.6% ± 113.6 SD. There was no significant association between *CYP3A4*, *CYP3A5*, *CYP2C19* and *CYP2C9* genotypes and the mean percent reduction in eggs count at 3 weeks' post-treatment ($p > 0.05$) (**Supplementary Table 1**). On negative binomial regression analysis, *CYP2C9*, *CYP2C19*, *CYP3A4* or *CYP3A5* genotypes were not significant predictors of mean percent reduction in eggs count at 3 weeks' post-treatment ($p > 0.05$) (**Supplementary Table 2**).

The Effect of CYP450 Genotypes on Treatment-Associated Adverse Events

In total, 26.8% (91/340) of the treated children experienced at least one treatment-associated adverse event within 4 h post-treatment. Abdominal pain (26.5%, 90/340) and vomiting (1.8%, 6/340) were the observed adverse event among the treated children. There was no significant association of *CYP2C9*, *CYP2C19*, or *CYP3A4*, or genotypes with treatment-associated adverse events as presented in **Table 4**. However, children carrying *CYP3A5* defective alleles *(*3, *6, *7)* had more incidence of adverse events than those who are wild type (*CYP3A5 *1/*1*) ($p = 0.048$) (**Table 4** and **Table 6**).

On multivariate logistic regression analysis, *CYP3A4*, *CYP3A5*, *CYP2C19* and *CYP2C9* genotypes were not significant predictors of adverse events. Baseline infection intensity was the only significant predictor of treatment-associated adverse events ($p < 0.05$). Children with heavy infections had a significantly higher incidence of adverse events compared to those children with light and moderate infections. The model was a good fit with the Hosmer and Lemeshow test for the goodness of fit for multivariate analysis $\chi^2 = 4.43$ and $p = 0.73$ (**Table 6**).

DISCUSSION

We investigated the effect of pharmacogenetics variations on PZQ pharmacokinetics and its treatment outcomes (efficacy and adverse events) among schistosomiasis infected school-aged children. The genotype and alleles frequencies of *CYP3A4*1B*,

CYP3A5 (*3, *6, *7), *CYP2C19* (*2, *3, *17), and *CYP2C9* (*2, *3) observed in this study were similar to what was reported previously in Tanzanian populations (Dandara et al., 2001; Mutagonda et al., 2017). Our key findings include 1) significant association of *CYP2C19* genotype with plasma PZQ concentrations and its metabolic ratio (*trans*-4-OH-PZQ/PZQ) and 2) no significant effect of *CYP3A4*, *CYP3A5*, *CYP2C19*, and *CYP2C9* genotypes on schistosomiasis treatment efficacy at 3-weeks post-treatment, 3) a borderline significant association of *CYP3A5* genotype with treatment-associated adverse events, being higher among carriers of defective variant alleles (*3, *6 and *7). Studies on the effect of CYP genotypes on plasma PZQ concentrations, metabolic ratio and schistosomiasis treatment outcomes are currently lacking (Zdesenko et al., 2020). To the best of our knowledge, this is the first study to investigate the effect of pharmacogenetics variations on plasma PZQ, *trans*-4-OH-PZQ concentrations and metabolic ratio (*trans*-4-OH-PZQ/PZQ) as well as treatment efficacy and safety.

PZQ, a racemic mixture of *R* and *S* enantiomers, is metabolized by CYP3A4, CYP3A5, CYP2C19, and CYP2C9 (Wang et al., 2014). We found a significant association of *CYP2C19* genotype with PZQ concentration; significantly higher plasma PZQ concentration among children carrying *CYP2C19* defective variant alleles than *CYP2C19*1/*1* and *CYP2C19 *17* carriers (ultra-rapid metabolizers). We also found a significant association between CYP2C19 genotype and metabolic ratio (*trans*-4-OH-PZQ/PZQ), where the metabolic ratio was higher among *CYP2C19 *17* carriers than *CYP2C19* (*2, *3) carriers (**Table 3**). These findings may indicate that CYP2C19 but not CYP3A4, CYP3A5, or CYP2C9 is a major metabolic pathway for the formation of *trans*-4-OH-PZQ metabolite. Our results are in line with a previous *in vitro* study that reported CYP2C19 as a major metabolic pathway for the formation of 4-OH-PZQ metabolite (Li et al., 2003). A recent study by Nleya et al., reported CYP3A is responsible for the formation of X-OH-PZQ and not 4-OH-PZQ (Nleya et al., 2019), which further supports the findings of our study.

Our study found no significant effect of *CYP3A4*, *CYP3A5*, *CYP2C19*, and *CYP2C9* genotypes on schistosomiasis treatment efficacy (**Table 4** and **5** and **Supplementary Tables 1,2**). CYP3A4 is a major metabolizing enzyme for most drugs used in tropical infectious diseases, including PZQ. In this study, CYP3A4 genotype was not significantly associated with schistosomiasis treatment efficacy. Although not statistically significant, higher cure rates among *CYP3A4*1B* carriers than *CYP3A4*1/*1* genotype were observed (**Tables 4** and **5**). Likewise, although the association between *CYP3A4* genotype and PZQ concentrations was not statistically significant, those carrying *CYP3A4* defective alleles had high PZQ concentrations than those with wild type *(CYP3A4*1/*1)* (**Table 3**) and a high cure rate. In line with our observation, a low CYP3A4 enzyme activity has been reported previously in the Tanzanian population carrying *CYP3A4* defective alleles (Mirghani et al., 2006; Diczfalusy et al., 2008). Furthermore, a recent study conducted among the Tanzanian population reported a linkage disequilibrium (LD) between *CYP3A4 *1B* and *CYP3A5 *1*, which resulted in a low CYP3A4 enzyme activity (Mutagonda et al., 2017), which may explain the observed high cure rate in children carrying *CYP3A4* defective alleles.

CYP3A5 is highly expressed among African populations than any other population, and its genotype determines the total CYP3A enzyme activity among black Africans (Gebeyehu et al., 2011; Ngaimisi et al., 2014). The *CYP3A5* defective alleles (*3, *5, *7) are associated with a low CYP3A enzyme activity in Tanzanian (Diczfalusy et al., 2008) and other African populations (Gebeyehu et al., 2011). In this study, *CYP3A5* genotype was not significantly associated with schistosomiasis treatment efficacy, although children carrying *CYP3A5* defective alleles (*3, *6, *7) were more cured than those with wild type genotype (CYP3A5 *1/*1) (**Tables 4** and **5**).

Despite a significant association between *CYP2C19* genotype and PZQ concentration and its metabolic ratio, *CYPC19* genotype was not significantly associated with schistosomiasis treatment efficacy among infected Tanzanian children following PZQ treatment. Although not statistically significant, children who carry *CYP2C19* defective alleles (*2, *3) were more cured than those who were *CYP2C19 *17* carriers (ultra-rapid metabolizers) (**Table 4**). The observed genotypes and alleles frequencies of *CYP2C19* were similar to previous studies conducted among Tanzania populations (Dandara et al., 2001). Similarly, *CYP2C9* genotype was not significantly associated with schistosomiasis treatment efficacy in the study population. The frequencies of *CYP2C9* defective alleles (*2, *3) were found to be very low (<1%), similar to reports from other African populations (Bains, 2013). Since defective variant alleles of both *CYP2C9* and *CYP2C19* occur at a lower frequency in the black African population, larger sample size studies are needed to explore further the impact of genetic variation on schistosomiasis treatment outcome in the sub-Sharan Africa population.

Previous studies reported the importance of pharmacogenetic variations for treatment-associated adverse events among HIV and Tuberculosis infected (Mugusi et al., 2012; Ngaimisi et al., 2013; Yimer et al., 2014) or cancer patients (Ahmed et al., 2019) in Sub-Saharan Africa. Factors such as age, sex and pre-treatment infection intensity have been reported previously to affect schistosomiasis treatment outcomes (Zwang et al., 2017). In our study, baseline infection intensity and not *CYP3A4*, *CYP2C19* and *CYP2C9* genotypes was a significant predictor of adverse events following PZQ treatment. Baseline infection intensity was a significant predictor of adverse events following PZQ treatment similar to what was reported in previous studies (Erko et al., 2012; Mnkugwe et al., 2019). Heavily infected children experienced significantly more incidence of adverse events than children with light and moderate infections (**Table 6**). Unlike previous studies we found no significant association of anaemia or age with adverse events following PZQ treatment (Zwang et al., 2017; Mnkugwe et al., 2019). Interestingly in a univariate analysis, carriers of *CYP3A5* defective variant alleles had significantly higher adverse events (**Tables 4** and **6**). Children carrying *CYP3A5* defective alleles (*3, *6, *7) had more incidence of adverse events than those CYP3A5*1/*1 genotype (**Table 4**).

We recently reported significantly higher plasma exposure of S-PZQ than R-PZQ following treatment of PZQ in school children

(Minzi et al., 2021). S-PZQ, the non-active component of PZQ, is the main contributor of the unpleasant taste of the drug causing nausea and vomiting in children (Meyer et al., 2009). We found no significant association of *CYP3A5* genotype with PZQ concentration or *trans*-4-OH-PZQ, the main metabolite of R-PZQ. Association of *CYP3A5* defective variant allele with a higher incidence of adverse events may indicate the importance of CYP3A for the metabolism of S-PZQ or other metabolites of R-PZQ not quantified in the present study, and hence our study limitation. Other CYP3A-dependent monohydroxy PZQ metabolites such as X-OH-PZQ reported recently (Nleya et al., 2019) may be responsible for the observed association of *CYP3A5* genotype with adverse events in our study. Future studies involving quantification of both R- and S-PZQ with their respective metabolites is needed to further explore the relevance of pharmacogenetic variation for treatment-associated adverse events.

CONCLUSIONS

We report a significant association of *CYP2C19* genotype with plasma PZQ exposure and its metabolic ratio (*trans*-4-OH-PZQ/PZQ) in schistosomiasis infected children. Although no significant effect of *CYP3A4*, *CYP2C19* and *CYP2C9* genotypes was observed on schistosomiasis treatment efficacy and adverse events, the borderline association of *CYP3A5* genotype with treatment-associated adverse events requires further investigation. For the first time, our study highlights the importance of pharmacogenetic variation for pharmacokinetics and treatment outcomes of schistosomiasis, a neglected tropical disease affecting millions of children in sub-Sharan Africa.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the National Institute for Medical Research (NIMR), Tanzania (Ref. No. NIMR/HQ/R.8a/Vol.IX/2343), Muhimbili University of Health and Allied Sciences (Ref. No. 2016-5-25/AEC/Vol.X/03) and Stockholm Ethics Committee (Ref.No. 2020-00845). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

OM, AK, and EA participated in funding acquisition; RM, SK, OM, AK, and EA participated in protocol development; RM, SK, OM, AK, and EA data collection, formal data analysis and interpretation; RM wrote the original draft; RM, SK, OM, AK, and EA review and editing of the manuscript; all authors have read and approved the final version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.712084/full#supplementary-material.

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Organic Anion Transporting Polypeptide 2B1 (OATP2B1) Genetic Variants: *In Vitro* Functional Characterization and Association With Circulating Concentrations of Endogenous Substrates

Samantha Medwid[1,2], *Hayley R. Price*[1], *Daniel P. Taylor*[1], *Jaymie Mailloux*[1,2], *Ute I. Schwarz*[1,2], *Richard B. Kim*[1,2,3] *and Rommel G. Tirona*[1,2]*

[1]*Department of Physiology & Pharmacology, University of Western Ontario, London, ON, Canada,* [2]*Division of Clinical Pharmacology, Department of Medicine, University of Western Ontario, London, ON, Canada,* [3]*Department of Oncology, Schulich School of Medicine, University of Western Ontario, London, ON, Canada*

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***Correspondence:**
Rommel G. Tirona
rommel.tirona@schulich.uwo.ca

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Organic anion transporting polypeptide 2B1 (OATP2B1, gene *SLCO2B1*) is an uptake transporter that is thought to determine drug disposition and in particular, the oral absorption of medications. At present, the clinical relevance of *SLCO2B1* genetic variation on pharmacokinetics is poorly understood. We sought to determine the functional activity of 5 of the most common missense OATP2B1 variants (c.76_84del, c.601G>A, c.917G>A, c.935G>A, and c.1457C>T) and a predicted dysfunctional variant (c.332G>A) *in vitro*. Furthermore, we measured the basal plasma concentrations of endogenous OATP2B1 substrates, namely estrone sulfate, dehydroepiandrosterone sulfate (DHEAS), pregnenolone sulfate, coproporphyrin I (CPI), and CPIII, and assessed their relationships with *SLCO2B1* genotypes in 93 healthy participants. Compared to reference OATP2B1, the transport activities of the c.332G>A, c.601G>A and c.1457C>T variants were reduced among the substrates examined (estrone sulfate, DHEAS, CPI, CPIII and rosuvastatin), although there were substrate-dependent effects. Lower transport function of OATP2B1 variants could be explained by diminished cell surface expression. Other OATP2B1 variants (c.76-84del, c.917G>A and c.935G>A) had similar activity to the reference transporter. In the clinical cohort, the *SLCO2B1* c.935G>A allele was associated with both higher plasma CPI (42%) and CPIII (31%) concentrations, while *SLCO2B1* c.917G>A was linked to lower plasma CPIII by 28% after accounting for the effects of age, sex, and *SLCO1B1* genotypes. No association was observed between *SLCO2B1* variant alleles and estrone sulfate or DHEAS plasma concentrations, however 45% higher plasma pregnenolone sulfate level was associated with *SLCO2B1* c.1457C>T. Taken together, we found that the impacts of OATP2B1 variants on transport activities *in vitro* were not fully aligned with their associations to plasma concentrations of endogenous substrates *in vivo*. Additional studies are required to determine whether circulating endogenous substrates reflect OATP2B1 activity.

Keywords: drug transporter, genetic variant, endogenous substrates, organic anion transporting polypeptide 2B1 (OATP2B1), pharmacogenenomics and personalised medicine

INTRODUCTION

Organic anion transporting peptide 2B1 (OATP2B1, previously known as OATP-B, gene name *SLCO2B1*) is a member of the solute transporting carrier (SLC) superfamily. OATP2B1 is involved in the cellular uptake of a wide variety of drugs including 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-Co-A) reductase inhibitors and fexofenadine (Kobayashi et al., 2003; Nozawa et al., 2004), as well as endogenous compounds such as steroid hormone conjugates (estrone sulfate, dehydroepiandrosterone sulfate (DHEAS), and pregnenolone sulfate), coproporphyrins (CP) and thyroid hormones (Tamai et al., 2000; Kullak-Ublick et al., 2001; Pizzagalli et al., 2003; Grube et al., 2006a; Bednarczyk and Boiselle, 2016; Shen et al., 2016; Meyer Zu Schwabedissen et al., 2018). OATP2B1 is ubiquitously expressed throughout the body in organs including intestine, liver, kidney, brain, heart, skeletal muscle, lung, placenta, pancreas and macrophages (Tamai et al., 2000; Kullak-Ublick et al., 2001; St-Pierre et al., 2002; Grube et al., 2006b; Niessen et al., 2009; Seki et al., 2009; Knauer et al., 2010; Hussner et al., 2015; Kim M. et al., 2017; Nakano et al., 2019). It is generally appreciated that intestinal OATP2B1 is involved in the oral absorption of medications as its inhibition by fruit juices is thought to reduce the bioavailability of substrate drugs including fexofenadine and celiprolol in humans (Dresser et al., 2002; Lilja et al., 2003). Indeed, pharmacokinetic studies in OATP2B1 knockout mice convincingly revealed a role of this transporter in the oral absorption of some substrate drugs, as well as a target of food- and drug-drug interactions (Medwid et al., 2019; Chen et al., 2020). Although there is significant experimental support for the relevance of intestinal OATP2B1 to drug absorption (McFeely et al., 2019), the impact of this transporter on drug distribution and elimination in other tissues where it is also expressed, remains significantly less understood (Kinzi et al., 2021).

Genetic variations and in particular, nonsynonymous single nucleotide variants (SNV) in drug transporters can be responsible for interindividual differences in drug response (Yee et al., 2018). Indeed, a SNV in the liver-specific OATP1B1 transporter (*SLCO1B1* c.521T>C), has become an established clinical pharmacogenetic marker that predicts systemic drug exposure (Niemi et al., 2011) and in some instances, treatment outcomes (SEARCH Collaborative Group et al., 2008; Trevino et al., 2009). For the most part, *in vitro* studies have consistently shown that the OATP1B1 c.521T>C (*5) variant has reduced activity (Tirona et al., 2001), which is mechanistically in keeping with the well-recognized influence on clinical pharmacokinetics and drug responses. In contrast, the pharmacological and therapeutic relevance of *SLCO2B1* genetic variation is less clear despite numerous clinical and *in vitro* studies examining the potential impacts.

Associations between the pharmacokinetics or responses of OATP2B1 substrate drugs for the most common *SLCO2B1* missense SNVs, c.935G>A and c.1457C>T (global mean allelic frequencies of 17.6 and 8.6%, respectively), have been reported in many studies, however their results have not always been consistent. For instance, with the most common *SLCO2B1* c.935G>A variant (*3 allele), montelukast plasma concentrations were lower in participants carrying the variant allele in some studies (Mougey et al., 2009; Mougey et al., 2011) but not others (Kim et al., 2013; Tapaninen et al., 2013). The *SLCO2B1* c.935G>A variant did not associate with plasma rosuvastatin concentrations in some studies (DeGorter et al., 2013; Kim TE. et al., 2017), although this genetic marker was linked to reduced lipid lowering effects. (Kim TE. et al., 2017). In prostate cancer patients undergoing androgen deprivation therapy, *SLCO2B1* c.935G>A variant carriers were compellingly shown to have shorter time to progression in different cohorts (Yang et al., 2011; Fujimoto et al., 2013; Wang et al., 2016; Hahn et al., 2019).

With respect to the *SLCO2B1* c.1457C>T variant allele and pharmacokinetic associations, contradicting studies have also been reported. For example, the *SLCO2B1* c.1457C>T variant was associated with having higher, lower or no impact on systemic exposures of fexofenadine (Akamine et al., 2010; Imanaga et al., 2011; Kashihara et al., 2017). Moreover, in one study the *SLCO2B1* c.1457C>T variant was linked to lower circulating concentrations of celiprolol (Ieiri et al., 2012) but no association was observed in another report (Kashihara et al., 2017). In a recent study, 22% lower concentration of the 3S-5R-fluvastatin enantiomer was observed in subjects with the *SLCO2B1* c.1457C>T variant, per allele (Hirvensalo et al., 2019).

In vitro studies have similarly provided heterogeneous results for the transport activity of OATP2B1 genetic variants. The OATP2B1 c.935G>A variant has mostly been associated with reduced transport activity, but its functional impact appears to be highly substrate- and experimental model-dependent (Nozawa et al., 2002; Ho et al., 2006; Yang et al., 2011; Nies et al., 2013; Yang et al., 2020). With the OATP2B1 c.1457C>T variant, *in vitro* studies are also conflicting with some reporting reduced transport activity (Nozawa et al., 2002; Nies et al., 2013), while for others, there was enhanced function (Ho et al., 2006; Yang et al., 2020), again with substrate-dependent effects. Taken together, because of all the divergent and inconsistent findings from clinical and biochemical studies, the potential impacts of *SLCO2B1* genetic variation to transporter activity remains to be understood.

The circulating concentrations of certain endogenous drug transporter substrates have become clinical biomarkers of transporter activity, especially in the context of predicting altered pharmacokinetics with drug-drug interactions and disease states (Rodrigues and Rowland, 2019). Indeed, coproporphyrin I (CPI) is a validated endogenous biomarker of OATP1B (OATP1B1 and OATP1B3) activity (Lai et al., 2016; Shen et al., 2016). Interestingly however, is that individuals homozygous for the reduced function *SLCO1B1* c.521T>C variant have about 2-fold higher baseline plasma CPI concentrations (Yee et al., 2018; Mori et al., 2019; Suzuki et al., 2021). Furthermore, there is 1.4-fold higher plasma estrone sulfate concentration in carriers of the *SLCO1B1* c.521T>C variant allele (van der Deure et al., 2008). These findings with *SLCO1B1* raise the possibility that the *in vivo* relevance of *SLCO2B1* genetic variation can be addressed by examining the concentrations of its circulating endogenous substrates.

In this report, we evaluated the *in vitro* transport activity of the most common OATP2B1 genetic variants in global populations [c.935G>A (*3), c.1457C>T, c.76_84del, c.917G>A, and c.601G>A] with the motivation of clarifying whether these cause functional effects. Furthermore, we explored the possibility that genetic variations in *SLCO2B1* are associated with the plasma concentrations of its endogenous substrates, namely, estrone sulfate, DHEAS, pregnenolone sulfate, CPI and CPIII. Our key findings are that circulating CPI and CPIII concentrations are greater in healthy individuals carrying the common *SLCO2B1* c.935G>A variant allele. However, there was a lack of significant impact of the OATP2B1 c.935G>A variant on substrate transport activity when tested *in vitro*.

MATERIALS AND METHODS

Reagents

Rosuvastatin, d5-estrone sulfate, d5-DHEAS, $^{15}N_4$-CPI, d8-CPIII and d6-rosuvastatin were purchased from Toronto Research Chemicals (Toronto, ON, Canada). CPI and CPIII were obtained from Frontier Specialty Chemicals (Logan, UT, United States). All other chemicals, unless otherwise stated, were obtained from Sigma-Aldrich (St. Louis, MO).

Variant OATP2B1 Plasmid Construction

hOATP2B1 reference sequence in pcDNA3.1 (Invitrogen, Carlsbad, CA, United States) expression plasmid was prepared using methods outlined previously (Tirona et al., 2003). OATP2B1 variant expression plasmids were created using QuikChange II site-directed mutagenesis kit (Agilent Technologies; Santa Clara, CA, United States) and primers found in **Supplementary Table S1**, following manufacturer's protocol. Reference OATP2B1 sequence and presence of polymorphisms were verified by Sanger sequencing.

Transient Overexpression of OATP2B1 and Variants in Cultured Cells

Human embryonic kidney type T (HEK293T) cells were purchased from American Type Culture Collection (Manassas, VA, United States) for use in transient transfection studies. HEK293 cells are routinely used in drug transporter studies as they are efficiently transfected and express relatively low basal expression of drug transporters (Ahlin et al., 2009). Previous studies which examined the transport function of OATP2B1 genetic variants have used the HEK293 cell line for transporter overexpression (Nozawa et al., 2002; Nies et al., 2013; Yang et al., 2020). HEK293T cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Thermo Scientific, Grand Island, NY, United States) supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, and 2 mM L-glutamine (Invitrogen), at 37°C, 5% CO_2. For HEK293T cell transport experiments, cells were grown on poly-L-lysine-coated 24-well plates. After 24 h, cells were transfected with blank insert expression plasmids (vector control) or expression plasmids containing transporter cDNA inserts (1 μg DNA/well) using Lipofectamine 3000 (Invitrogen), according to our previously described method (Medwid et al., 2019). Cells were incubated with transfection plasmids for 16 h prior to experiments.

Solute Transport by Reference OATP2B1 and Variants in Vitro

HEK293T cells were plated onto 24-well culture plates for solute uptake experiments. Estrone sulfate, DHEAS, CPI, CPIII or rosuvastatin (each at 1 μg/ml final concentration) was dissolved in modified Krebs-Henseleit buffer (KHB) (1.2 mM $MgSO_4$, 0.96 mM KH_2PO_4, 4.83 mM KCl, 118 mM NaCl, 1.53 mM $CaCl_2$, 23.8 mM $NaHCO_3$, 12.5 mM 4-[2-hydroxyethyl]-1-piperazineethanesulfonic acid, 5 mM glucose) at pH 6. Cultured cells were treated with substrates (200 μl) for 10–30 min at 37°C, 5% CO_2. Thereafter, cells were washed three times rapidly with ice-cold phosphate-buffered saline (PBS). Cells were lysed using 200 μl of acetonitrile (for estrone sulfate, DHEAS, or rosuvastatin analyses) or 12 M formic acid (for CPI and CPIII analyses) spiked with internal standards (d5-estrone sulfate 100 ng/ml, d5-DHEAS 100 ng/ml, $^{15}N_4$-CPI 100 nM, or d6-rosuvastatin 20 ng/ml). Cell lysates were centrifuged for 10 min at 13,500 rpm in a microcentrifuge and supernatants were dried in a SpeedVac (Thermo Fisher) at 45°C and resuspended in 100–200 μl mobile phase. Residues were analyzed for estrone sulfate, DHEAS, CPI, CPIII and rosuvastatin by liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods described below. The specific functional activity of transfected wildtype OATP2B1 and its variants were determined after subtraction of the cellular substrate uptake of blank vector control transfected cells.

Cell Surface Protein Biotinylation

After transfection of HEK293T cells, sulfo-NHS-SS-Biotin (Thermo Scientific) diluted 0.5 mg/ml/well in PBS containing 100 μM $CaCl_2$ and 2.12 mM $MgCl_2$ (PBS/Mg/Ca) was added to cells and incubated for 1 h at 4°C. Cells were then washed with ice-cold PBS/Mg/Ca containing 50 μM glycine (PBS/Mg/Ca/glycine) 3 times followed by a 20-min incubation with PBS/Mg/Ca/glycine. Cells were lysed in RIPA buffer [10 mM Tris, 150 mM NaCl, 1.27 mM EDTA, 0.1% (w/v) SDS, and 10% (v/v) Triton X-100] containing protease inhibitors and cell lysate was sonicated. Streptavidin-agarose (Thermo Scientific) was added to a proportion of cells and rocked for 1 h at room temperature. The remaining cell lysate in RIPA buffer was used to determine total protein concentrations. Streptavidin-agarose samples were then centrifuged for 3 min at 18,000 g and the pellet washed 3 times with ice-cold RIPA buffer. Pellets were subsequently rocked with 4X LDS sample buffer (Invitrogen) containing 5% 2-β-mercaptoethanol and protease inhibitors for 30 min. Thereafter, samples were centrifuged and supernatant was collected and stored until sodium dodecylsulfate - polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting.

Western Blot

Cell surface biotinylated and total protein samples were analyzed by SDS-PAGE using 4–12% gradient gels (NuPage, Invitrogen). After transfer to polyvinylidene difluoride membranes, blots were

probed with human OATP2B1 (Cat. No. H-189, Santa Cruz, Dallas, TX), GAPDH (Cat. No. sc-4772, Santa Cruz) or Na+/K+ ATPase (Cat. No. 3010S, Cell Signalling, Danvers, MA, United States) and visualized using horseradish-peroxidase labeled anti-mouse or anti-rabbit antibodies (Cell Signaling) and chemiluminescence reagent (Amersham ECL Select, GE Healthcare) on an ImageQuant LAS 500 (GE Healthcare, Mississauga, ON, Canada).

Participants and Plasma Samples

Morning (~8 am) blood samples were obtained after overnight fast from 93 healthy participants recruited from previously reported studies (Woolsey et al., 2016; McLean et al., 2018; Tirona et al., 2018). These studies were approved by the Human Research Ethics Board at University of Western Ontario (London, ON, Canada) and all participants provided informed written consent. Participant demographics can be found in **Table 3**.

Liquid Chromatography-Tandem Mass Spectrometry

Estrone Sulfate, Pregnenolone Sulfate and DHEAS Assay. Plasma samples (100 µl) were combined with internal standard solution (300 µl) containing d5-estrone sulfate (100 ng/ml) and d5-DHEAS (100 ng/ml) in acetonitrile. Samples were vortexed and centrifuged at 13,000 g and 4°C for 15 min. The resulting supernatant was transferred to a microcentrifuge tube for drying in a SpeedVac. The residue was reconstituted in mobile phase (100 µl) containing 0.1% ammonium hydroxide in water and 0.1% ammonium hydroxide (90%/10%) for injection into the liquid chromatograph. Analytes were separated by liquid chromatography (Agilent 1200; Agilent; San Clara, CA, United States) using a Hypersil Gold column (50 × 3 mm, 5 µm, Thermo Fisher Scientific) following 60 µl sample injection. A mobile phase of 0.1% v/v ammonium hydroxide in water (A) and 0.1% v/v ammonium hydroxide in acetonitrile (B) was used, with an elution gradient of 10% B from 0–1.0 min, 10–90% B from 1.0–4.5 min, 90% B from 4.5–5.25 min, 90–10% B from 5.25–5.8 min and 90% B from 5.8–6.0 min, for a run time of 6 min and flow rate of 0.5 ml/min. The heated electrospray ionization source of the triple quadrupole mass spectrometer (Thermo TSQ Vantage; Thermo Fisher Scientific) was operated in negative mode (4000 V, 350°C) with collision energy set at 25 V. Additional ionization source conditions used were as follows: 40 arbitrary units for sheath gas pressure, 15 arbitrary units for auxiliary gas pressure and 350°C for capillary temperature. Selected reaction monitoring for estrone sulfate, d5-estrone sulfate, DHEAS, d5-DHEAS, and pregnenolone sulfate was performed using mass transitions 349.2→268.3 m/z, 354.1→273.4 m/z, 367.1→97.0 m/z, 372.1→98.0 m/z, and 395.1→97.0 m/z, respectively. Estrone sulfate/d5-estrone sulfate, DHEAS/d5-DHEAS and pregnenolone sulfate had retention times of 2.84, 2.91, and 3.13 min, respectively. Calibration samples containing estrone sulfate 0–4 ng/ml, pregnenolone sulfate 0–4,000 ng/ml and DHEAS 0–4,000 ng/ml were prepared in PBS from ethanol stock solutions and processed similarly as above.

CPI and CPIII Assay. CPI concentrations were measured according to a published method (Lai et al., 2016) with modifications. Plasma samples (200 µl) were combined with internal standard solution (100 µl) containing d8-CPIII 1.5 µmol/ml in 12 M formic acid. Ethyl acetate (1 ml) was combined, and samples were vortexed for 1 min and centrifuged at 13,000 g and 4°C for 15 min. The resulting organic layer (760 µl) was transferred to a microcentrifuge tube for drying in a SpeedVac. The residue was reconstituted in mobile phase (100 µl) containing 0.1% formic acid in water and 0.1% formic acid in acetonitrile (80%/20%) for injection into the liquid chromatograph. Solutes were separated on a Zorbax Eclipse Plus C18 column (100 mm × 2.1 mm, 1.8 µm). A mobile phase of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) was used, with an elution gradient of 20% B from 0–0.5 min, 20–71% B from 0.5–9 min, 71–98% B from 9–10 min, 98% B from 10–10.25, 98–20% B from 10.25-5–11.25 min and 20% B from 11.25–12.5 min, for a run time of 12.5 min and flow rate of 0.2 ml/min. Mass spectrometry detection was carried out on a TSQ Vantage triple-quadrupole instrument set in positive mode for detection of CPI/CPIII, d8-CPIII and $^{15}N_4$-CPI with transitions 655.4 → 596.4 m/z, 659.3 → 600.3 m/z and 663.0 → 602.4 m/z. CPI/$^{15}N_4$-CPI and CPIII/d8-CPIII eluted at 8.29 and 8.54 min, respectively. Calibration samples containing CPI 0–10 nM and CPIII 0–1 nM were prepared in PBS from DMSO stock solutions and processed similarly as above. All experiments and analytical procedures involving CPI and CPIII were performed under low light conditions.

Rosuvastatin Assay. Analytes were separated by liquid chromatography using Hypersil Gold (50 × 3 mm, 5 µm) following 50 µl sample injection. A mobile phase of 0.1% v/v formic acid in water (A) and 0.1% v/v formic acid in acetonitrile (B) was used, with an elution gradient of 25% B from 0–1.0 min, 25–40% B from 1.0–6.0 min, 40–25% B from 6.0–7.0 min, and 25% B from 7.0–8.0 min, for a run time of 8 min and flow rate of 0.5 ml/min. The heated electrospray ionization source of the TSQ Vantage triple-quadrupole mass spectrometer was operated in positive mode (4500 V, 350°C) with collision energy set at 25 V. Additional ionization source conditions used were as follows: 40 arbitrary units for sheath gas pressure, 15 arbitrary units for auxiliary gas pressure and 350°C for capillary temperature. Selected reaction monitoring for rosuvastatin and d6-rosuvastatin was performed using mass transitions 482.1→258.2 m/*z*, and 488.0→264.3 m/*z*, respectively. Rosuvastatin and d6-rosuvatatin had a retention time of 4.6 min.

Genotyping

Volunteers were genotyped by TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA, United States) for *SLCO2B1* c.76_84del (rs72408262; C_99453792_10), *SLCO2B1* c.601G>A (rs35199625; C_25606765_20), *SLCO2B1* c.917G>A (rs78825186; C_105413676_20), *SLCO2B1* c.935G>A rs12422149; C_3101331_10), *SLCO2B1* c.1457C>T (rs2306168; C_16193013_20), *SLCO1B1* c.388A>G (rs2306283; C_1901697_20), *SLCO1B1* c.521C>T (rs4149056; C_30633906_10), *ABCG2* (Breast Cancer Resistance Protein,

TABLE 1 | *SLCO2B1* SNVs studied and their allele frequencies.

Designation	Nucleotide change	Protein change	CADD score[a]	REVEL score[b]	MetaLR score[b]	Minor allele frequency[c]			
						Global	Caucasian	African	East asian
rs60113013	c.76_84del	p.Glu26_Thr28del	14.9	NA	NA	0.0293	0.0187	0.0032	0.1021
rs142693902	c.332G>A	p.Arg111Gln	27.8	0.632	0.436	0.0014	0.0007	0.0001	0.0000
rs35199625	c.601G>A	p.Val201Met	22.8	0.149	0.01	0.0198	0.0124	0.0022	0.0577
rs78825186	c.917G>A	p.Arg306His	9.4	0.111	0.141	0.0146	0.0226	0.0036	0.0001
rs12422149	c.935G>A	p.Arg312Gln	7.7	0.026	0	0.1759	0.1036	0.0910	0.3261
rs2306168	c.1457C>T	p.Ser486Phe	12.0	0.022	0	0.0826	0.0244	0.3365	0.2250

[a]*Combined Annotation Dependent Depletion (CADD) score was obtained from https://cadd.gs.washington.edu.*
[b]*Rare Exome Variant Ensemble Learner (REVEL) and MetaLR score was obtained from https://m.ensembl.org.*
[c]*Allele frequencies sourced from gnomAD database, https://gnomad.broadinstitute.org.*
NA, not applicable.

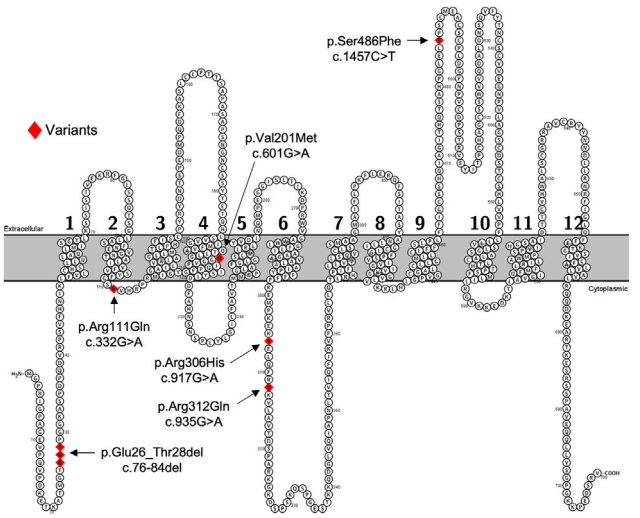

FIGURE 1 | Predicated 2-D structure of OATP2B1 full length transcriptional variant. Genetic variants of interest are highlighted in red and indicated by arrows with residue number and amino acid change. The predicted 2-dimensional membrane topology model of OATP2B1 was generated using Protter interactive protein visualization software (https://wlab.ethz.ch/protter/start/).

BCRP) c.421>A (rs2231141; C_15854163_70), *CYP* (Cytochrome P450) *2C9*2* (rs1799853; C_25625805_10), *CYP2C9*3* (rs1057910; C_27104891_10), *ABCC2* (Multidrug Resistance Protein 2, MRP2) c.1249G>A (rs2273697; C_22272980_20) and *ABCC2* c.-24C>T (rs717620; C_2814642_10).

Statistics

Unpaired, two-tailed, student's t-test was used to assess differences between the transport activities of variants and reference OATP2B1. Univariate analysis with unpaired student's t-test was used to compare plasma endogenous OATP2B1 substrate concentrations among wildtype and variant carriers (heterozygous and homozygous). Multiple linear regression was used to determine the contributions of participant genotypes and demographic variables to the log-transformed plasma endogenous OATP2B1 substrate concentrations. *A priori* statistical significance was set at a p-value of <0.05. All statistical analysis was conducted using GraphPad Prism 9 (La Jolla, CA, United States).

RESULTS

Transport Activity of OATP2B1 Genetic Variants

We selected the five most common nonsynonymous OATP2B1 genetic variants with global allelic frequencies greater than 1% for *in vitro* functional assessment: c.76_84del, c.601G>A, c.917G>A, c.935G>A, and c.1457C>T (**Table 1**; **Figure 1**). Predicted deleteriousness or pathogenicity for the common OATP2B1 genetic variants based on computational ensemble models are shown in **Table 1**. The Combined Annotation Dependent Depletion (CADD) scores range in value from 0 to 100, with greater values reflecting higher probability of deleteriousness of a variant. The Rare Exom Variant Ensemble Learner (REVEL) and Meta-Logistic-Regression (MetaLR) models provide scores with values ranging from 0 to 1, with higher values predicting pathogenicity/deleteriousness. We included another rare genetic variant, OATP2B1 c.332G>A (global allelic frequency 0.0014) in the *in vitro* study as a potential positive (deleterious) control with high CADD, REVEL and MetaLR scores (**Table 1**). The OATP2B1 c.601G>A variant was the only other variant that the *in silico* models predict to be potentially deleterious/pathogenic. The transport activities of the OATP2B1 variants were determined by assessing cellular accumulation of the endogenous substrates estrone sulfate, DHEAS, CPI, CPIII as well as the substrate drug rosuvastatin, in transiently transfected cells. OATP2B1-mediated cellular accumulation of substrates was evidenced by 9.5-, 1.5-, 2.0-, 5.2--and 6.5-fold greater cellular uptake for estrone sulfate, DHEAS, CPI, CPIII and rosuvastatin, respectively, when compared to blank vector control cells (**Figure 2**). The following summarizes the OATP2B1 variants with altered transport compared to wildtype according to substrate. OATP2B1-mediated estrone sulfate transport was significantly lower with OATP2B1 variants c.332G>A (79.2%) and c.1457C>T (29.3%) (**Figure 2A**). The variants c.332G>A, c.601G>A and c.1457C>T had lower OATP2B1-mediated DHEAS cellular accumulation by 43.4, 45.9 and 45.1%, respectively (**Figure 2B**). OATP2B1-mediated CPI uptake was lower by 75.9% with the c.1457C>T variant compared to

reference (**Figure 2C**). For CPIII, there was lower OATP2B1-mediated transport for variants c.76-84del (18.2%), c.332G>A (77.4%), c.601G>A (32.5%), c.1457C>T (45.6%) compared to reference (**Figure 2D**). OATP2B1 c.76-84del had greater OATP2B1-mediated rosuvastatin cellular accumulation by 25%, while c.332G>A, c.601G>A, c.935G>A and c.1457C>T had lower transporter-mediated rosuvastatin cellular accumulation by 28.3, 45.0, 9.9, and 31.6%, respectively (**Figure 2E**). Across all substrates, the OATP2B1 c.1457C>T variant was found to have reduced transport activity compared to OATP2B1 reference. Lower transport activity was also generally observed for the OATP2B1 c.332G>A and c.601G>A variants, however, this was not statistically significant for all substrates. Overall, the OATP2B1 c.76-84del, c.917G>A and c.935G>A variants were not particularly different in transport activity compared to the reference transporter.

Estrone Sulfate and CPIII Transport Kinetics by OATP2B1 Genetic Variants

OATP2B1-mediated transport kinetics were further evaluated for the nonsynonymous variants with estrone sulfate and CPIII. Correcting for cellular accumulation of solutes in the vector control cells, the maximal uptake rates (V_{max}), affinities (K_m) and estimated uptake clearance (V_{max}/K_m) for OATP2B1 reference and variants are shown in **Table 2**. With estrone sulfate transport, the V_{max} and K_m values for OATP2B1 variants c.332G>A and c.1457C>T could not be determined as saturable kinetics were not evident. Assuming non-saturable, linear OATP2B1 transport, the c.332G>A and c.1457C>T variants had markedly reduced uptake clearance than reference OATP2B1. For CPIII, the OATP2B1 c.332G>A variant had clearly altered transport kinetics compared to reference OATP2B1, with a reduction of V_{max} by 73%.

Cell Surface Expression of OATP2B1 Variants

Total and cell surface protein expression of OATP2B1 reference and variants in transfected HEK293T cells were examined by western blot. Cell-surface expression of OATP2B1 was absent in blank vector transfected HEK293T cells (**Supplementary Figure S1**). When normalized to Na^+/K^+ ATPase, cell surface protein expression of OATP2B1 c.332G>A, c.601G>A, c.935G>A and c.1457C>T were decreased significantly by 51, 72, 37, and 83% compared to OATP2B1 reference, respectively (**Figure 3**; **Supplementary Figure S1**).

Study Cohort for Circulating OATP2B1 Substrates

Plasma samples were obtained from 93 healthy volunteers for analysis. The median age was 25, 40.9% were male and the mean weight was 69.8 kg. Of the 93 participants, 69 were Caucasian, 20 East Asian, and 4 African. Allelic frequencies of each *SLCO2B1* variant in the cohort were 0.027, 0.016, 0.027, 0.123, and 0.118 for c.76-84del, c.601G>A, c.917G>A, c.935G>A and c.1457C>T, respectively (**Table 3**). No deviations from Hardy-Weinberg were seen for *SLCO2B1* genotypes. The allelic frequencies for *SLCO2B1* variants in the study cohort differed by race (**Table 3**) and were comparable to that reported in the Genome Aggregation Database (gnomAD) database (Karczewski et al., 2020) (**Table 1**). For example, the *SLCO2B1* c.935G>A and c.1457C>T variants were more frequent in East Asian than Caucasian participants (**Table 3**).

Effects of Demographic Factors on Plasma Endogenous OATP2B1 Substrate Concentrations

Median plasma concentrations (range) of estrone sulfate, DHEAS, pregnenolone sulfate, CPI and CPIII were 0.73 ng/ml (0.04–3.74 ng/ml), 1826 ng/ml (82–6,515 ng/ml), 52.1 ng/ml (9.4–112.3 ng/ml), 0.92 nM (0.29–3.25 nM) and 0.12 nM (0.04–0.21 nM), respectively (**Figure 4**). Univariate analyses were performed to compare OATP2B1 endogenous substrate concentrations with demographic factors (age, sex, race). Estrone sulfate concentrations were not associated with age, sex, or race (**Figure 4A**). Lower DHEAS concentrations were observed with increasing age as was for female compared to male sex, and for Caucasian compared to East Asian race (**Figure 4B**). Similarly, younger age and male sex was associated with greater concentrations of pregnenolone sulfate (**Figure 4C**). Lastly, CPI and CPIII concentrations were not associated with age, however, the levels of both compounds were greater in males compared to females, and in East Asians compared to Caucasians (**Figures 4D,E**).

Univariate Analysis of Genetic Variations on Plasma Endogenous OATP2B1 Substrate Concentrations

We examined whether *SLCO2B1* variants c.76-84del, c.601G>A, c.917G>A, c.935G>A, and c.1457C>T were associated with plasma concentrations of OATP2B1 endogenous substrates. The *SLCO2B1* variant c.332G>A was not genotyped in this cohort because the expected minor allelic frequency was less than 0.01% (**Table 1**). Pairwise comparisons showed greater plasma DHEAS (by 40%) and pregnenolone sulfate (by 57%) concentrations in participants carrying *SLCO2B1* c.1457C>Talleles (**Table 4**). The *SLCO2B1* c.935G>A allele was associated with higher plasma concentrations of CPI and CPIII by 43 and 46%, respectively (**Table 4**). Additionally, the *SLCO2B1* c.917G>A allele was associated with a 33% lower CPIII plasma levels (**Table 4**).

Since the OATP2B1 endogenous substrates (estrone sulfate, DHEAS, CPI or CPIII) measured in plasma are also substrates of other transporters (e.g., OATP1B1, MRP2 and BCRP) or subject to drug metabolism (e.g., CYP2C9), we examined their possible associations with common SNPs in these genes (Zhai et al., 2011; Dudenkov et al., 2017; Muller et al., 2018) by pairwise comparisons. *SLCO1B1* c.388A>G was associated with higher pregnenolone sulfate levels (by 47%) but not significantly for estrone sulfate, DHEAS, CPI, or CPIII concentrations (**Supplementary Table S2**). Likewise, *SLCO1B1* c.521T>C, *ABCG2* (BCRP) c.421C>A, *CYP2C9*2*, *CYP2C9*3*, *ABCC2* (MRP2) c.1248G>A and *ABCC2* c.-24C>T were not significantly associated with any of the endogenous substrates investigated (**Supplementary Table S2**).

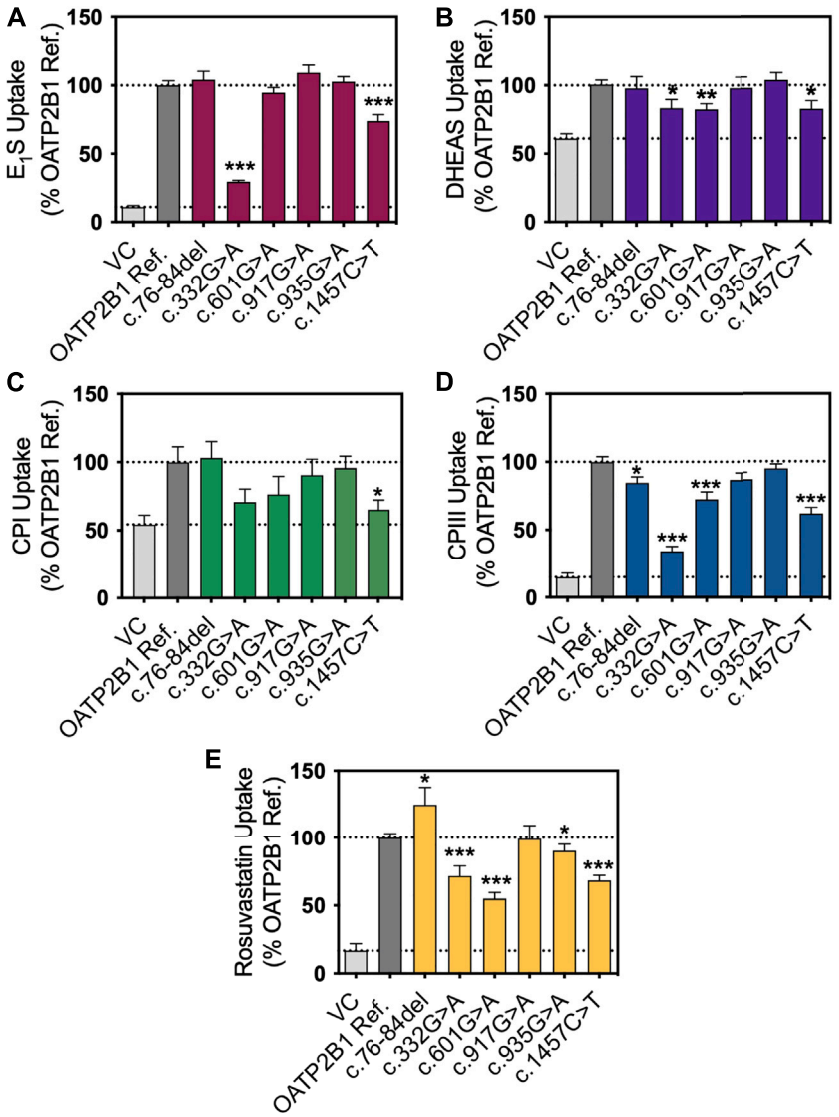

FIGURE 2 | *In vitro* transport activity of OATP2B1 genetic variants with substrates. Cellular accumulation of **(A)** estrone sulfate, (E_1S) (1 μg/ml, $n = 3$), **(B)** dehydroepiandrosterone sulfate (DHEAS) (1 μg/ml, $n = 4$), **(C)** coproporphyrin (CP) I (1 μg/ml, $n = 3$), **(D)** CPIII (1 μg/ml, $n = 3$) and **(E)** rosuvastatin (1 μg/ml, $n = 3$) in HEK293T cells were transiently transfected with vector control (VC), OATP2B1 reference and OATP2B1 variants after incubation for 10 min (E_1S, DHEAS, CPIII and rosuvastatin) or 30 min (CPI) in Krebs-Henseleit buffer (KHB) at pH 6. Results are shown as mean ± SEM, $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Multivariable Analysis of *SLCO2B1* Genetic Variations on Plasma Endogenous OATP2B1 Substrate Concentrations

Multivariable linear regression analyses were performed to determine whether *SLCO2B1* variant were associated with plasma concentrations of each of the OATP2B1 endogenous substrates. For each model, demographic variables were included such as sex, race and age, particularly when associations were found in univariate analyses. Furthermore, the clinically relevant *SLCO1B1* c.388A>G and *SLCO1B1* c.521C>T alleles were included into models because the measured solutes are also OATP1B1 substrates and for some solutes (e.g., estrone sulfate and CPI), associations with these genotypes have been previously reported. The final models with parameter estimates are shown in **Table 5**.

In the model for estrone sulfate, there was an association of the *SLCO1B1* c.521C>T allele with 62% higher plasma concentrations ($p = 0.053$) when the model was adjusted for sex and included other *SLCO2B1/SLCO1B1* genotypes. It is notable that variables included in the model poorly explained the interindividual variability in circulating estrone sulfate as R^2 was 0.047.

For DHEAS, 49% of variation in circulating concentrations could be explained by a model that includes the variables of sex, age, and *SLCO2B1/SLCO1B1* genotypes. Sex and age were variables that were significantly associated with DHEAS concentrations. The model predicts males have 94% higher

TABLE 2 | Estrone Sulfate and CPIII transport kinetics by OATP2B1 and its genetic variants.

	Variant	V_{max}[a] (pmol·mg protein^{-1}·min^{-1})	K_m[a] (μM)	CL (V_{max}/K_m) (μL·mg protein^{-1} min^{-1})
Estrone Sulfate	OATP2B1 Ref	91.6 ± 5.2	5.9 ± 1.2	15.6
	c.76-84del	70.2 ± 8.1	4.1 ± 1.8	17.0
	c.332G>A	N.D.	N.D.	0.25[b]
	c.601G>A	68.1 ± 6.8	4.0 ± 1.6	17.1
	c.917G>A	46.2 ± 3.9	1.9 ± 0.7	24.8
	c.935G>A	63.9 ± 5.1	2.8 ± 1.0	22.5
	c.1457C>T	N.D.	N.D.	0.38[b]
CPIII	OATP2B1 Ref.	25.5 ± 1.5	0.034 ± 0.011	743
	c.76-84-del	54.8 ± 5.2	0.051 ± 0.025	1,069
	c.332G>A	6.8 ± 0.8	0.055 ± 0.034	125
	c.601G>A	40.4 ± 4.9	0.052 ± 0.033	775
	c.917G>A	62.7 ± 8.0	0.058 ± 0.038	1,077
	c.935G>A	40.8 ± 3.1	0.066 ± 0.027	629
	c.1457C>T	40.5 ± 4.1	0.062 ± 0.032	649

[a]*Mean ± standard error of estimate.*
[b]*Estimated uptake clearance based on linear regression;*
N.D., not determined.

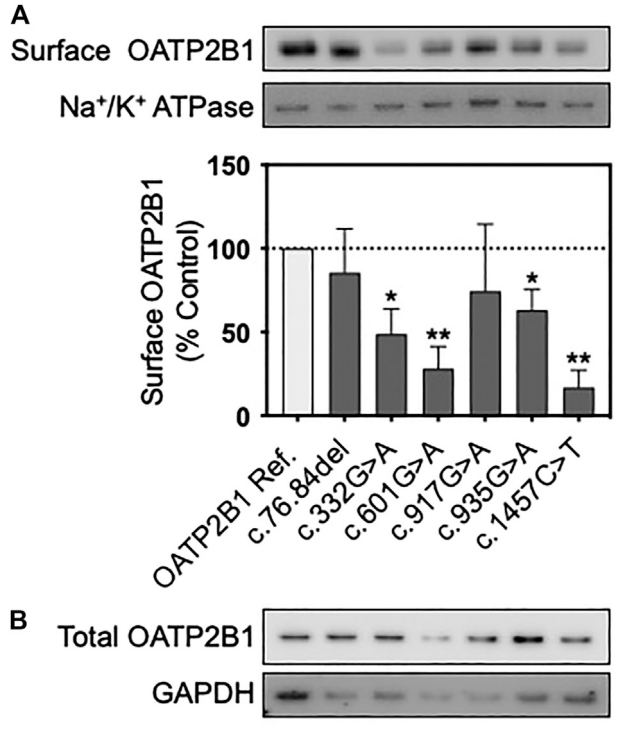

FIGURE 3 | Protein expression of OATP2B1 genetic variants. Representative western blots of **(A)** cell surface and **(B)** total OATP2B1 protein expression in HEK293T cells transfected with OATP2B1 reference and OATP2B1 genetic variants. Western blot analysis of surface OATP2B1 protein expression was normalized to Na^+/K^+ ATPase. Results are shown as mean ± SEM (n = 3), *p < 0.05, **p < 0.01.

DHEAS concentrations, while advancing age results in decreasing plasma DHEAS, with a 22% lower level for each decade. Although *SLCO2B1* c.1457C>T was associated with DHEAS concentrations in univariate analysis, this was no longer found when adjusting for sex and age.

About 45% of the variability in circulating pregnenolone sulfate concentration was explained by a model that considers sex, age and *SLCO2B1/SLCO1B1* genotypes. Males are predicted to have 31% greater pregnenolone concentrations than females (p = 0.012) and increasing age significantly contributes to decreasing circulating levels (p < 0.0001). The *SLCO1B1* c.388A>G variant did not associate with pregnenolone sulfate concentrations as previously found in univariate analysis when adjusting for other variables. Interestingly, *SLCO2B1* c.1457C>T variant carriers continue to be associated with higher (45%, p = 0.014) pregnenolone sulfate concentrations with the multivariable model.

In the multivariable model for CPI, male sex is predicted to have 32% higher circulating concentrations than female sex (p = 0.006). Carriers of the *SLCO2B1* c.935G>A variant are predicted to have 42% greater plasma CPI levels (p = 0.009). There was no longer a significant association with race that was found in the univariate analysis for CPI concentrations. Furthermore, the *SLCO1B1* c.521T>C was not significantly associated with CPI levels. Altogether, approximately 27% of the variability in CPI could be explained by the model.

With the multivariable model for CPIII, female sex was significantly associated with lower CPIII concentrations by 22%. Again, race no longer was associated with circulating CPIII with multivariable regression analysis as was previously noted in the simple pairwise comparison. The *SLCO2B1* c.935G>A variant is predicted to result in 31% greater plasma CPIII (p = 0.006), while possession of the *SLCO2B1* c.917G>A variant was associated with 28% lower CPIII (p = 0.037). Approximately 35% of the variability in plasma CPIII could be explained by the model.

DISCUSSION

OATP2B1 is considered an emerging transporter with clinical importance according to the International Transporter

TABLE 3 | Study participant demographics (*n* = 93).

Age, median (range)	25 (18–62)			
Sex, N (%)				
Male	38 (40.9)			
Female	55 (59.1)			
Weight (kg) (range)[a]	69.8 (43.3–108.9)			
Race, N				
African	4			
East Asian	20			
Caucasian	69			
Allelic Frequency	Entire Cohort	African	East Asian	Caucasian
SLCO2B1 c.76-84del	0.027	0.000	0.050	0.022
SLCO2B1 c.601G>A	0.016	0.000	0.050	0.007
SLCO2B1 c.917G>A	0.027	0.000	0.000	0.036
SLCO2B1 c.935G>A	0.123	0.250	0.350	0.051
SLCO2B1 c.1457C>T	0.118	0.250	0.450	0.014
SLCO1B1 c.388A>G	0.387	0.375	0.400	0.399
SLCO1B1 c.521T>C	0.172	0.125	0.075	0.203

[a]*Obtained for 79 of 93 participants.*

Consortium (Zamek-Gliszczynskiet al., 2018) and it has been argued that this transporter is deserving of greater attention (McFeely et al., 2019; Kinzi et al., 2021). Indeed, OATP2B1 seems to be involved in the oral absorption of medications and is the target of drug interactions in the intestine (McFeely et al., 2019; Medwid et al., 2019). Nevertheless, additional evidence to support or refute roles for OATP2B1 in drug disposition and in physiological functions is needed (Bednarczyk and Sanghvi, 2020; Kinzi et al., 2021). For several drug transporters such as OATP1B1, Organic Cation Transporter 1 (OCT1) and BCRP, the occurrence of functional genetic variations that influence drug and endobiotic disposition has helped to firmly establish their clinical relevance. But for OATP2B1, there have been many inconsistencies in the effects of common missense genetic variants on the plasma concentrations of presumed substrate drugs. Furthermore, the effects of these nonsynonymous genetic variants on OATP2B1 transport function *in vitro* have also been heterogeneous. The key limitations of studies that aim to determine a potential clinical role for OATP2B1 in drug disposition have been the lack of transporter-selective OATP2B1 substrates or inhibitors for use as pharmacological tools. Furthermore, it is possible that the *in vivo* pharmacokinetic effects of functional OATP2B1 genetic variations have been masked or complicated by the fact that altered transport activities in the gut that change oral drug bioavailability may be offset by impacts in other tissues that alter biodistribution and clearance.

In this report we aimed to provide additional insights into the functional consequences of relatively common genetic variants in OATP2B1/*SLCO2B1* by examining potential impacts to endogenous substrate disposition both *in vitro* and *in vivo*. We have shown that the common OATP2B1 c.1457C>T variant has reduced transport activity towards a range of endogenous compounds and a prototypical drug. Importantly, we found associations with the *SLCO2B1* c.935G>A variant with higher plasma concentrations of the endogenous substrates, CPI and CPIII, as well as with greater circulating pregnenolone sulfate levels in individuals carrying the *SLCO2B1* c.1457C>T variant.

In transiently transfected cells, the OATP2B1 c.332G>A, c.601G>A, c.1457C>T variants had the most pronounced effects on OATP2B1 substrate transport, with decreased the cellular accumulation of estrone sulfate, DHEAS, CPI, CPIII and rosuvastatin compared to OATP2B1 wildtype (**Figure 2**). However, there were substrate-dependenteffects, particularly with the OATP2B1 c.601G>A variant. Reduced transport function of OATP2B1 c.332G>A, c.601G>A and c.1457C>T could be explained by their decreased cell surface expression of OATP2B1 (**Figure 3**). The OATP2B1 c.332G>A and the c.601G>A variants possessed the highest CADD/REVEL/MetaLR scores (**Table 1**) among the variants examined and are predicted to change amino acids near or within transmembrane spanning domains of OATP2B1 involved in the substrate translocation pore (**Figure 1**). Therefore, our results for these variants could be somewhat expected. In the context of previous studies, our observations are consistent with some that found reduced activity of the OATP2B1 c.332G>A and/or c.601G>A variants towards several substrates (Ho et al., 2006) but not with another report that observed no functional effects of the c.601G>Avariant (Nies et al., 2013). On the other hand, the OATP2B1 c.1457C>T variant results in a missense change in an amino acid residue in the large 5th extracellular loop and has a relatively low CADD/REVEL/MetaLR scores. However, we found that the OATP2B1 c.1457C>T variant had reduced transport function *in vitro* which was similar to other studies (Nozawa et al., 2002; Nies et al., 2013). But in contrast, two other studies found increased activity of OATP2B1 c.1457C>T (Ho et al., 2006; Yang et al., 2020). Lastly, we found that the most common OATP2B1 variant, namely c.935G>A, had rather benign functional consequences for substrates, except for a very slight reduction in rosuvastatin transport activity. Such a result would be in keeping with its low CADD/REVEL/MetaLR scores. However, our findings for the OATP2B1 c.935G>A variant contrast with others that find a reduction in transport function for some substrates (Yang et al., 2011; Nies et al., 2013; Yang et al., 2020).

There has been significant interest in circulating endogenous substrates of drug transporters and their potential utility as

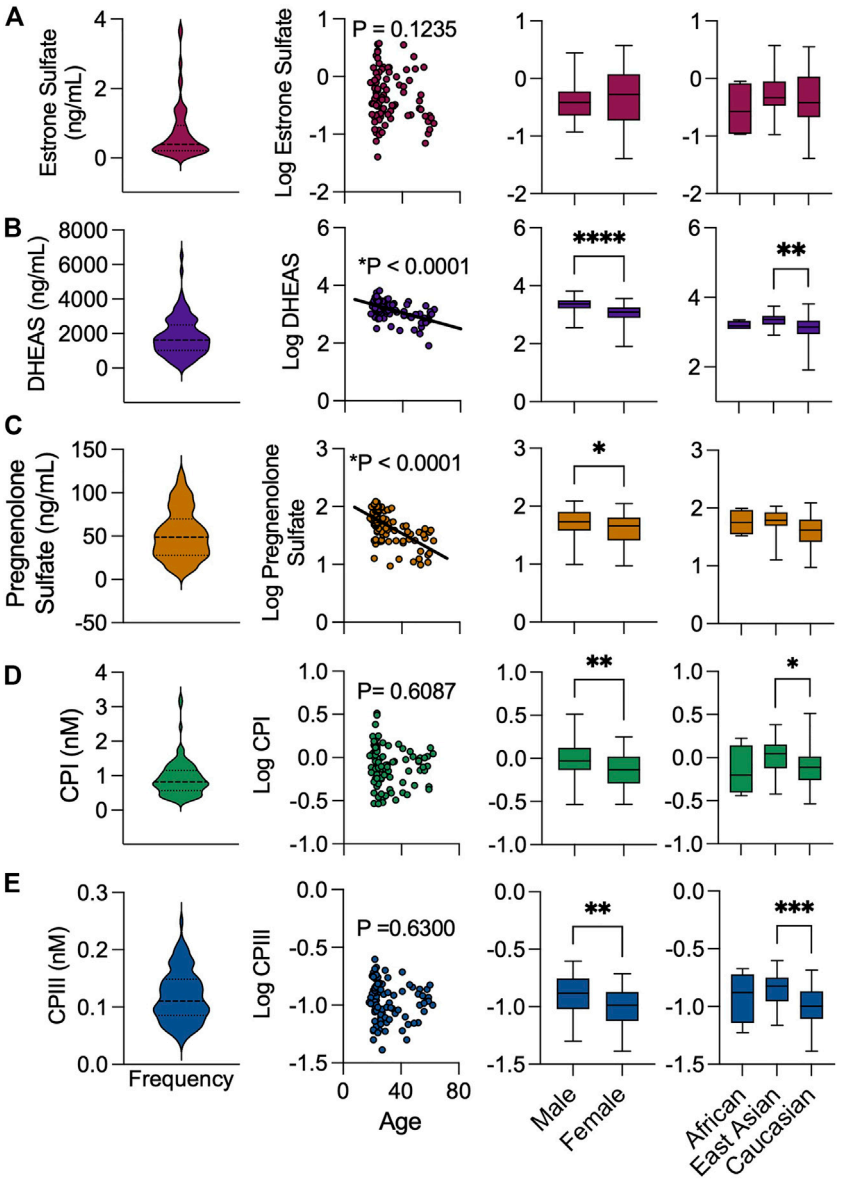

FIGURE 4 | Cohort distribution of endogenous biomarkers levels by baseline demographics. Frequency distribution of **(A)** estrone sulfate, **(B)** DHEAS, **(C)** pregnenolone sulfate **(D)** CPI and **(E)** CPIII. Association of endogenous substrates with age, sex, and ethnicity. Box and whiskers plots are shown as mean (line), 25th and 75th percentile (box) and range (whiskers) $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

biomarkers of altered transporter activity. For instance, plasma concentrations of CPI, pyridoxic acid and N1-methylnicontinamide can serve to monitor the activities of OATP1B1/1B3, organic anion transporters (OATs) and organic cation transporters (OCTs), Multidrug And Toxin Extrusion (MATEs), respectively (Ito et al., 2012; Lai et al., 2016; Shen et al., 2019). Pharmacological inhibition or reduced function genetic polymorphisms of these drug transporters could result in elevated plasma concentrations of the endogenous biomarkers through a reduction in systemic clearance conferred by decreased transporter activities in the liver and kidney. Similarly for OATP2B1, we propose that higher concentrations of its endogenous substrates in circulation would signify reduced activity of a OATP2B1 genetic variant.

Estrone sulfate, the most abundant circulating estrogen, is taken up by cells from blood and converted to active estradiol for physiological endocrine function. Estrone sulfate is a well-studied substrate of OATP2B1, however it is also a substrate of many transporters including other OATPs, Na^{+}-taurocholate co-transporting polypeptide (NTCP), OATs, organic solute transporter alpha-beta(OSTαβ), BCRP, and MRPs. Consequently, *SLCO2B1* genetic variants were not associated with estrone sulfate plasma concentrations in our cohort of

TABLE 4 | Univariate Analyses of *SLCO2B1* gene variants with circulating endogenous substrate concentrations.

Variant	*SLCO2B1* c.76-84del		*SLCO2B1* c.601G>A		*SLCO2B1* c.917G>A		*SLCO2B1* c.935G>A		*SLCO2B1* c.1457C>T	
Carrier status[a]	**NC**	**C**	**NC**	**C**	**NC**	**C**	**NC**	**C**	**NC**	**C**
N	88	5	90	3	88	5	72	21	76	17
Estrone Sulfate (ng/ml)	0.71 (±0.08)	1.00 (±0.69)	0.71 (±0.08)	1.40 (±1.17)	0.74 (±0.09)	0.50 (±0.09)	0.75 (±0.09)	0.66 (±0.18)	0.68 (±0.08)	0.95 (±0.29)
DHEAS (ng/ml)	1836 (±123)	1,652 (±355)	1827 (±120)	1792 (±584)	1856 (±122)	1299 (±337)	1721 (±138)	2149 (±209)	1701 (±121)	**2388*** (±318)
Pregnenolone Sulfate (ng/ml)	52 (±3)	56 (±16)	52 (±3)	68 (±26)	53 (±3)	32 (±5)	49 (±3)	63 (±7)	47 (±3)	**74**** (±6)
CPI (nM)	0.92 (±0.06)	0.95 (±0.22)	0.92 (±0.05)	1.04 (±0.39)	0.93 (±0.06)	0.76 (±0.21)	0.84 (±0.06)	**1.20**** (±0.10)	0.92 (±0.06)	0.94 (±0.10)
CPIII (nM)	0.12 (±0.01)	0.12 (±0.02)	0.12 (±0.01)	0.15 (±0.02)	0.12 (±0.01)	**0.08*** (±0.01)	0.11 (±0.00)	**0.16**** (±0.01)	0.11 (±0.01)	0.14 (±0.01)

[a]*NC, non-carrier; C, Carrier (heterozygotes + homozygotes).*
Mean ± S.E.M.
*p < *0.05*, **p < *0.01*.

TABLE 5 | Multivariable linear regression models for circulating endogenous substrates of OATP2B1.

Model	Variable	Coefficient	*p*-Value
Log (Estrone Sulfate) (R^2 = 0.047)	Intercept	−0.3522	
	Sex[a]	−0.05838	0.5320
	SLCO2B1 c.935G>A[b]	−0.02600	0.8227
	SLCO2B1 c.1457C>T[b]	0.07600	0.5559
	SLCO1B1 c.388A>G[b]	−0.07181	0.5051
	SLCO1B1 c.521T>C[b]	0.2094	**0.0525**
Log (DHEAS) (R^2 = 0.491)	Intercept	3.321	
	Sex[a]	0.2882	**<0.0001**
	Age	−0.01091	**<0.0001**
	SLCO2B1 c.935G>A[b]	0.06765	0.2898
	SLCO2B1 c.1457C>T[b]	0.09664	0.1906
	SLCO1B1 c.388A>G[b]	0.05940	0.3426
	SLCO1B1 c.521T>C[b]	−0.01988	0.7349
Log (Pregnenolone Sulfate) (R^2 = 0.451)	Intercept	1.809	
	Sex[a]	0.1168	**0.0121**
	Age	−0.01001	**<0.0001**
	SLCO2B1 c.935G>A[b]	0.02807	0.6161
	SLCO2B1 c.1457C>T[b]	0.1624	**0.0135**
	SLCO1B1 c.388A>G[b]	0.05564	0.1635
	SLCO1B1 c.521T>C[b]	0.07223	0.3116
Log (CPI) (R^2 = 0.271)	Intercept	−0.2455	
	Sex[a]	0.1197	**0.0063**
	Race[c]	0.1109	0.1298
	SLCO2B1 c.935G>A [b]	0.1512	**0.0093**
	SLCO2B1 c.1457C>T[b]	−0.06612	0.3692
	SLCO1B1 c.388A>G[b]	0.05354	0.2836
	SLCO1B1 c.521T>C[b]	0.07340	0.1387
Log (CPIII) (R^2 = 0.340)	Intercept	−1.038	
	Sex[a]	0.1084	**0.0007**
	Race[c]	0.07965	0.1410
	SLCO2B1 c.917G>A[b]	−0.1452	**0.0370**
	SLCO2B1 c.935G>A[b]	0.1164	**0.0057**
	SLCO2B1 c.1457C>T[b]	−0.004622	0.9303
	SLCO1B1 c.388A>G[b]	0.006791	0.8509
	SLCO1B1 c.521T>C[b]	−0.01293	0.7182

[a]*Males compared to reference (Females).*
[b]*Carriers (heterozygotes + homozygotes) compared to reference (Non-carriers).*
[c]*East Asian compared to reference (Caucasians).*

healthy volunteers. This is despite that there was reduced estrone sulfate transport activity with the OATP2B1 c.1457C>T variant *in vitro* and that 17 of 93 participants in the study carried this allele (5 homozygote, 12 heterozygote). However, we did confirm greater estrone sulfate concentrations in individuals with the *SLCO1B1* c.521T>C allele as was previously reported (van der Deure et al., 2008). But the multivariate model for plasma estrone sulfate concentrations was not particularly effective in explaining interindividual variability (R^2 = 0.047) indicating other genetic and biological factors are important (Platia et al., 1984; Feofanova et al., 2020).

DHEAS and pregnenolone sulfate are circulating sex steroid precursors of androgens and progesterone that are synthesized in the adrenal glands. Intact DHEAS and pregnenolone sulfate are neurosteroid hormones that functionally interact with neurotransmitter receptors and ion channels in the central nervous system (Grube et al., 2018). We observed the well-known and strong relationships between sex and age with plasma DHEAS and pregnenolone sulfate concentrations (Orentreich et al., 1984). DHEAS and pregnenolone sulfate are substrates of similar membrane transporters as estrone sulfate. Indeed, DHEAS is a substrate of OATP1B1/1B3, although previous studies in healthy volunteers found that treatment with rifampin, a potent inhibitor of OATP1B1/1B3, did not affect plasma DHEAS levels (Shen et al., 2017; Takehara et al., 2017). Likewise, we did not find that the reduced function *SLCO1B1* c.521C>T allele was associated with DHEAS (or pregnenolone sulfate) concentrations. But DHEAS and pregnenolone sulfate plasma levels were associated with the *SLCO2B1* variant c.1457C>T in univariate analysis (**Table 4**). After multivariate regression including the factors of age and sex, DHEAS plasma levels were no longer associated with *SLCO2B1* c.1457C>T. This may be due to the lower age for *SLCO2B1* c.1457C>T carriers compared to those with wildtype *SLCO2B1*. However, with adjustment for age and sex, pregnenolone sulfate concentrations were still predicted to be higher in those carrying *SLCO2B1* c.1457C>T alleles (**Table 5**). Higher plasma pregnenolone sulfate levels would be consistent with the generally reduced transport activity of the OATP2B1 c.1457C>T variant in our *in vitro* studies.

CPI and CPIII are by-products of heme synthesis that are cleared from the body by biliary and renal excretion, with elimination in bile being the predominant pathway. The hepatocyte uptake of both CPI and CPIII are determined by the actions of OATP1B1, OATP1B3 and OATP2B1, while efflux into bile and blood are dependent on MRP2 and MRP3, respectively (Moriondo et al., 2009; Bednarczyk and Boiselle, 2016; Shen et al., 2016; Kunze et al., 2018). It is notable that while CPI is a good substrate of both OATP1B1 and OATP1B3, it is poorly transported by OATP2B1 (Bednarczyk and Boiselle, 2016; Shen et al., 2016). On the other hand, CPIII is capably transported by OATP1B1, OATP1B3 and OATP2B1 (Bednarczyk and Boiselle, 2016). We also find that OATP2B1 more efficiently transports CPIII than CPI (**Figure 2**). Genetic mutations that cause combined deficiencies in OATP1B1/OATP1B3 (Rotor Syndrome), result in redirection of CPI and CPIII elimination from bile to urine and an increase in CPI/CPIII urinary ratio (Wolkoff et al., 1976; van de Steeg et al., 2012). Unlike CPI, basal CPIII concentrations in the blood do not appear to be associated with the reduced function *SLCO1B1* c.521T>C allele (Yee et al., 2019). Based on this evidence, we speculated that although CPI and CPIII are both OATP2B1 substrates, circulating CPIII would be more sensitive to the impacts of OATP2B1 genetic variation.

In our cohort of healthy participants, we found that both CPI and CPIII plasma concentrations were significantly influenced by sex and race, but not age. Males had greater concentrations of CPI and CPIII than females by 31 and 28%, respectively. The sex dependency on circulating CPI was previously reported in a cohort of Japanese subjects (Mori et al., 2019) and is thought to be related to differences in synthesis rate (Takita et al., 2020). In univariate analyses, East Asians had greater concentrations of CPI and CPIII compared to Caucasians (**Figure 4**). However, with multivariable regression, race was no longer an independent predictor of circulating CPI and CPIII (**Table 5**). It is likely that other covariates, particularly the differing allelic frequencies of *SLCO2B1* variants (c.917G>A, c.935G>A and c.1457C>T) between the subgroups of East Asians and Caucasians (**Table 3**), largely contributed to the observed racial differences in coproporphyrin concentrations.

The key novel findings of our study are that circulating concentrations of both CPI and CPIII are greater in individuals carrying the most common *SLCO2B1* c.935G>A variant (**Table 4**). This association was maintained in multiple linear regression when adjusting for other covariates including sex, race, and *SLCO1B1* genotype (**Table 5**). These results suggest that the *SLCO2B1* c.935G>A variant is a reduced transport function allele *in vivo*. However, this notion is in contrast with the lack of significant functional effects of the OATP2B1 c.935G>A variant observed *in vitro* (**Figure 2**). We also found that the *SLCO2B1* c.917G>A allele was associated with lower CPIII concentrations (**Tables 4**, **5**). Again, this *in vivo* association was not consistent with our observations of no change in OATP2B1 c.917G>A transport activity *in vitro* (**Figure 2**). However, it must be cautioned that there were relatively few participants (5 out of 93) with the *SLCO2B1* c.917G>A variant. Another unexpected finding was that the *SLCO2B1* c.935G>A variant was associated with higher plasma CPI concentrations given that CPI is a relatively poor substrate of OATP2B1 and that the absolute hepatic expression of OATP2B1 is approximately one-third of the more efficient CPI transporter, OATP1B1 (Badee et al., 2015). Additionally, we found CPI plasma concentrations were similar between *SLCO1B1* wildtype and *SLCO1B1* c.521T>C variant carriers (TC and CC genotypes), despite other studies having reported increased CPI with the variant allele (Mori et al., 2019; Yee et al., 2019; Suzuki et al., 2021). This difference is likely due to the fact that only one study participant had the homozygous *SLCO1B1* c.521CC genotype, which was previously noted to have the most prominent impacts on CPI levels (Yee et al., 2019; Suzuki et al., 2021). Taken together, our findings imply that both plasma CPI and CPIII are sensitive to alterations in OATP2B1 activity that would be manifest with the possession of functional genetic polymorphisms and during inhibitory drug interactions. It follows that variation in circulating CPI and CPIII concentrations may not distinguish alterations in OATP2B1 activity apart from those occurring for OATP1B1. Finally, it is tempting to speculate that assessment of renal clearance of CPIII could better serve as a selective measure of (renal) OATP2B1 activity since CPIII is highly secreted by the

kidney (Lai et al., 2016; Feng et al., 2021), in contrast to CPI which is eliminated mostly by glomerular filtration, and OATP2B1 is expressed in the proximal tubules (Ferreira et al., 2018).

We focused on relatively common missense variants in OATP2B1 to evaluate potential impacts on transporter function both *in vitro* and *in vivo*. However, a recent analysis indicates that rare variation in the *SLCO2B1* gene may account for 11.6% of functional variability in OATP2B1 (Zhang and Lauschke, 2019). Therefore, targeted *in vitro* biochemical evaluation of rare OATP2B1 variants and high-throughput, deep mutational scanning techniques (Zhang et al., 2021), together with case- and population-based association studies are necessary to provide a more complete understanding of the relevance of OATP2B1 genetic variation.

In conclusion, we found that basal circulating concentrations of several endogenous substrates of OATP2B1 were associated with common non-synonymous genetic variations in the transporter in healthy individuals. These genetic associations were poorly aligned with the observed functional activities of the OATP2B1 variants *in vitro*, as well as with predictions from *in silico* algorithms. Additional studies are required to establish whether endogenous substrates may serve as biomarkers of OATP2B1 activity.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/**Supplementary Material**, further inquiries can be directed to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Human Subject Research Ethics Board, University of Western Ontario. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

SM, HP, DT, JM, and RT performed the experiments. SM, US, RK, and RT were involved in study design. SM and RT drafted the manuscript. All authors reviewed the draft and final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.713567/full#supplementary-material

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Effects of Cytochrome P450 and Transporter Polymorphisms on the Bioavailability and Safety of Dutasteride and Tamsulosin

Gonzalo Villapalos-García[1†], *Pablo Zubiaur*[1†], *Marcos Navares-Gómez*[1†], *Miriam Saiz-Rodríguez*[2†], *Gina Mejía-Abril*[1], *Samuel Martín-Vílchez*[3], *Manuel Román*[1,3], *Dolores Ochoa*[1,3] *and Francisco Abad-Santos*[1,3,4*†]

[1]*Clinical Pharmacology Department, School of Medicine, Hospital Universitario de La Princesa, Instituto Teófilo Hernando, Instituto de Investigación Sanitaria La Princesa (IP), Universidad Autónoma de Madrid, Madrid, Spain,* [2]*Research Unit of Hospital Universitario de Burgos (HUBU), Burgos, Spain,* [3]*Unidad de Investigación Clínica y Ensayos Clínicos (UICEC) Hospital Universitario de La Princesa, Platform SCReN (Spanish Clinical Research Network), Instituto de Investigación Sanitaria La Princesa (IP), Madrid, Spain,* [4]*Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBERehd), Instituto de Salud Carlos III, Madrid, Spain*

Dutasteride and tamsulosin are one of the first-line combination therapies for the management of benign prostatic hyperplasia (BPH). Despite being more effective than monotherapies, they produce frequent adverse drug reactions (ADRs). Institutions such as Food and Drug Administration and European Medicines Agency recommend precaution with *CYP2D6* poor metabolizers (PMs) that receive *CYP3A4* inhibitors and tamsulosin. However, no specific pharmacogenetic guideline exists for tamsulosin. Furthermore, to date, no pharmacogenetic information is available for dutasteride. Henceforth, we studied the pharmacokinetics and safety of dutasteride/tamsulosin 0.5 mg/0.4 mg capsules according to 76 polymorphisms in 17 candidate pharmacogenes. The study population comprised 79 healthy male volunteers enrolled in three bioequivalence, phase-I, crossover, open, randomized clinical trials with different study designs: the first was single dose in fed state, the second was a single dose in fasting state, and the third was a multiple dose. As key findings, *CYP2D6* PMs (i.e., *4/*4 and *4/*5 subjects) and intermediate metabolizers (IMs) (i.e., *1/*4, *1/*5, *4/*15 individuals) presented higher AUC ($p = 0.004$), higher $t_{1/2}$ ($p = 0.008$), and lower Cl/F ($p = 0.006$) when compared with NMs (*1/*1 individuals) and UMs (1/*1 × 2 individuals) after multiple testing correction. Moreover, fed volunteers showed significantly higher t_{max} than fasting individuals. Nominally significant associations were observed between dutasteride exposure and *CYP3A4* and *CYP3A5* genotype and between tamsulosin and *ABCG2*, *CYP3A5*, and *SLC22A1* genotypes. No association between the occurrence of adverse drug reactions and genotype was observed. Nonetheless, higher incidence of adverse events was found in a multiple-dose clinical trial. Based on our results, we suggest that dose adjustments for PMs and UMs could be considered to ensure drug safety and effectiveness, respectively. Further studies are warranted to confirm other pharmacogenetic associations.

Keywords: dutasteride, tamsulosin, pharmacogenetics, pharmacokinetics, *CYP2D6*, *CYP3A4*

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Elena De Mattia, Centro di Riferimento per l'oncologia di Aviano (IRCCS), Italy
Umamaheswaran Gurusamy, University of California San Francisco, United States
Volker Martin Lauschke, Karolinska Institutet (KI), Sweden

***Correspondence:**
Francisco Abad-Santos
francisco.abad@uam.es

†ORCID ID:
Gonzalo Villapalos-García
orcid.org/0000-0002-4849-3268
Pablo Zubiaur
orcid.org/0000-0002-6150-4320
Marcos Navares-Gómez
orcid.org/0000-0003-2501-6845
Miriam Saiz-Rodríguez
orcid.org/0000-0002-1660-3135
Francisco Abad-Santos
orcid.org/0000-0002-6519-8885

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INTRODUCTION

Dutasteride and tamsulosin are one of the first-line combination therapies for the management of benign prostatic hyperplasia (BPH). Combination therapy is frequent in BPH patients, due to difficulties in reaching effectiveness with single treatments (Lerner et al., 2021a; Lerner et al., 2021b).

Dutasteride belongs to 5-α reductase inhibitors (5-ARIs), which prevent dihydrotestosterone production and, consequently, delay prostatic tissue growth. It is administered by oral route. It presents 60% oral bioavailability, and its median time to reach maximum plasma concentration (t_{max}) is around 3 h (1–10 h range) after the administration of 0.5 mg single dose. Dutasteride shows a volume of distribution (Vd) of 300–500 L and a high plasma protein binding (>99.5%). Its elimination is dose-dependent. At single doses lower than 5 mg, dutasteride clearance is rapidly performed, with a shorter half-life ($t_{1/2}$) of 3–9 days. However, at 0.5 mg daily doses, the elimination is slower, reaching a $t_{1/2}$ of 3–5 weeks. It is extensively metabolized by cytochrome P450 (*CYP*) isoforms *CYP3A4* and *CYP3A5* into four major metabolites: two of them less active than dutasteride and two other that are similarly active to the parent drug. They are primarily excreted in stools and marginally in urine; only between 1 and 15.4% of the dutasteride dose is excreted unmetabolized in feces. It has been also reported that dutasteride is not metabolized *in vitro* by human cytochrome P450 isoenzymes *CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6*, and *CYP2E1* (FDA, 2010).

Tamsulosin belongs to α-1 receptor antagonists (ARAs). This family of drugs reduces the sympathetic tone of smooth muscle in the prostate and bladder neck, facilitating urine expulsion. Tamsulosin is likewise administered orally and presents >90% oral bioavailability, linear pharmacokinetics, Vd of 16 L, and exhibits high plasmatic protein binding (94–99%). After the administration of 0.4 mg single-dose, the median t_{max} is 6 h (2–24 h range). It has a median $t_{1/2}$ of 10–13 h both in single-dose and multiple-dose regimens. It is 90% metabolized by mainly *CYP3A4* and *CYP2D6*. The remaining unaltered tamsulosin (i.e., approximately 10% of the administered dose) is excreted in urine [Agencia Española del Medicamento y Productos Sanitarios (AEMPS), 2015].

Despite being more effective than monotherapy, combination therapies usually cause greater adverse drug reactions (ADRs) (Greco and McVary, 2008). Dutasteride/tamsulosin treatment may produce dizziness, erectile dysfunction, decreased libido, retrograde ejaculation, and breast alterations [Agencia Española del Medicamento y Productos Sanitarios (AEMPS), 2015]. In addition to the adverse events, underdosing can determine a lack of treatment effectiveness. It is, therefore, important to improve the effectiveness and tolerability of current therapies by means of individualized approaches. Genetic variants in genes encoding for drug metabolizing enzymes, transporters, or drug targets affect drug pharmacokinetics and pharmacodynamics, which relates to ADR occurrence and drug exposure. Notably, tamsulosin Food and Drug Administration (FDA) drug label includes an assortment according to patient pharmacogenetics. Particularly, caution should be exercised for *CYP2D6* poor metabolizers (PMs) treated with *CYP3A4* moderate inhibitors, for the risk of elevated drug blood levels (FDA, 2010). No other high level of evidence pharmacogenetic information is available for tamsulosin. Additionally, no pharmacogenetics information related to dutasteride is known.

Hence, our goal was to conduct a candidate gene pharmacogenetic study evaluating 76 polymorphisms in 17 pharmacogenes, including *CYP1A2, CYP2A6, CYP2B6, CYP2C19, CYP2C8, CYP2C9, CYP2D6, CYP3A4, CYP3A5,* and *CYP4F2* and transporters such as *ABCB1, ABCC2, ABCG2, SLC22A1, SLC28A3, SLCO1B1,* and *UGT1A1* in healthy volunteers participating in bioequivalence clinical trials.

MATERIALS AND METHODS

Study Population

The study population was enrolled in three bioequivalence clinical trials testing two different formulations of dutasteride/tamsulosin 0.5 mg/0.4 mg hard capsules. Each clinical trial comprised 36 individuals. The number of volunteers who completed the three clinical trials and that provided their informed consent for the pharmacogenetic study was 88 out of 108. Nine of them were duplicates, i.e., they participated in two out of the three clinical trials, and were thence excluded from the repeated pharmacogenetic studies. Thus, the total number of volunteers that participated in this work was 79.

The clinical trials were performed at the Clinical Trial Unit of Hospital Universitario de La Princesa (UECHUP) (Madrid, Spain). Inclusion and exclusion criteria were common to the three clinical trials. They involved healthy males aged from 18 to 55 years old, who were either surgically sterile or that agreed to use double efficient contraceptive methods and that committed to avoid sperm donation for at least 6 months after the first administration of the drug. Exclusion criteria comprised any organic or psychic condition, previous use of prescription pharmacological treatment, body mass index (BMI) outside of the 18–30 kg/m^2 range, consumption of abuse drugs, alcohol, or tobacco, blood donation in the previous month before starting the trial, and history of swallowing problems.

Study Design

The reference formulation used in the clinical trials was Duodart® (tamsulosin/dutasteride 0.5/0.4 mg, GlaxoSmithKline, England), which was also used for the pharmacogenetic study. The three clinical trials presented different study designs. They were bioequivalence, phase-I, crossover, open, randomized clinical trials. They were blinded for the analytical determination of dutasteride and tamsulosin plasma levels. They differed in the dose regimen and the feeding conditions. In the first one, a single dose was administered under fed conditions (S1) (**Supplementary Figure S1**); in the second, a single dose was administered under fasting conditions (S2) (**Supplementary Figure S2**); in the last one, eight doses were administered during eight consecutive days under fed conditions (M) (**Supplementary Figure S3**).

S1 (fed-state) and S2 (fasting-state) studies consisted of a single oral dose of Duodart® or a test formulation administered in two periods to 36 subjects, respectively (n = 72). Both formulations contained dutasteride 0.5 mg/tamsulosin 0.4 mg. Volunteers were hospitalized from 10 h before to 24 h after dosing. Administration of the drug was done by investigators in the Clinical Trials Unit of the Hospital Universitario de la Princesa (UECHUP), and individuals were checked each time they swallowed the capsule. Three of them were excluded as did not complete the second period (n = 69). A 28-day washout period was scheduled between periods. Drug administration was established 10 min after a high-fat breakfast in S1 and 10 h after their last meal and 5 h before their next in S2. After drug intake, 23 blood samples were collected from each volunteer at 0 h (before receiving the drug), 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 20, 24, 32, 48, and 72 h after the administration of the drug. Tamsulosin and dutasteride plasma concentrations were quantified. Likewise, M (multiple-dose) comprised 36 subjects who received Duodart® or a test formulation during eight days. 10 h before the last drug administration (i.e., the eighth dose), they were hospitalized until 24 h after dosing. Only tamsulosin plasma concentrations were quantified in this multiple-dose study. Two volunteers were excluded from the bioequivalence analysis as they did not complete the second period (n = 34). Periods were separated by a 7-day washout period. Every day, the volunteers visited the UECHUP to provide a trough blood sample (i.e., a total of seven blood samples) and to receive a standard breakfast and the dose. Drug intake was established 10 min after having breakfast. Afterward, on day 8, they were hospitalized. They received the drug 30 min before dosing and after fasting for 10 h. Then, 23 blood samples were obtained from each volunteer at 0 h (before receiving the drug), 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 20, 24, 32, 48, and 72 h after the administration of the drug.

Clinical laboratory analyses and dutasteride and tamsulosin plasma level determinations were outsourced in the three clinical trials. During periods, samples were frozen at −20°C until their shipment to an external laboratory. Drug determinations were performed after liquid-liquid extraction by high-performance liquid chromatography coupled with mass spectrometry (LC-MS) with a lower limit of quantification (LLOQ) of 50.00 pg/ml for dutasteride and 99.80 pg/ml for tamsulosin.

The race or biogeographic origin variable was self-reported by healthy volunteers as well as their biological sex and age. Weight and height were measured during the screening to assess inclusion criteria.

Pharmacokinetic Analyses

Pharmacokinetic parameters were calculated using CERTARA Phoenix WinNonlin Professional software version 7.0 (Certara United States, Princeton, NJ, United States) with a noncompartmental method for both drugs in S1 and S2 trials and for tamsulosin in M. In S1 and S2, the area under the curve (AUC) between 0 and 72 h (AUC_t) was calculated with the linear trapezoidal rule. The AUC between 72 h and infinite (AUC_∞) was estimated as C_t/k_e (C_t being the drug plasma concentration at 72 h and k_e being the terminal rate constant, calculated by linear regression of the log-linear part of the concentration–time curve). The AUC between 0 and ∞ was calculated as $AUC_t + AUC_{t\text{-}\infty}$ (AUC_∞). In M, the AUC at steady state, i.e., between the eighth drug administration and 24 h later (AUC_τ), was similarly calculated with the linear trapezoidal rule. In the three clinical trials, the maximum plasma concentration (C_{max}) and time to reach C_{max} (t_{max}) were observed directly; the half-life ($t_{1/2}$) was calculated as $\ln 2/k_e$; clearance (Cl) was calculated as dose divided by AUC_∞ or AUC_τ; and volume of distribution (Vd) was estimated as Cl/k_e. The minimum concentration in the steady state (C_{min}) was directly observed in the multiple-dose clinical trial.

Genotyping

DNA extraction from peripheral venous blood was performed in a MagNa Pure System (Roche Applied Science, United States). DNA concentration was measured with a Qubit 3.0 Fluorometer (ThermoFisher, United States). The genotyping was performed with a custom TaqMan® OpenArray® panel (Thermo Fisher Scientific, United States) in a QuantStudio 12k Flex real-time PCR system (Thermo Fisher Scientific, United States). Volunteers were genotyped for variants in genes potentially related to dutasteride/tamsulosin absorption, distribution, metabolism, and excretion, based on most important pharmacogenes and the literature (FDA, 2010): cytochrome P450 isoforms *CYP1A2* (*1B, rs2470890; *1C, rs2069514; *1F, rs762551), *CYP2A6* (*9, rs28399433), *CYP2B6* (*4, rs2279343; *5, rs3211371; *9, rs3745274; *18, rs28399499; *22, rs34223104; rs4803419), *CYP2C19* (*2, rs4244285; *3, rs4986893; *4, rs28399504; *5, rs56337013; *6, rs72552267; *7, rs72558186; *8, rs41291556; *9, rs17884712; *17, rs12248560; *35, rs12769205), *CYP2C8* (*2, rs11572103; *3, rs10509681 and rs11572080; *4, rs1058930), *CYP2C9* (*2, rs1799853; *3, rs1057910; *5, rs28371686; *8, rs7900194 and rs9332094; *11, rs28371685), *CYP2D6* (*3, rs35742686; *4, rs3892097; *6, rs5030655; *7, rs5030867; *8, rs5030865A; *9, rs5030656; *10, rs1065852; *12, rs5030862; *14, rs5030865T; *15, rs77467110; *17, rs28371706; *19, rs72549353; *29, rs59421388; *41, rs28371725; *56, rs72549347; *59, rs79292917; rs1135840), *CYP3A4* (*2, rs55785340; *3, rs4986910; *6, rs4646438; *18, rs28371759; *22, rs35599367), *CYP3A5* (*3, rs776746; *6, rs10264272; *7, rs41303343), and *CYP4F2* (*3, rs2108622); transporters such as *ABCB1* (C1236T, rs1128503; C3435T, rs1045642; G2677T/A, rs2032582), *ABCC2* (rs2273697), *ABCG2* (rs2231142), *SLC22A1* (*2, rs72552763; *3, rs12208357; rs34059508), *SLC28A3* (rs7853758), and *SLC O 1B1* (*1B, rs2306283; *2, rs56101265; *5, rs4149056; *6, rs55901008; *9, rs59502379; *10, rs56199088; *13, rs56061388; *17/*21, rs4149015; rs11045879); and other drug metabolizing enzymes such as *UGT1A1* (*6, rs4148323; *80, rs887829). A *CYP2D6* copy number variation assay (CNV) was performed in the same thermal cycler with a 96-well thermal block, performed with TaqMan® technology as previously described (Belmonte et al., 2018).

Haplotyping and Phenotyping

Genotypes were used to infer haplotypes which define phenotypes or diplotypes. The genotyping technique used does

not allow knowing with complete certainty whether or not two polymorphisms are located on the same chromosome. This is important in order to correctly define alleles. However, the location of these polymorphisms can be inferred with sufficient confidence from the allele frequency data available. Consequently, allele assignment was conducted according to Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines for *CYP2C9* and nonsteroidal anti-inflammatory drugs (Caudle et al., 2014), *CYP2C19* and voriconazole (Moriyama et al., 2017), *CYP2D6* and opioids (Crews et al., 2021), *CYP3A5* and tacrolimus (Birdwell et al., 2015), *SLC O 1B1* and simvastatin (Ramsey et al., 2014, 1), and *UGT1A1* and atazanavir (Gammal et al., 2016, 1). The possible phenotypes were ultrarapid (UM), rapid (RM), normal (NM), intermediate (IM) and poor metabolizer (PM) for drug-metabolizing enzymes, and normal (NF) and intermediate function (IF) for transporters. *CYP3A5* phenotype can be denoted either by using the CPIC nomenclature, namely, NM, IM, and PM, or by using the traditional nomenclature of *CYP3A5* "expressors" and "nonexpressors." In this work, the CPIC nomenclature is used to be consistent with the rest of the genes. NMs are equivalent to expressors (i.e., *1/*1); IMs are equivalent to heterozygotes with one expressor allele (i.e., *1) and one nonexpressor allele (i.e., *3, *6, and *7), and PMs are nonexpressors (i.e., *3/*3 and *3/*6). *CYP2D6* phenotype that resulted ambiguous after CNV (e.g., *1/*4 individuals with three copies that could be interpreted as *1 × 2/*4 or NM and *1/*4 × 2 or IM) was excluded from the analysis. Despite *UGT1A1**80 function is unknown, it is in very high linkage disequilibrium with *28, which are decreased function variants. Thus, *1/*1 individuals were considered NMs, *1/*80 subjects were considered IMs, and*80/*80 individuals were considered PMs. *CYP2C8* allele functionality is not defined. Thus, individuals were grouped into diplotypes. For *ABCB1*, following a similar methodology previously published (Zubiaur et al., 2021), individuals were grouped according to their total number of mutations: group 1 was considered any individual with no allelic variants, group 2 consists of those with 1–3 allelic variants, and group 3 consists of those with 4–6 allelic variants. Otherwise, genetic variants were individually analyzed for each gene. The reference SNP number (rs) was named, when available, following the allelic nomenclature following the PharmVar nomenclature [Pharmacogene Variation Consortium (PharmVar), 2018 at www.PharmVar.org (Gaedigk et al., 2018, CPT 103:399; Gaedigk et al., 2019, CPT 105:29)]. A summary table of the correspondence between diplotypes and phenotypes is provided in **Supplementary Table S1**.

Safety

During hospitalization, volunteers were asked about treatment tolerability in several occasions. Adverse events (AEs) reported after open questions as well as self-reported AEs were registered in volunteers' data collection logbook. The causality between drug administration and the occurrence of adverse events (AEs) was evaluated following Karch–Lasagna (Karch and Lasagna, 1977) algorithm for S1 and the algorithm of Spanish Pharmacovigilance System (Aguirre and García, 2016) for S2 and M clinical trial. Only definite, probable, or possible adverse events were considered adverse drug reactions (ADRs) and counted for the present study.

Statistical Analysis

From 76 initial polymorphisms, 19 final genetic variables were tested (17 genes, but three *CYP1A2* alleles were analyzed independently). Race and clinical trial were added as covariates, and dose/weight correction was applied as control confounding variables. Hardy–Weinberg equilibrium was calculated by χ^2 test comparing observed and expected allele distributions. Regarding the pharmacokinetics analysis, Vd and Cl were adjusted for bioavailability (i.e., divided by weight) becoming Vd/F and Cl/F, respectively. AUC_{∞}, AUC_{τ}, C_{max}, and C_{min} were adjusted for the dose-weight ratio (DW). Tamsulosin data were obtained both from multiple- and single-dose studies. Since AUC_{∞} after a single dose and AUC_{τ} are equivalent (i.e., they correspond to the total AUC resulting from a drug administration), both variables were merged into a single "AUC" variable. Normality was analyzed by quantile–quantile plots. Homoscedasticity was tested by Levene's test. For homoscedastic normal variables, differences in means were studied by *t*-test (two categories within a variable) or ANOVA (three or more categories within a variable) with logarithmically transformed pharmacokinetic parameters (e.g., LnAUC), in order to achieve normal distribution. For those variables with three or more groups, a pairwise comparison Bonferroni post hoc analysis was performed. For heteroscedastic variables, differences in means were studied by Welch's *t*-test (two categories within a variable) or Welch's ANOVA (three or more categories within a variable). A multivariate analysis was performed by means of linear regression. The significant variants from the univariate analysis and the study design were considered the independent variables for the multivariate analysis of all pharmacokinetic parameters, which were established as dependent variables. Benjamini and Hochberg correction for multiple comparisons was performed, i.e., false discovery rate (FDR) after multivariate analysis (Benjamini and Hochberg, 1995) for 61 tests for tamsulosin and 44 for dutasteride. p values lower than 0.05 after FDR correction were considered statistically significant; p values lower than 0.05 before FDR correction were considered nominally significant. Concerning treatment safety, the incidence of ADRs depending on phenotypes, genotypes, self-reported race, and clinical trial design was analyzed by χ^2 test, and the risk of developing those ADRs was calculated by logistic regression. For the ANOVA or t-test, the p value is shown for nominally significant relationships (p_{ANOVA}). For the multivariate analysis, significance ($p < 0.05$) was indicated with the unstandardized β-coefficient, R^2 value, p of multivariate analysis (p_{MV}), and p after FDR (p_{FDR}). All calculations were computed in R version 4.0.3 software (R Core Team, 2020).

Ethics

The protocol and informed consent for the three clinical trials were approved by the Independent Ethics Committee (IECCR, CEIm) of Hospital Universitario de la Princesa and the Spanish

TABLE 1 | Significant relationships between dutasteride pharmacokinetics and clinical trial design, volunteers self-reported race, and genotype.

		AUC (ng·h/mL)	C_{max} (ng/ml)	t_{max} (h)	$t_{1/2}$(h)	Vd/F (L/kg)	Cl/F (ml/h·kg)
SRR	Caucasian (*n* = 38)	44.21 (49.22%)	2.49 (37.21%)	2.74 (57.1%)	61.95 (45.03%)	9.39 (66.19%)	138.42 (103.53%)
	Latin (*n* = 14)	42.87 (45.69%)	2.56 (27.41%)	3.68 (48.16%)	49.36 (41.09%)	7.58 (34.56%)	144.97 (93.54%)
CT	Fed (S1) (*n* = 25)	49.53 (48.12%)	2.76 (30.37%)	**3.68 (44.53%)*¹**	56.25 (47.96%)	**7.32 (50.29%)*¹†**	142.22 (120.99%)
	Fasting (S2) (*n* = 27)	38.59 (43.54%)	2.28 (36.86%)	2.35 (60.65%)	60.7 (43.4%)	10.37 (62.86%)	138.3 (76.17%)
CYP3A4	*1/*1 (*n* = 50)	42.17 (43.51%)	2.49 (32.62%)	2.98 (55.49%)	58.56 (46.14%)	9.09 (60.9%)	143.79 (98.44%)
	*1/*22 + *22/*22 (*n* = 2)	85.76 (56.8%)	3.11 (74.06%)	3.25 (76.15%)	58.54 (1.52%)	**4.23 (47.72%)*¹†**	49.91 (46.37%)
CYP3A5	NM + IM (*n* = 9)	38.17 (52.17%)	2.17 (33.36%)	**3.89 (49.7%)*¹**	45.04 (27.19%)	8.81 (42.07%)	168.31 (92.21%)
	PM (*n* = 43)	45.04 (47.23%)	2.58 (34.25%)	2.8 (55.49%)	61.39 (45.37%)	8.92 (65.65%)	134.3 (102.64%)
SLC28A3	rs7853758 A/A (*n* = 4)	37.33 (67.95%)	2.65 (35.92%)	2 (28.87%)	55.52 (48.76%)	9.65 (42.55%)	207.06 (115.38%)
	A/G (*n* = 14)	35.51 (57.83%)	2.26 (39.61%)	2.91 (51.24%)	50.08 (46.7%)	11.03 (77.17%)	200.91 (94.4%)
	G/G (*n* = 31)	**49.27 (39.89%)*²†**	2.63 (31.92%)	3.16 (57.37%)	63.6 (43.32%)	**7.64 (34.9%)†**	**98.26 (63.7%)*²**

*Data is presented as mean (coefficient of variation). SRR: Self-reported race, CT: Clinical Trial, S1: single-dose feeding conditions trial, S2: single-dose fasting conditions trial, NM: Normal metabolizer, IM: Intermediate metabolizer, PM: Poor metabolizer. *¹: p < 0.05 after ANOVA, *²: p < 0.05 after ANOVA and Bonferroni post-hoc vs A/G, †: nominal p < 0.05 after multivariate analysis. No variable remained statistically significant after FDR correction. Bold data represents significant results.*

Drug Agency (AEMPS). S1 (EUDRA-CT number: 2017-001592-23), S2 (EUDRA-CT number: 2017-003227-29), and M (EUDRA-CT number: 2017-003244-21) clinical trials were performed according to Spanish regulation, ICH guidelines for Good Clinical Practices, and Revised Declaration of Helsinki (World Medical Association, 1991).

RESULTS

Demographic Results

The study population comprised 79 male healthy volunteers, defined by mean ± standard deviation, with a median age of 24 ± 6.7 years old, mean height of 1.76 ± 0.07 m, mean weight of 76.87 ± 8.72 kg, and body max index (BMI) of 24.86 ± 2.26 m/kg^2. The population was composed of 52 (74%) Caucasians and 18 (26%) Latin individuals. No significant differences in demographics were found between these two groups.

All polymorphisms analyzed were in Hardy–Weinberg equilibrium, except for *CYP1A2* *1C (rs2069514), *CYP2A6* *9 (rs28399433), *CYP2B6* *4 (rs2279343), *ABCB1* rs2032582, *CYP2C8* *8 (rs1058930), and *CYP3A4* *22 (rs35599367).

Dutasteride

All the analyzed variables presented normal distributions after logarithmic adjustment. All variables presented homoscedastic distribution except t_{max} for *CYP2A6* *9 and *SLC22A1* *2, $t_{1/2}$ for *CYP1A2* *1B and *CYP2C9* phenotype, and Vd/F for *CYP2C9* phenotype.

Fed conditions presented higher t_{max} (p_{ANOVA} = 0.002) and higher Vd/F than fasting conditions (p_{ANOVA} = 0.006) after univariate analysis. Moreover, *CYP3A4**22 allele carriers showed lower Vd/F than *1/*1 individuals (p_{ANOVA} = 0.023). Additionally, *SLC28A3* rs7853758 A/G and A/A subjects presented lower AUC (p_{ANOVA} = 0.012) and higher Cl/F (p_{ANOVA} = 0.043) than G/G. *SLC28A3* rs7853758 (β = −0.51, R^2 = 0.15, p_{MV} = 0.011, and p_{FDR} = 0.065) maintained significance in multivariate analysis for AUC. Food (β = 0.3, R^2 = 0.39, p_{MV} = 0.016, and p_{FDR} = 0.087), *CYP3A4* genotype (β = −0.7, p_{MV} = 0.024, and p_{FDR} = 0.11), and *SLC28A3* rs7853758 (β = 0.26, R^2 = 0.39, p_{MV} = 0.039, and p_{FDR} = 0.16) remained significant after multivariate analysis for Vd/F. Nonetheless, all of these variables lost significance after FDR correction (**Table 1**). Thus, no statistically significant effect was found for dutasteride.

Tamsulosin

All the analyzed variables presented normal distributions after logarithmic adjustment. All variables presented homoscedastic distribution, except: AUC and Cl/F for *CYP2A6* *9; Vd/F for *CYP1A2**1F and *CYP2A6* *9; $t_{1/2}$ for *CYP1A2**1F, *SLC22A1**3, and *CYP2C9* phenotype; and C_{max} for clinical trial design and *CYP2C9* phenotype.

Fasting conditions presented higher AUC (p_{ANOVA} = 0.011) than fasting and multiple dose. Fasting conditions and multidose administration exhibited lower t_{max} (β = −0.21, R^2 = 0.16, p_{MV} = 0.001, and p_{FDR} = 0.008) and higher C_{max} (β = 0.22, R^2 = 0.25, p_{MV} = 0.014, and p_{FDR} = 0.063) than fed conditions. Moreover,

TABLE 2 | Significant relationships between tamsulosin pharmacokinetics and clinical trial design, volunteers self-reported race and genotype.

			AUC (ng·h/mL)	C_{max} (ng/ml)	C_{min} (ng/ml)
SRR		Caucasian (*n* = 56)	176.13 (47.37%)	12.28 (36.83%)	3.26 (65.53%)
		Latin (*n* = 23)	160.16 (44.27%)	12.84 (37.39%)	2.43 (51.48%)
CT		Fed (S1) (*n* = 25)	148.51 (49.05%)	**10.35 (36.44%)*²**	—
		Fasting (S2) (*n* = 27)	**202.57 (47.75%)*¹**	13.85 (41.83%)	—
		Multiple dose (M) (*n* = 27)	161.66 (35.39%)	12.98 (24.04%)	2.98 (63.78%)
ABCG2	rs2231142	G/G (*n* = 65)	177.70 (47.41%)	12.67 (36.58%)	3.17 (65.42%)
		G/T (*n* = 14)	142.62 (33.15%)	11.38 (38.01%)	2.33 (42.27%)
CYP2D6		UM (*n* = 11)	131.02 (28.5%)	10.37 (27.41%)	1.81 (51.59%)
		NM (*n* = 37)	145.63 (41.7%)	11.53 (34.92%)	2.54 (49.92%)
		IM (*n* = 23)	**221.00 (43.3%)*⁵†³‡**	14.87 (36.24%)	3.38 (35.58%)
		PM (*n* = 6)	**223.07 (36.11%)†³‡**	12.66 (36.2%)	5.11 (90.43%)
CYP3A5		NM + IM (*n* = 16)	139.47 (49.29%)	10.59 (33.14%)	2.23 (47.41%)
		PM (*n* = 63)	179.61 (45.06%)	12.91 (36.59%)	3.24 (64.06%)
SLC22A1	rs12208357	*1/*1 (*n* = 73)	165.11 (45.47%)	12.07 (36.95%)	2.77 (69.1%)
		*1/*3 (*n* = 6)	**248.97 (41.24%)*⁴**	**17.01 (22.15%)*⁴**	**4.68 (4.14%)*⁴**

			t_{max} (h)	$t_{1/2}$(h)	Vd/F (ml/kg)	Cl/F (ml/h·kg)
SRR	Caucasian (*n* = 56)		6.41 (25.16%)	11.94 (32.55%)	556.69 (29.83%)	109.93 (43.07%)
	Latin (*n* = 23)		6.52 (25.58%)	11.7 (23.15%)	617.16 (38.7%)	115.7 (42.44%)
CT	Fed (S1) (*n* = 25)		**7.36 (27.91%)*²†¹‡**	10.04 (22.95%)	557.64 (29.69%)	127.18 (42.17%)
	Fasting (S2) (*n* = 27)		5.83 (21.13%)	11.86 (32.09%)	**498.61 (31.98%)*¹‡**	**97.44 (44.79%)*¹**
	Multiple dose (M) (*n* = 27)		6.2 (17.64%)	**13.58 (26.10%)*³†²**	665.4 (31.28%)	111.36 (38.13%)
ABCG2	rs2231142	G/G (*n* = 65)	6.21 (29.56%)	11.87 (29.34%)	548.25 (30.13%)	107.63 (40.83%)
		G/T (*n* = 14)	6.49 (24.33%)	11.89 (34.41%)	**695.21 (36.49%)*⁴**	130.09 (46.48%)
CYP2D6	UM (*n* = 11)		6.14 (24.2%)	10.42 (23.81%)	667.2 (34.99%)	137.35 (39.51%)
	NM (*n* = 37)		6.55 (28.7%)	10.85 (24.58%)	595.42 (31.71%)	123.68 (39.44%)
	IM (*n* = 23)		6.35 (20.49%)	**12.89 (23.56%)*⁵†³‡**	**491.02 (30.97%)*⁵†³**	**86.76 (38.68%)*⁵†³‡**
	PM (*n* = 6)		7 (18.07%)	**16.98 (36.68%)†³‡**	**577.06 (25.43%)†³**	**83.69 (39.13%)†³‡**
CYP3A5	NM + IM (*n* = 16)		6.75 (27.18%)	11.4 (23.38%)	**686.97 (34.88%)***	**137.7 (41.26%)***
	PM (*n* = 63)		6.37 (24.61%)	11.99 (31.46%)	545.68 (30.48%)	104.98 (41%)
SLC22A1	rs12208357	*1/*1 (*n* = 73)	6.47 (25.85%)	11.73 (30.76%)	583.65 (32.68%)	114.3 (41.3%)
		*1/*3 (*n* = 6)	6.17 (12.21%)	**13.57 (20.12%)*⁴**	**460.44 (34.76%)*⁴†⁴**	**78.81 (55.1%)*⁴†⁴**

Data are presented as mean (coefficient of variation). SRR: Self-reported race, CT: Clinical Trial, S1: single-dose feeding conditions trial, S2: single-dose fasting conditions trial, M: multiple-dose feeding conditions trial, UM: Ultrarrapid metabolizer, NM: Normal metabolizer, IM: Intermediate metabolizer, PM: Poor metabolizer. ^{*1}*:* $p < 0.05$ *after ANOVA and Bonferroni post-hoc analysis vs S2 vs M.* ^{*2}*:* $p < 0.05$ *after ANOVA and Bonferroni post-hoc analysis vs S2 vs M.* ^{*3}*:* $p < 0.05$ *after ANOVA and Bonferroni post-hoc analysis vs S1 vs S2.* ^{*4}*:* $p < 0.05$ *after ANOVA.* ^{*5}*:* $p < 0.05$ *after ANOVA and Bonferroni post-hoc analysis vs UM and NM.* $^{\dagger 1}$*: nominal* $p < 0.05$ *after multivariate analysis vs S2 and M.* $^{\dagger 2}$*: nominal* $p < 0.05$ *after multivariate analysis vs S1 and S2.* $^{\dagger 3}$*: nominal* $p < 0.05$ *after multivariate analysis vs UM and NM.* $^{\dagger 2}$*: nominal* $p < 0.05$ *after multivariate analysis vs S1 and S2.* $^{\dagger 3}$*: nominal* $p < 0.05$ *after multivariate analysis vs UM and NM.* $^{\dagger 2}$*: nominal* $p < 0.05$ *after multivariate analysis vs S1 and S2.* $^{\dagger 4}$*: nominal* $p < 0.05$ *after multivariate analysis ‡:* $p < 0.05$ *after FDR.* C_{min} *was only calculated in M clinical trial. Bold data represents significant results.*

multivariate analysis also revealed that single-dose administration showed lower Vd/F (β = −0.22, R^2 = 0.31, p_{MV} = 0.002, and p_{FDR} = 0.008) and lower $t_{1/2}$ (β = −0.20, R^2 = 0.30, p_{MV} = 0.001, and p_{FDR} = 0.008) than single-dose trials. *ABCG2* rs2231142 C allele carriers presented higher Vd/F (p_{ANOVA} = 0.014) than G/G individuals. Univariate and multivariate analysis also revealed that *CYP2D6* UMs and NMs presented lower AUC than PMs and IMs (β = −0.34, R^2 = 0.36, p_{MV}<0.001, and p_{FDR} = 0.004). Additionally, UMs and NMs had lower $t_{1/2}$ (β = −0.20, R^2 = 0.30, p_{MV} = 0.002, and p_{FDR} = 0.008), higher Vd/F (β = 0.14, R^2 = 0.31, p_{MV} = 0.046, and p_{FDR} = 0.19), and lower Cl/F (β = 0.33, p_{MV} = 0.009, and p_{FDR} = 0.006) than PMs and IMs. *CYP3A5* NMs and IMs presented higher Vd/F (p_{ANOVA} = 0.019) and Cl/F (p = 0.027) than PM. Finally, *SLC22A1* *1/*3 individuals presented higher AUC (p_{ANOVA} = 0.020), higher C_{max} (p_{ANOVA} = 0.017), higher C_{min} (p_{ANOVA} = 0.038), and lower Cl/F (p_{ANOVA} = 0.026) than *1/*1 volunteers (**Table 2**).

After FDR, the *CYP2D6* phenotype remained statistically significant for tamsulosin AUC, Cl/F, $t_{1/2}$, and t_{max}, and clinical trial design remained the statistically significant variables for tamsulosin Vd/F, $t_{1/2}$, and t_{max}.

Safety

No serious ADR was reported. The ADRs reported comprised dizziness, testicular pain, epididymo-orchitis, headache, ejaculation disorder, hypotension symptomatic, retrograde ejaculation, libido decreased, and abnormal urine odor. Eight volunteers presented at least one ADR. The most frequent ADRs were headache (n = 3) and retrograde ejaculation (n = 3), followed by libido decrease (n = 2) and ejaculation disorder (n = 2). The remaining ADRs were only observed in one volunteer. Participants in the multiple-dose clinical trial were related to higher incidence of ADR than participants in single-dose (7 ADR vs. 1 ADR, respectively; p < 0.05). No relationship between polymorphisms or race with ADR occurrence was found.

Nonsignificant results are provided in **Supplementary Table S2**.

DISCUSSION

Dutasteride and tamsulosin are widely used drugs effective for the treatment of BPH. However, drug underexposure can lead to a lack of effectiveness, and overexposure, to the occurrence of ADRs; as mentioned earlier, both circumstances may lead to drug discontinuation. In order to achieve safe and effective responses to pharmacological treatments, pharmacogenetic-based dose adjustments are proposed for different drugs by institutions such as CPIC (Amstutz et al., 2018; Crews et al., 2021) and DPGW (Dutch Pharmacogenetics Working Group Pharmacogenetic Recommendations and 2019) or regulatory agencies such as FDA or EMA. In particular, FDA and EMA drug labels for tamsulosin 0.4 mg and combined formulations (e.g., Duodart®) warrant precaution for *CYP2D6* PMs using concomitant *CYP3A4* inhibitors (FDA, 2010b; Agencia Española del Medicamento y Productos Sanitarios (AEMPS), 2015). Subjects with this phenotype may be overexposed to tamsulosin, and ADRs may occur. Nonetheless, no additional pharmacogenetic guideline or dose adjustment recommendation is available for tamsulosin. Neither is there any prescribing information available for dutasteride. The latter is consistent with the scarcity of well-designed observational pharmacogenetic studies for both drugs, especially for dutasteride. Thus, our intention in this study is to further elucidate the effects of pharmacogenetics on these two drugs.

The observed dutasteride pharmacokinetic parameters were in general congruent with the literature, e.g., AUC of 39.6 ± 23.1 ng·h/ml and C_{max} of 2.14 ± 0.77 ng/ml, compared with 43.03 ± 20.73 ng·h/ml and of 2.46 ± 0.89 ng/ml, respectively (Agencia Española del Medicamento y Productos Sanitarios (AEMPS), 2015). No significant difference was found between the two groups of race and any pharmacokinetic parameter.

Feeding is important for absorption velocity of orally administered drugs. Meals (especially high-fat meals) delay gastric emptying, augmenting the transit time to the small intestine and, subsequently, delaying the absorption into the systemic circulation (McLachlan and Ramzan, 2006). As expected, fed individuals presented higher dutasteride t_{max} than fasting volunteers and lower Vd/F. Despite not being statistically significant, a 34% higher AUC and 22% higher C_{max} were observed in fed individuals compared to fasting volunteers, which is consistent with the nominally significant differences observed in the Vd/F. Nonetheless, these results did not remain significant after multiple testing corrections, which is congruent with previous bioequivalence clinical trials that reported no differences in dutasteride pharmacokinetics (Kurczewski et al., 2017).

Consistent with the well-known pharmacokinetic profile of dutasteride, pharmacokinetic variability was significantly related to *CYP3A4* and *CYP3A5* polymorphism (Agencia Española del Medicamento y Productos Sanitarios (AEMPS), 2015). *CYP3A4* *22 allele carriers showed more than double AUC than *1/*1 carriers. However, this difference was not significant, likely due to the reduced number of volunteers carrying the *22 allele (n = 2). Similar to the explanation for the feeding conditions, these volunteers consistently presented significantly lower Vd/F. Moreover, *CYP3A5* NMs and IMs showed a higher t_{max} compared to PMs. This might reflect a reduced rate of elimination by PMs. As the elimination rate decreases, it requires less time for the drug to accumulate and to reach peak concentration. Consistently, we observed a 30% greater $t_{1/2}$ in PMs compared to NM + IMs (however, this difference was not statistically significant). However, as these results did not remain significant after multiple testing corrections, they might be spurious. Lastly, *SLC28A3* rs7853758 A/G and A/A subjects presented lower AUC, lower $t_{1/2}$, and higher Cl/F than G/G. Nonetheless, none of these associations remained significant after FDR correction. Further studies are warranted to confirm whether *CYP3A* or *SLC28A3* polymorphism affects dutasteride pharmacokinetics.

The observed tamsulosin pharmacokinetic parameters under single-dose after fed conditions were similarly consistent with the literature, for example, AUC of 187.2 ± 95.7 ng·h/ml and C_{max} of 11.3 ± 4.44 ng/ml compared to 147.4 ± 72.8 ng·h/ml and 10.35 ±

3.77 ng/ml, respectively (Agencia Española del Medicamento y Productos Sanitarios (AEMPS), 2015) (JALYN). No significant difference was found between the two groups of race and any pharmacokinetic parameter.

As mentioned before, food alters drug absorption and, therefore, pharmacokinetic parameters linked to it (e.g., t_{max} or C_{max}). This is likely caused by the different solubility of a drug based on the stomach pH and the transit time to the small intestine. Previous works state that fasting conditions are related to faster and greater tamsulosin absorption (FDA, 1997). Consistently, in this work, fasting volunteers exhibited nominally significant higher AUC (29%) and lower t_{max} (15%) compared to fed volunteers. Congruent with literature, a 16% higher C_{max} was also observed in fasting volunteers; nonetheless, the association was not significant (Agencia Española del Medicamento y Productos Sanitarios (AEMPS), 2015). As expected, we found a statistically significant higher t_{max} when the drug was administered after a high-fat breakfast, compared to fasting conditions. Additionally, as expected, fed administration had lower C_{max} than multiple-dose administration. Finally, the nonexistent difference between the AUC of fed and multiple dose is consistent because, under the same conditions, the AUC_{∞} is equivalent to the AUC_{τ}. Multiple-dose $t_{1/2}$ was found significantly higher than fed $t_{1/2}$. Theoretically, for drugs with linear pharmacokinetics like tamsulosin, $t_{1/2}$ should remain constant regardless of the dose or administration regimen. However, we observed a greater $t_{1/2}$ in multiple dose compared to fed conditions. This difference is likely explained by the limitations of noncompartmental analysis and the possibility of a type-1 error. Nevertheless, both $t_{1/2}$ values coincided with the range provided in the literature (10–13 h) (Agencia Española del Medicamento y Productos Sanitarios (AEMPS), 2015).

Tamsulosin is 90% metabolized by *CYP3A4* and *CYP2D6*, but also by other cytochrome P450 isoforms to a lesser extent (Agencia Española del Medicamento y Productos Sanitarios (AEMPS), 2015). Previous studies reported a relationship between tamsulosin bioavailability and *CYP2D6* phenotype (Choi et al., 2012; Byeon et al., 2018; Kim et al., 2018). Our results confirm that tamsulosin pharmacokinetics is significantly altered by the *CYP2D6* phenotype: PMs and IMs exhibited a significantly higher bioavailability than NMs and UMs. Although we did not observe differences in ADR incidence due to the limitations in our study design and we had no effectiveness data, our results indicate that UMs will likely be underexposed and PMs overexposed leading to ineffectiveness and worse tolerability, respectively. Considering that ADR occurrence was significantly related to the multiple dose clinical trial, we can assume that an enhanced exposure to the drug relates to a higher incidence of ADRs. We suggest that a dose reduction for PMs or an increase for UMs could be beneficial. However, further studies are required to indicate the extent of such dose modifications. The only formulation strength available for tamsulosin (in combination) is 0.4 mg, both in Europe and United States (EMA and FDA, respectively) (FDA, 2010b; Agencia Española del Medicamento y Productos Sanitarios (AEMPS), 2015). There is, therefore, a need for the marketing of formulations that facilitate the individualization of pharmacotherapy (e.g., dutasteride-tamsulosin 0.5/0.3 mg and 0.5/0.5 mg strengths). Nevertheless, further studies are warranted to confirm the clinical relevance of our conclusions. Whether patients may benefit or not from dose adjustments based on *CYP2D6* phenotype should be demonstrated prior to routine implementation. Entities such as CPIC, SEFF, or DPWG may eventually publish clinical guidelines supporting or rejecting the need for a pharmacogenetic-guided prescription.

CYP3A5 PMs showed lower Cl/F than NMs and IMs. Considering that tamsulosin is a *CYP3A4* substrate, it would be expected that *CYP3A5* metabolized it. This association suggests that tamsulosin is a *CYP3A5* substrate and that its phenotype contributes to its pharmacokinetic variability. However, previous research studies (FDA, 2010b; Agencia Española del Medicamento y Productos Sanitarios (AEMPS), 2015) reported no relationship between *CYP3A5* genotype and tamsulosin pharmacokinetic variability (Kim et al., 2018). Moreover, the association lost significance after applying FDR correction. To the best of our knowledge, this is the first work to suggest a similar association. However, further studies are required to replicate our observation.

ABCG2 encodes for the Breast Cancer Resistant Protein (BCRP). It is an ATP-binding cassette transporter and plays a major role in multidrug resistance, specially involved in the response to mitoxantrone and anthracycline (Bethesda, 2004). The impact of rs2231142 is controversial. T/T individuals were associated with decreased clearance of sulfasalazine in healthy individuals as compared to genotypes GG + GT (Gotanda et al., 2015). In this study, conversely, G/T individuals presented a significantly higher Vd/F than G/G individuals and approximately 18% lower AUC (not significant) and 23% higher Cl/F (not significant). This suggests, on the contrary, that tamsulosin is a BCRP substrate and that rs2231142 is related to lower exposure. As these results did not remain significant after multiple testing corrections, further studies should investigate the impact of this polymorphism and whether tamsulosin is a BCRP substrate.

SLC22A1 encodes for the organic cationic transporter 1 (OCT1), one of the three similar polyspecific cationic transporters mediating the uptake of many organic cations from the blood. It has substrate selectivity for a variety of endogenous ligands (dopamine, serotonin, and choline) as well as cationic drugs, such as metformin, cimetidine, imatinib, oxaliplatin, and tramadol and agmatine. OCT1 carries drugs into the liver and kidneys, where the compound is metabolized and excreted (Whirl-Carrillo et al., 2012). *SLC22A1* *1/*3 individuals presented significantly higher AUC, C_{max}, C_{min}, $t_{1/2}$, and lower Vd/F and Cl/F than *1/*1. This suggests that tamsulosin might be an OCT1 substrate. The potential reduced function of the transporter could reduce drug's hepatic uptake and, consequently, the elimination of tamsulosin, thus incrementing its bioavailability. Considering that this association did not remain significant after FDR correction, these findings could be considered spurious. Nevertheless, further studies would be necessary to confirm if tamsulosin pharmacokinetics is impacted by *SLC22A1* polymorphism.

Nonetheless, this study presents several limitations. First, the sample size is small. To address this issue, three different clinical

trials were analyzed. This leads to the second limitation: merging of three different study designs complicates the statistical analysis. The study design was analyzed as a covariate, but despite this, the statistical power is more limited than in a unique study design. Furthermore, the incidence of *CYP2D6* UMs is significantly higher than expected from literature (14 vs. 7%, respectively). We are confident with the robustness of our genotyping, but we must note this limitation. Thus, further studies are required to confirm the results here obtained.

CONCLUSION

CYP2D6 phenotype severely affected tamsulosin pharmacokinetics. PMs and IMs presented twice higher exposure to tamsulosin than UMs and NMs. The results were consistent with the literature and the guidelines of regulatory institutions, such as FDA and EMA, which do not include specific dose adjustment recommendations. Here, we suggest that a dose adjustment could improve tamsulosin effectiveness and safety. Further studies are warranted to confirm whether this adjustment would be beneficial for the patient. Alternatively, dutasteride pharmacokinetics was not altered based on genotypes or drug dose regimen. To the best of our knowledge, this is the largest study analyzing tamsulosin pharmacogenetics (n = 79) and the first study of this type for dutasteride. Additionally, new potential associations were proposed regarding *ABCG2, CYP3A4, CYP3A5*, and *SLC22A1*. However, the main limitation of this study is the limited sample size. Consequently, further prospective studies should be addressed to confirm such associations.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation. The data is the property of the promoter and will be made available upon reasonable request.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Independent Ethics Committee (IECCR, CEIm) of Hospital Universitario de la Princesa and the Spanish Drug Agency (AEMPS). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

GV-G, PZ, and FA-S conceptualized the study; GV-G and PZ contributed to methodology; GV-G provided software; FA-S and PZ validated the study; GV-G and PZ were responsible for formal analysis; GV-G, PZ, MN-G, MS-R, GM-A, MR, SM-V, DO, and FA-S investigated the study; FA-S provided resources; GV-G, MN-G, and PZ were responsible for data curation; GV-G prepared the original draft; GV-G, PZ, and FA-S reviewed and edited the manuscript; GV-G and PZ visualized the study; PZ and FA-S supervised the study; PZ and FA-S were responsible for project administration; FA-S acquired funding. All authors have read and agreed to the published version of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.718281/full#supplementary-material

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Experience of a Strategy Including CYP2C19 Preemptive Genotyping Followed by Therapeutic Drug Monitoring of Voriconazole in Patients Undergoing Allogenic Hematopoietic Stem Cell Transplantation

Irene García-García[1], Irene Dapía[2], Jaime Montserrat[1], Lucía Martinez de Soto[1], David Bueno[3], Lucía Díaz[1], Javier Queiruga[1], Amelia Rodriguez Mariblanca[1], Pilar Guerra-García[3], Elena Ramirez, Jesus Frías[1], Antonio Pérez Martínez[3], Antonio J. Carcas-Sansuan[1] and Alberto M. Borobia[1]**

[1]Clinical Pharmacology Department, IdiPAZ, La Paz University Hospital School of Medicine, Autonomous University of Madrid, Madrid, Spain, [2]Medical and Molecular Genetics Institute (INGEMM), La Paz University Hospital, Madrid, Spain, [3]Paediatric Haemato-oncology Department, University Hospital La Paz, Madrid, Spain

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Youssef Daali,
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Yalda H. Ardakani,
Tehran University of Medical Sciences, Iran
Howard L McLeod,
University of South Florida, United States

***Correspondence:**
Antonio J. Carcas-Sansuan
antonio.carcas@uam.es
Alberto M. Borobia
alberto.borobia@salud.madrid.org

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Many factors have been described to contribute to voriconazole (VCZ) interpatient variability in plasma concentrations, especially CYP2C19 genetic variability. In 2014, Hicks et al. presented data describing the correlation between VCZ plasma concentrations and CYP2C19 diplotypes in immunocompromised pediatric patients and utilized pharmacokinetic modeling to extrapolate a more suitable VCZ dose for each CYP2C19 diplotype. In 2017, in our hospital, a clinical protocol was developed for individualization of VCZ in immunocompromised patients based on preemptive genotyping of CYP2C19 and dosing proposed by Hicks et al., Clinical Pharmacogenetics Implementation Consortium (CPIC) clinical guidelines, and routine therapeutic drug monitoring (TDM). We made a retrospective review of a cohort of 28 immunocompromised pediatric patients receiving VCZ according to our protocol. CYP2C19 gene molecular analysis was preemptively performed using PharmArray®. Plasma trough concentrations were measured by immunoassay analysis until target concentrations (1–5.5 µg/ml) were reached. Sixteen patients (57.14%) achieved VCZ trough target concentrations in the first measure after the initial dose based on PGx. This figure is similar to estimations made by Hicks et al. in their simulation (60%). Subdividing by phenotype, our genotyping and TDM-combined strategy allow us to achieve target concentrations during treatment/prophylaxis in 90% of the CYP2C19 Normal Metabolizers (NM)/Intermediate Metabolizers (IM) and 100% of the Rapid Metabolizers (RM) and Ultrarapid Metabolizers (UM) of our cohort. We recommended modifications of the initial dose in 29% (n = 8) of the patients. In RM ≥12 years old, an increase of the initial dose resulted in 50% of these patients achieving target concentrations in the first measure after initial dose adjustment based only on PGx information. Our experience highlights the need to improve VCZ dose predictions in

children and the potential of preemptive genotyping and TDM to this aim. We are conducting a multicenter, randomized clinical trial in patients with risk of aspergillosis in order to evaluate the effectiveness and efficiency of VCZ individualization: VORIGENIPHARM (EudraCT: 2019-000376-41).

Keywords: voriconazole, pharmacogenetic, preemptive, therapeutic drug monitoring, CYP2C19

INTRODUCTION

Voriconazole (VCZ) is a second-generation triazole antifungal agent with broad-spectrum activity against a variety of fungal infections. It is indicated for the treatment of invasive aspergillosis, candidaemia in nonneutropenic patients, fluconazole-resistant invasive infections of *Candida*, and severe fungal infections of *Scedosporium* spp. and *Fusarium* spp. In the case of invasive aspergillosis, VCZ appears as first-line therapy in the treatment guidelines (Patterson et al., 2016). Additionally, VCZ is commonly used as a prophylaxis agent in immunocompromised patients, highly susceptible to invasive fungal infections (IFIs) (Hicks et al., 2014; Solano et al., 2017). VCZ is characterized by nonlinear pharmacokinetics and wide interpatient variability in serum concentrations, especially in pediatric population (Hicks et al., 2014; Boast et al., 2016), which is directly related to both VCZ efficacy and the occurrence of adverse drug reactions (ADRs) (Park et al., 2012). In this context, the early achievement of VCZ therapeutic plasma concentrations is essential in order to avoid hepatotoxicity and neurotoxicity (Park et al., 2012) without compromising VCZ antifungal activity.

Many factors have been described to contribute to this variability, especially *CYP2C19* genetic variability, age, drug interactions, and liver disease (Miyakis et al., 2010; Wang et al., 2014a). VCZ has an extensive hepatic metabolism mainly through CYP2C19 and a small contribution of CYP3A4, CYP2C9, and FMO3 (Whirl-Carrillo et al., 2012). It has been well reported that the *CYP2C19* genotype is related to CYP2C19 enzymatic activity and interindividual variability in VCZ plasma concentrations (Hicks et al., 2014). CYP2C19 Ultrarapid or Rapid Metabolizers (UM or RM) phenotypes have been related to lower VCZ plasma concentrations than Normal Metabolizers (NM) and Intermediate or Poor Metabolizers (IM or PM) to higher VCZ plasma concentrations. In this context, *CYP2C19* genotyping for CYP2C19 phenotype inference represents a good tool for the individualization of VCZ therapy. Moreover, the Clinical Pharmacogenetics Implementation Consortium (CPIC) (Moriyama et al., 2017) and the Royal Dutch Association for the Advancement of Pharmacy Pharmacogenetics Working Group (DPWG) (Swen et al., 2011) have developed clinical guidelines for VCZ dose adjustment based on *CYP2C19* genotype. Up to 35 variant star (*) alleles along the *CYP2C19* gene have been described by the Pharmacogene Variation (PharmVar) Consortium (www.PharmVar.org) related to absent, reduced, or increased enzymatic CYP2C19 activities.

Although clinical guidelines for VCZ dose adjustment based on *CYP2C19* genotype could be of enormous help to individualize VCZ treatment, the existing recommendations make no relevant distinction between adult and pediatric patients and are not very specific. CPIC guideline for VCZ and CYP2C19 recommends selecting other antifungal agents in adult and pediatric UM and in adult RM; in PM, they also recommend selecting another antifungal agent, except in those patients in which VCZ is considered to be the most appropriate treatment, where they propose a preferably lower than standard dosage with therapeutic drug monitoring (TDM) (Moriyama et al., 2017). The DPWG suggests a dose adjustment for UM and PM but does not differentiate between adults and children (Swen et al., 2011).

In 2014, Hicks et al. performed a retrospective review focusing on immunocompromised patients with cancer prescribed VCZ for either antifungal prophylaxis or treatment of an IFI at the St Jude Children's Research Hospital in order to describe the association between *CYP2C19* genotype and VCZ trough concentrations. In those patients carrying the CYP2C19*17 allele, related to increased enzymatic activity, the number of patients achieving VCZ target concentrations was lower than in the other CYP2C19 phenotypic groups. In contrast, VCZ plasma concentrations in those patients carrying CYP2C19*2 allele, related to decreased enzymatic activity, were generally higher. Taking into account these observations, this group developed a second approach consisting of the calculation of an extrapolated VCZ daily dose for each *CYP2C19* group that would allow increasing the number of patients achieving the VCZ therapeutic range. This study proposed that dose modifications based on pharmacogenetic (PGx) information could be an interesting tool for VCZ therapy optimization and individualization.

In addition, due to its nonlinear pharmacokinetics (PK), some studies recommend routine VCZ TDM as a useful strategy to increase the number of patients that achieve therapeutic plasma concentrations and therefore increase VCZ efficacy and safety. Generally, VCZ though the therapeutic range is set between 1 and 5.5 or 6 μg/ml measured in the first 5 days after drug administration and regularly thereafter (Ashbee et al., 2014; Boast et al., 2016; Moriyama et al., 2017).

Despite the fact that TDM is of great help to achieve therapeutic levels, initial low plasma concentration may increase the risk of mortality, even if TDM is subsequently used to achieve target concentrations (Park et al., 2012). There is evidence showing that obtaining VCZ therapeutic levels in the first week of treatment is related to the clinical outcome of fungal infection, as well as to the tolerability to the treatment, decreasing the dose-dependent adverse effects (Ashbee et al., 2014).

Taking into account the reported studies, in 2017, we included VCZ in our strategy for the implementation of pharmacogenetics in our hospital (Luong et al., 2016a). This strategy is framed

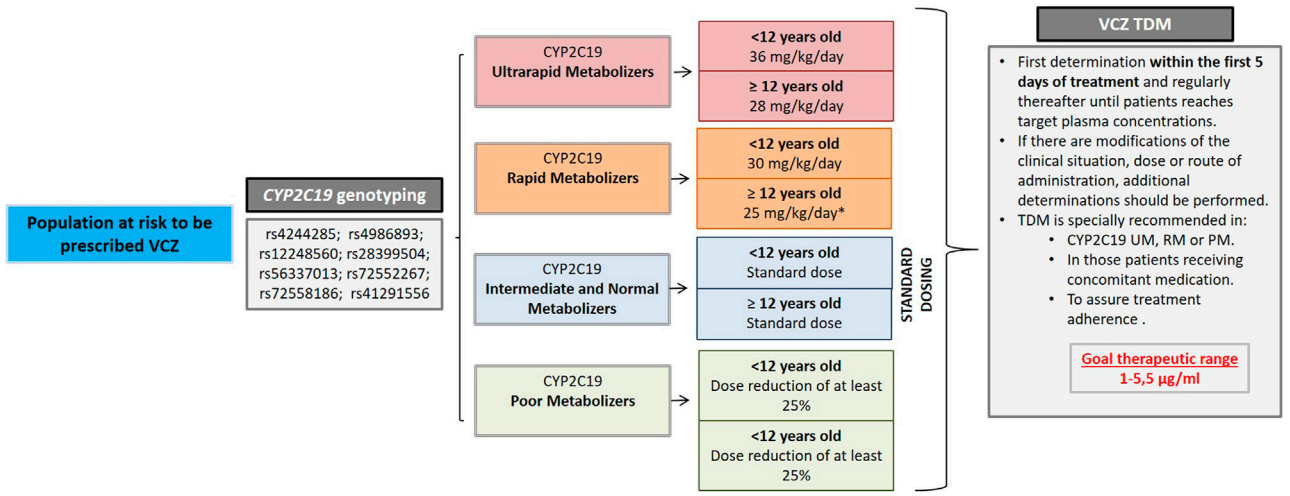

FIGURE 1 | Strategy implemented in La Paz University Hospital (HULP) for the individualization of VCZ therapy in 2017. Dose adjustments based on PGx information were derived from Hicks et al.'s study (Hicks et al., 2014) and CPIC guidelines for CYP2C19 and VCZ therapy (Moriyama et al., 2017) except dose adjustment for Rapid Metabolizer (RM) patients ≥12 years old (*), which were based on literature, and observations made in our hospital Hicks et al. (2014) proposed standard dosing for these patients. For poor Metabolizers, strict controls every 24 h are recommended, as the risk of toxicity is elevated. If ADRs are detected, choose an alternative agent. TDM recommendations were based on TDM guidelines of the British Society for Medical Mycology (Ashbee et al., 2014).

within the Clinical Pharmacogenetics Unit of La Paz University Hospital (HULP) and therefore a clinical protocol was developed in collaboration with the Pediatric Oncology and Hematology Department for individualization of VCZ therapy in immunosuppressed patients. Our strategy consisted of a combination of both preemptive genotyping of *CYP2C19* (for optimizing initial dosing) and routine TDM in hematological patients undergoing allogeneic hematopoietic stem cell transplantation (alloHSCT) with a high risk of developing IFI and who will receive VCZ as either prophylaxis or treatment (**Figure 1**).

We aimed to provide information about our experience implementing a strategy to individualize VCZ treatment including *CYP2C19* preemptive genotyping and TDM in immunocompromised pediatric patients. Also, our objective is to compare our results with the standard care results obtained by Hicks et al. and those expected in their simulation of VCZ dosing based on PGx (Hicks et al., 2014) as a measure to evaluate its efficacy for (a) incrementing the number of patients within therapeutic range 5 days after VCZ administration and (b) reducing the time required to achieve therapeutic plasma concentrations during treatment/prophylaxis.

METHODS

Patients and Study Design

The study was designed as a single-center, retrospective study, focusing on immunocompromised pediatric patients. The patients selected were managed according to the protocol described in **Figure 1** that was implemented in routine care in 2017, prior to conducting this study. Patients genotyped for *CYP2C19* that eventually received VCZ and had at least one VCZ plasma trough concentration were eligible for this study. We selected a cohort of 28 immunocompromised patients with malignant conditions undergoing alloHSCT who were prescribed VCZ either as prophylaxis or treatment for a suspected IFI. All patients were pediatric, aged 1–18 years. All the participants/their parents or legal guardians (if applicable) provided written consent before the pharmacogenetics study.

According to our protocol, at the first clinical evaluation previous to alloHSCT, blood samples are collected and sent to the Clinical Pharmacogenetics Unit for preemptive genotyping of *CYP2C19*. Therefore, genetic results are available at the time of VCZ prescription through the Electronic Health Record (EHR). An initial sampling of VCZ concentrations is indicated within the first 5 days of treatment. Regular PK measures should be performed thereafter until patients reached target plasma concentrations (1–5.5 µg/ml) or until treatment or prophylaxis is completed (Park et al., 2012; Ashbee et al., 2014; Luong et al., 2016b). Prophylaxis is usually maintained for 100 days, but it can be extended to day 180 in patients with continuous immunosuppression or graft vs. host disease. Treatment is maintained until IFI completes remission.

This study is under the umbrella of a master protocol approved by the Ethics Committee (CEIm) of Hospital Universitario La Paz (Identifier: Clinical Ethical Approval No. PEI-2915) on September 21, 2017.

Pharmacogenetic Study

Molecular analysis was performed in all 28 patients for the selected SNPs of the *CYP2C19* gene: rs4244285 (c.681G > A), rs4986893 (c.636G > A), rs12248560 (c.–806C > T), rs28399504 (c.1A > G), rs56337013 (c.1297C > T), rs72552267 (c.395G > A), rs72558186 (c.819 + 2 T > A), and rs41291556 (c.358 T > C) using our custom SNP-array platform PharmArray®. Genotypes were

codified to the star-allele nomenclature (*) using the Haplotype Set IDs provided by PharmGKB (Whirl-Carrillo et al., 2012) and PhamVar (PA166128323) (Gaedigk et al., 2018). CYP2C19 phenotypes were inferred using the CPIC standardized allele definition and functionality tables (PA166124411) as well as specific clinical guidelines (Moriyama et al., 2016). The final molecular report was integrated in the EHR of each patient.

VCZ Initial Dose Adjustment Based on PGx Results

Initial dose adjustment recommendations were made by the Clinical Pharmacology Department and were mainly based on Hicks et al. calculations of extrapolated doses (Hicks et al., 2014) and CPIC clinical guidelines (Moriyama et al., 2017). Our individualization strategy included modification of dosage in CYP2C19*1/*17 patients ≥12 years old. These patients were assigned a dose of 14 mg/kg/day in Hicks et al. simulation and pediatric patients with this phenotype were recommended to initiate therapy with standard care dosing by CPIC clinical guidelines; however, there is evidence demonstrating that these patients are likely to have subtherapeutic trough concentrations when receiving standard doses (OwusuObeng et al., 2014; Hamadeh et al., 2017). Based on the literature and our previous experience, we considered that these patients required higher doses to achieve target concentrations and we recommended an initial dose of 25 mg/kg/day instead.

Figure 1 shows our VCZ therapy individualization strategy. Clinical recommendations based on genetic results were incorporated into the EHR.

Analysis of VCZ Plasma Concentrations and Dose Adjustment Based on TDM

VCZ plasma concentrations were measured at Hospital La Paz in the TDM Laboratory of the Clinical Pharmacology Department by immunoassay analysis: ARK voriconazole assay (Thermo Scientific) in a Dimension® EXL 200 de Siemens®. The lower limit of VCZ detection was 0.7 μg/ml and the upper limit of detection was 16.0 μg/ml.

The samples analyzed were trough concentration. Samples were sent to the TDM Laboratory following standard clinical procedure for hospital samples.

Plasma trough concentrations as per our protocol are recommended to be measured within the first 5 days of VCZ administration and regularly thereafter until target concentrations (1–5.5 μg/ml) are reached.

TDM recommendations were based on TDM guidelines of the British Society for Medical Mycology (Ashbee et al., 2014). TDM recommendations were also incorporated into the EHR.

Statistical Analysis

Descriptive statistics were calculated for all variables, with percentages being reported. The Shapiro Wilks test was used to contrast if the first concentration measure after initial dose adjustment based on PGx was normally distributed in our population. We rejected the null hypothesis in the test for normality ($p < 0.001$) concluding that concentration shows a nonnormal distribution. Statistical analyses were performed using R software (V 3.6.3). To assess for significant differences between phenotype and first concentration measure after initial dose adjustment based on PGx, the Mann–Whitney *U* test was applied.

RESULTS

Study Population Characteristics

The demographic and clinical data as well as *CYP2C19* diplotype frequencies found in our cohort are summarized in **Table 1**. Our population consisted of 28 immunocompromised pediatric patients undergoing allogeneic hematopoietic stem cell transplantation due to different malignancies and therefore at risk of invasive fungal disease. The study population was stratified by age (≤11 years and ≥12 years) to properly compare our results with those in Hicks et al.'s study (Hicks et al., 2014): 79% (N = 22) of the patients were 11 years old and younger and 21% (N = 6) were over 11 years old. A comparison between *CYP2C19* diplotype frequencies found in our cohort of the Spanish population and those found in Hicks et al.'s study (Hicks et al., 2014) is shown in **Table 1**. After molecular analysis, we recommended different initial doses of VCZ depending on the CYP2C19 phenotypic classification. The average time from molecular study request to the incorporation of the clinical recommendations into the EHR was 21.9 days. The final pharmacogenetic clinical report was always available at the time of prescription.

Based on the previously mentioned criteria (**Figure 1**), we found that standard dose modifications were indicated in 29% (N = 8) of the patients in our cohort. CYP2C19*1/*1, *1/*2, and *2/*17 patients (CYP2C19 NM and IM) were assigned standard initial doses, whereas CYP2C19*1/*17 and *17/*17 patients (CYP2C19 RM and UM) were recommended increased starting doses (**Figure 1**). No PM were found in our cohort.

Pharmacokinetic Evaluation of a Strategy for the Individualization of VCZ Treatment Based on PGx and TDM

The average VCZ trough concentration was 2.15 ± 2.62 μg/ml for all *CYP2C19* diplotype groups. There were no significant differences between groups.

We found that 57.14% (N = 16) of the patients achieved target VCZ concentrations in the first VCZ level determination after genetic results were available for initial dose adjustment. When subdividing by CYP2C19 phenotypic group, we can see that 65% (N = 13) of the CYP2C19 NM and IM (assigned with standard initial doses), 33.33% (N = 2) of the RM, and 50% (N = 1) of the UM achieved goal therapeutic range in the first VCZ level determination after genetic results were available for initial dose adjustment. (**Table 2.1A**). **Figure 2** shows VCZ trough concentrations at the first measure after initial dose adjustment based on PGx.

TABLE 1 | Patients characteristics (*N* = 28). Patients characteristics found in our cohort of the Spanish population are compared to those in Hicks et al.'s study (*N* = 33).

			All patients *N* = 28	Hicks et al. *N* = 33
Age	<12 years old, n(%)		22 (78.57%)	19 (58.58%)
	≥12 years old, n(%)		6 (21.43%)	14 (42.42%)
	Median (years) [range (years)]		9.5 [<1–17]	9.0 [1–19]
Gender, n (%)	Female		16 (57.14%)	14 (42.42%)
	Male		12 (42.86%)	19 (58.58%)
CYP2C19 dyplotype, n (%)	CYP2C19*17/*17		2 (7.14%)	4 (12.12%)
	CYP2C19*1/*17		6 (21.43%)	8 (24.24%)
	CYP2C19*2/*17		3 (10.71%)	0 (0%)
	CYP2C19*1/*1		13 (46.43%)	11 (33.33%)
	CYP2C19*1/*2		4 (14.29%)	9 (27.27%)
	CYP2C19*2/*2		0 (0%)	1 (3.03%)
Primary diagnosis, n (%)	Acute lymphoblastic leukemia		5 (17.9%)	12 (36.4%)
	Acute myeloid leukimia		6 (21.4%)	13 (39.4%)
	Non-Hodgkin lymphoma		1 (3.6%)	3 (9.1%)
	Other		16 (57.1%)	5 (15.1%)
		Severe aplastic anemia posthepatitis	2	
		Acute biphenotypic leukemia	1	
		Autoimmune lymphoproliferative syndrome due to CTLA4 haploinsufficiency	1	
		Fanconi anemia	1	
		Idiopathic aplastic anemia	4	
		Malignant infantile osteopetrosis	1	
		Combined immunodeficiency	4	
		Chronic granulomatous disease	1	
		Sickle cell anemia	1	

In the subgroup of patients under 12 years old, we found that 62.50% of the CYP2C19 NM and IM, 25% of the RM, and 50% of the UM achieved goal therapeutic range in the first measure after initial dose adjustment based on PGx (**Table 2.2A**). The number of patients ≥12 years old in our cohort is limited (*N* = 6). In this subgroup of patients, 75% of the CYP2C19 NM and IM, 50% of the RM, and 50% of the UM achieved goal therapeutic range in the first measure after initial dose adjustment based on PGx (**Table 2.3A**).

In those patients who were not able to achieve target concentrations in the first level measured, VCZ trough concentrations were regularly assessed in order to guide dose modifications and achieve the goal therapeutic range. In our study including all groups of age, we found that 90% of the CYP2C19 NM and IM and 100% of the CYP2C19 RM and UM achieved target concentrations during treatment/prophylaxis (**Table 2.1B**). The observed times required to achieve the goal therapeutic range for each patient are represented in **Figure 3**; 75% (*N* = 21) of the patients achieved target concentrations within the first 20 days of treatment.

DISCUSSION

Due to its great interindividual variability in plasma concentrations and clinical response, there is a growing interest in personalizing VCZ therapeutic strategies for each patient. To this aim, optimization of VCZ initial dosing and TDM have been reported as interesting tools for guiding VCZ treatment and prophylaxis (Park et al., 2012; Ashbee et al., 2014; Hicks et al., 2014; Boast et al., 2016). In this context, our group has designed a protocol for the individualization of VCZ therapy in immunocompromised patients pre-alloHSCT based on PGx (for the optimization of initial dosing) and routine TDM for further dose adjustments. We implemented a "preemptive genotyping strategy in a predefined risk population" (Luong et al., 2016a), where molecular analysis was requested in the first clinical evaluations pre-alloHSCT. Therefore, short response times were required, so molecular and clinical reports could be available at the time of VCZ prescription. The average response time in our study cohort was 21.9 days and met the required treating deadlines.

Taking into account molecular results and based on Hicks et al. simulation (Hicks et al., 2014), CPIC clinical guidelines, and previous own experience, we recommended modifications of initial standard dosing in 29% of the patients. We found that 57.14% of our patients achieved target VCZ concentrations in the first measure after initial dose adjustment based on PGx. In contrast, only 46.5% of VCZ troughs (obtained at a steady state) from the patients in Hicks et al.'s study, where all patients were treated with standard VCZ regimens, were within the therapeutic range (Hicks et al., 2014). In their simulation with extrapolated initial doses, Hicks et al. predicted that 60% of the VCZ troughs would be within the therapeutic range, similar to the results in our cohort. Results of our cohort stratified by phenotype and age can be found in **Table 2**. **Table 3** shows a comparison between our overall results and those found in Hicks et al.'s study. **Table 4** shows a comparison between our results and those found in Hicks et al.'s study subgrouped by phenotype.

In our cohort, CYP2C19*1/*1, *1/*2, and *2/*17 (CYP2C19 NM and IM) were assigned standard initial doses, resulting in

TABLE 2 | Percentage of patients in the goal therapeutic range (1–5,5 μg/ml) by *CYP2C19* diplotype in our study cohort including 1) all groups of age, 2) patients <12 years old, and 3) patients >12 years old. (A) First trough level measure after initial dose adjustment based on PGx. (B) Level within goal therapeutic range measured after the first trough.

CYP2C19 diplotype classification	CYP2C19 inferred phenotype	(A) % of patients in goal therapeutic range at first measure after initial dose adjustment based on PGx	(B) % of patients in goal therapeutic range at any level extracted after the first trough
(1) All groups of age (*N* = 28)		57.14%	92.86%
CYP2C19*1/*1, *1/*2, and *2/*17	CYP2C19 Normal and Intermediate Metabolizers (NM and IM)	65.00%	90%
CYP2C19*1/*17	CYP2C19 Rapid Metabolizers (RM)	33.33%	100%
CYP2C19*17/*17	CYP2C19 Ultrarapid Metabolizers (UM)	50.00%	100%
(2) Patients <12 years old (*N* = 22)			
CYP2C19*1/*1, *1/*2, and *2/*17	CYP2C19 NM and IM	62.50%	87.5%
CYP2C19*1/*17	CYP2C19 RM	25.00%	100%
CYP2C19*17/*17	CYP2C19 UM	50.00%	100%
(3) Patients >12 years old (*N* = 6)			
CYP2C19*1/*1, *1/*2, and *2/*17	CYP2C19 NM and IM	75.00%	100%
CYP2C19*1/*17	CYP2C19 RM	50.00%	100%
CYP2C19*17/*17	CYP2C19 UM	50.00%	100%

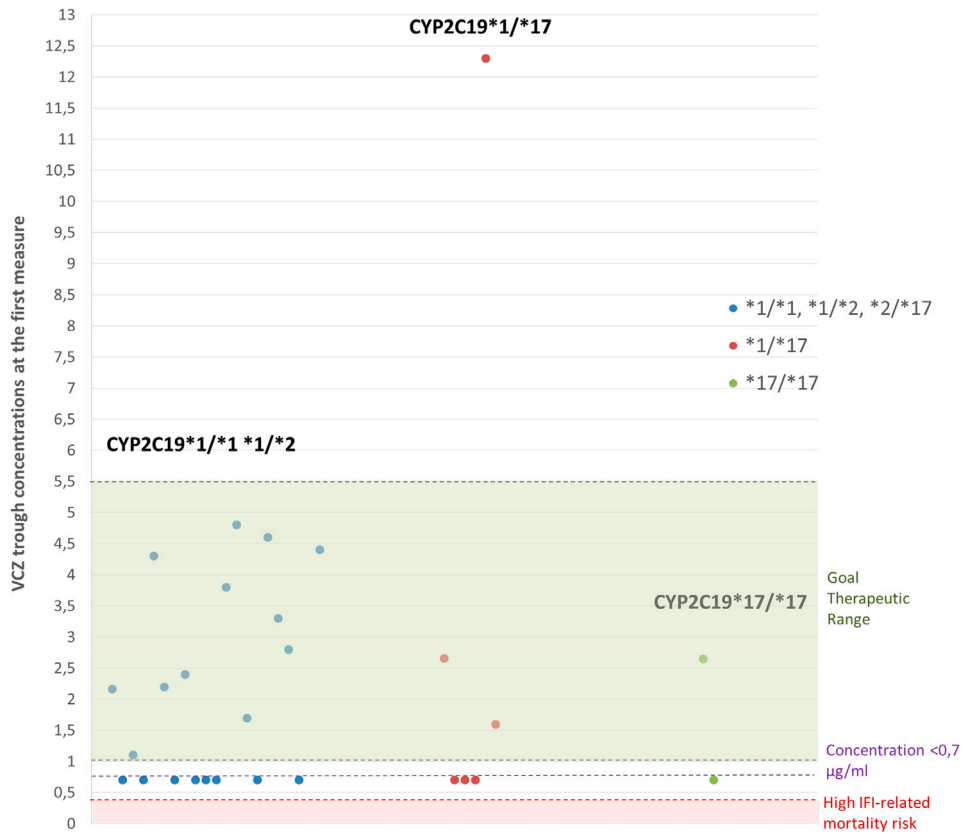

FIGURE 2 | VCZ trough concentrations obtained at the first measure after initial dose adjustment based on PGx. Green area represents target therapeutic range (1–5.5 μg/ml). The red area (<0.35 μg/ml) represents a high-risk area of IFI-related mortality (Miyakis et al., 2010). *N* = 28 patients.

65% of the patients achieving target concentrations in the first trough concentration determination. CYP2C19*1/*17 patients were recommended an initial standard dose of 25 mg/kg/day in patients ≥12 years old and 30 mg/kg/day in younger patients resulting in 33.33% of the patients achieving target concentrations in the first 5 days (**Table 2.1A**). Hicks et al.

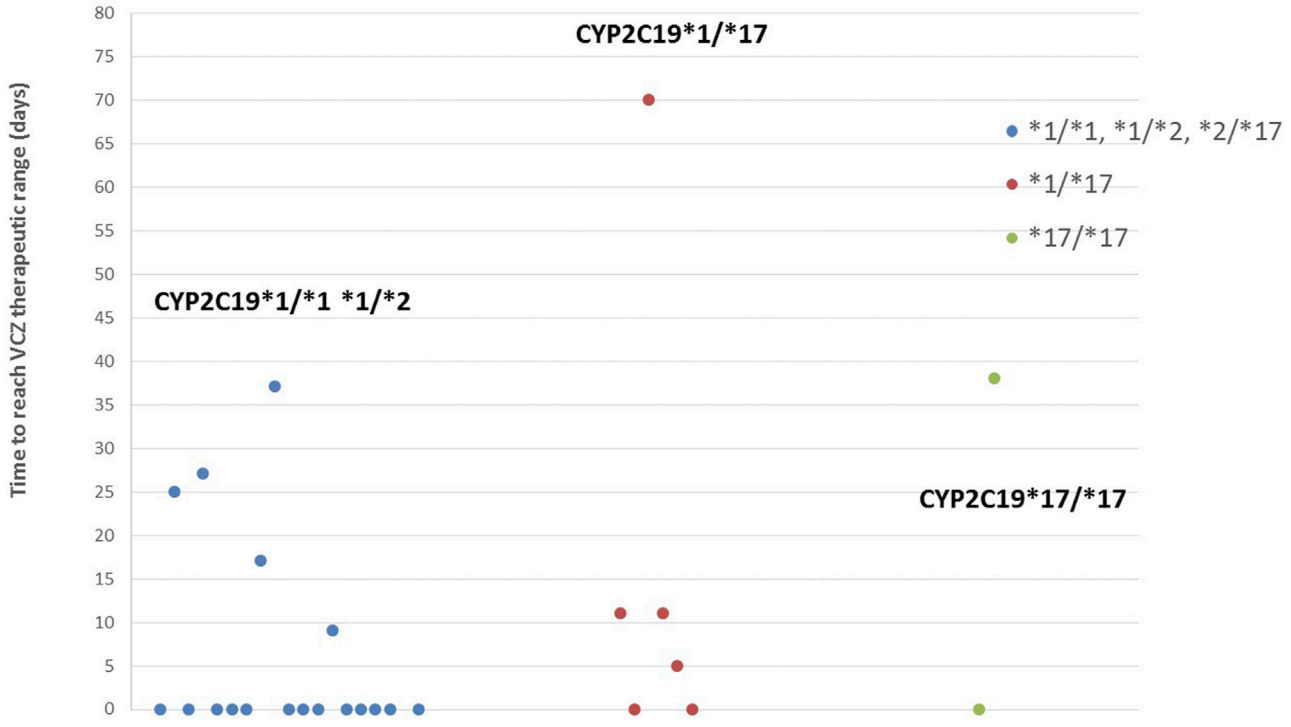

FIGURE 3 | Time to reach VCZ goal therapeutic range (1–5,5 μg/ml) with a combined PGx and TDM strategy. 75% of the patients achieved target concentrations within the first 20 days and 92.85% before the end of treatment/prophylaxis.

TABLE 3 | Percentage of patients[a]/troughs[b] within goal therapeutic range. Comparison of our results (A) with those found in Hicks et al.'s study with standard care and (B) simulation with extrapolated doses (C).

Study Cohort (*N* = 28)	Hicks et al. (*N* = 33)	
(A) % of patients in goal therapeutic range: first measure after initial dose adjustment based on PGx	**(B) % of voriconazole troughs within the goal therapeutic range (Hicks et al. standard care)**	**(C) % of voriconazole troughs within the goal therapeutic range (Hicks et al. simulation with extrapolated doses)**
57.14%	46.5%	60%

[a]*In our study, the percentage of patients within the goal therapeutic range were calculated.*
[b]*Hicks et al. calculated the proportion of voriconazole troughs within the therapeutic range. The proportion of patients within the goal therapeutic range could not be extracted from Hicks et al.'s data.*

reported that only 21% of the troughs in of RM < 12 years old were within concentration range with standard care (Hicks et al., 2014) (**Table 5.1B**). In our cohort (where 79% of the patients were <12 years old), guiding initial doses based on *P*Gx information increased the percentage of RM achieving VCZ therapeutic range: 25% of RM < 12 (**Table 5.1A**). Hicks et al. did not propose dose modifications for RM patients ≥12 years old and predicted that up to 57% of the patients could achieve therapeutic range with standard doses (Hicks et al., 2014). However, based on our previous clinical experience, we recommended an increase of standard initial doses also in older patients (25 mg/kg/day) resulting in 50% of RM ≥ 12 years old achieving target concentrations (**Table 5.2A**).

As we show in pediatric patients, papers by Hicks et al. (2020) and Patel et al. (2020) reporting adult data demonstrate that increased VCZ dosage in RM/UM leads to a drastic reduction of subtherapeutic concentrations' in adult patients with neutropenic acute myeloid leukemia (Hicks et al., 2020) and in prophylaxis after allogeneic hematopoietic cell transplant (Patel et al., 2020). In this context, we propose that CYP2C19 RM (and UM) dosing recommendations should be reviewed for a greater increase of the percentage of patients achieving goal therapeutic range, still low especially among younger patients.

Finally, PGx-guided initial dosing in our cohort allowed one of the CYP2C19*17/*17 patients to achieve VCZ therapeutic

TABLE 4 | Percentage of patients[a]/troughs[b] within goal therapeutic range subgrouped by phenotype. Comparison of our results (A) with those found in Hicks et al.'s study with standard care (B). All groups of age.

	(A) Study cohort (*N* = 28)	(B) Hicks et al. (*N* = 33)
CYP2C19 inferred phenotype	**% of patients in goal therapeutic range: first measure after initial dose adjustment based on PGx**	**% of voriconazole troughs within the goal therapeutic range[c] (Hicks et al.)**
CYP2C19 NM	69.23%	63.66%
CYP2C19 IM/Poor Metabolizers (PM)	57.14[e]	90%[d]
CYP2C19 RM	33.33%	50%
CYP2C19 UM	50.00%	0%

[a]*In our study, the percentage of patients within the goal therapeutic range was calculated.*
[b]*Hicks et al. calculated the proportion of voriconazole troughs within the therapeutic range. The proportion of patients within the goal therapeutic range could not be extracted from Hicks et al.'s data.*
[c]*Voriconazole trough concentrations are the mean voriconazole thought concentrations per patient obtained from a scatter plot from Hicks et al.'s manuscript (Hicks et al., 2014).*
[d]*This group in Hicks et al.'s study included Intermediate and Poor Metabolizers (IM and PM) (CYP2C19*1/*2A, CYP2C19*1/*2B, and CYP2C19*2A/*2A).*
[e]*This group in our study included only IM (CYP2C19*1/*2 and CYP2C19*1/*17). No PM were found in our study.*

TABLE 5 | Percentage of patients[a]/troughs[b] within goal therapeutic range subgrouped by phenotype and age. Comparison of our results (A) with those found in Hicks et al.'s study with standard care and (B) and simulation with extrapolated doses (C).

		Study cohort	Hicks et al.	
***CYP2C19* diplotype classification**	**CYP2C19 inferred phenotype**	**(A) % of patients in goal therapeutic range: first measure after initial dose adjustment based on PGx**	**(B) % of voriconazole troughs within the goal therapeutic range (Hicks et al. standard care)**	**(C) % of voriconazole troughs within the goal therapeutic range (Hicks et al. simulation with extrapolated doses)**
Patients <12 years old (1)		**(*N* = 22)**	**(*N* = 19)**	
CYP2C19*1/*1	CYP2C19 NM	66.67%	51%	54%
CYP2C19*1/*2	CYP2C19 IM	100%	65%	88%
CYP2C19*2/*17	CYP2C19 IM	33.33%	NA	NA
CYP2C19*1/*17	CYP2C19 RM[a]	25.00%	21%	52%
CYP2C19*17/*17	CYP2C19 UM[a]	50.00%	0%	50%
Patients ≥12 years old (2)		**(*N* = 6)**	**(*N* = 13)**	
CYP2C19*1/*1	CYP2C19 NM	50%	36%	36%
CYP2C19*1/*2	CYP2C19 IM	66.67%	63%	63%
CYP2C19*2/*17	CYP2C19 IM	NA	NA	NA
CYP2C19*1/*17	CYP2C19 RM	50%	57%	57%
CYP2C19*17/*17	CYP2C19 UM	NA	0%	100%

[a]*In our study, the percentage of patients within the goal therapeutic range was calculated.*
[b]*Hicks et al. calculated the proportion of voriconazole troughs within the therapeutic range. The proportion of patients within the goal therapeutic range could not be extracted from Hicks et al.'s data.*
NA: no data available. No patient with that phenotype was found in that cohort.

concentrations in the first measure after VCZ administration. The other UM patient had a first VCZ trough concentration of 0.7 μg/ml and eventually achieved target concentrations after 38 days thanks to TDM (**Figure 3**). In Hicks et al.'s study, all CYP2C19*17/*17 patients showed subtherapeutic concentrations and contrary to our cohort, none of them achieve concentrations within the therapeutic range. All UM patients in our cohort were under 12 years old (**Tables 5.1A, B**). Dose adjustments based on preemptive genotyping improved the percentage of patients carrying the CYP2C19*17 achieving promptly target concentrations; however, as mentioned before, dosing recommendations in this population should be reviewed for greater results. Previous studies have reported that supratherapeutic concentrations (>5.5 μg/ml) can be related to the occurrence of adverse effects, especially neurotoxicity (Miyakis et al., 2010; Park et al., 2012). In the first measures after VCZ administration, we only found one CYP2C19 RM patient <12 years old with a VCZ trough concentration of 12 μg/ml (**Figure 2**). This patient developed voriconazole-induced phototoxicity. However, this was rapidly corrected after TDM and therapeutic VCZ concentrations were achieved in 5 days. This could be due to the presence of drug interactions, nonlinear PK unpredicted variability or a rare *CYP2C19* variant, or genetic variations in other genes involved in the metabolic pathway not detected by our genotyping panel. However, the group of patients more likely to show VCZ plasma concentrations >5.5 μg/ml are CYP2C19*2*/*2 patients, with no representation in this study.

The second tool used in our individualization strategy was TDM for guiding VCZ dose adjustments in those patients that did not achieve therapeutic range in the first measure since VCZ administration. **Table 3** shows the percentage of patients that achieve the VCZ therapeutic range before the end of treatment/prophylaxis. We found that 90% of the CYP2C19 NM and IM and 100% of the CYP2C19 RM and UM achieved target

concentrations during treatment/prophylaxis and therefore the potential of this strategy to improve dose adjustment. Treatment failure has been reported to occur within the first 35 days of treatment (Miyakis et al., 2010). Implementing our combined *P*Gx and TDM strategy, 75% of the patients achieved target concentrations within the first 20 days of treatment.

The main limitation of our study is that the data were collected retrospectively from medical records and some information was not available. However, data concerning genetic results, dose recommendations based on phenotype, and at least one VCZ plasma trough level were available for all the patients. According to our clinical protocol, an initial sampling of VCZ concentration should be obtained within the first 5 days of treatment; however, not all the clinicians followed this recommendation. Another limitation is that, in those patients who did not achieve therapeutic range in the first measure since VCZ administration, dose adjustment recommendations were based on VCZ trough concentrations; however, final dose modifications were performed at the discretion of the treating specialist. Also, another limitation of the study is the lack of a control group in which dose adjustments are based only on TDM. Finally, probably due to relatively small sample size, PM were not represented in our cohort; in spite of this, our protocol includes dose recommendations for these patients: dose reduction of at least 25% of standard dose and early and strict TDM to minimize the risk of concentrations above the therapeutic range (Scholz et al., 2009; Lee et al., 2012; Wang et al., 2014b; Hicks et al., 2014). Despite the fact that our study is not exempt from limitations, it provides relevant information about VCZ individualization based on PGx.

Herein, we have described our strategy for VCZ individualization based on PGx and TDM. Unfortunately, the implementation of similar strategies in the clinical practice still faces different challenges: lack of large population-based studies, insufficiency of cost-effectiveness evidence, and the general barriers to pharmacogenetics implementation.

In this context, we are developing a multicenter, randomized clinical trial to evaluate the effectiveness and efficiency of a preemptive genotyping strategy for VCZ, including an economic evaluation from the perspective of the Spanish National Health System. (Lee et al., 2012; MonserratVillatoro et al., 2020).

CONCLUSION

Taking into account these results, we can see that there is a need to improve VCZ dose predictions and that PGx represents a helpful tool for initial dose adjustment and optimization, especially in patients with extreme phenotypes, as it helps to increase the number of patients within goal therapeutic range and decreases the time required to achieve target concentrations when compared with standard care. However, due to the VCZ nonlinear pharmacokinetics resulting in unpredictable and unanticipated changes in drug exposure, TDM is extremely important for guiding dose modifications over treatment and prophylaxis. In our experience, a combination of both strategies can be of great benefit for the patients.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary materials; further inquiries can be directed to the corresponding author/s.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of La Paz University Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

Trial conceptualization was performed by IG, ID, AC, and AB. IG and AB developed the study methodology. IG, ID, JM, LM, DB, LD, JQ, AR, and PG were responsible for the investigation. ER, JF, AP, AC, and AB contributed to study supervision. IG, AB, and AC contributed to writing the original draft, and all authors contributed to the article review and editing.

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REVIEW
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Reviewing Data Integrated for PBPK Model Development to Predict Metabolic Drug-Drug Interactions: Shifting Perspectives and Emerging Trends

Kenza Abouir[1,2], *Caroline F Samer*[1,3], *Yvonne Gloor*[1], *Jules A Desmeules*[1,2,3] *and Youssef Daali*[1,2,3]*

[1]Division of Clinical Pharmacology and Toxicology, Department of Anesthesiology, Pharmacology, Intensive Care and Emergency Medicine, Geneva University Hospitals, Geneva, Switzerland, [2]Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), University of Geneva, Geneva, Switzerland, [3]Faculty of Medicine, University of Geneva, Geneva, Switzerland

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***Correspondence:**
Youssef Daali
Youssef.daali@hcuge.ch

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Physiologically-based pharmacokinetics (PBPK) modeling is a robust tool that supports drug development and the pharmaceutical industry and regulatory authorities. Implementation of predictive systems in the clinics is more than ever a reality, resulting in a surge of interest for PBPK models by clinicians. We aimed to establish a repository of available PBPK models developed to date to predict drug-drug interactions (DDIs) in the different therapeutic areas by integrating intrinsic and extrinsic factors such as genetic polymorphisms of the cytochromes or environmental clues. This work includes peer-reviewed publications and models developed in the literature from October 2017 to January 2021. Information about the software, type of model, size, and population model was extracted for each article. In general, modeling was mainly done for DDI prediction via Simcyp® software and Full PBPK. Overall, the necessary physiological and physio-pathological parameters, such as weight, BMI, liver or kidney function, relative to the drug absorption, distribution, metabolism, and elimination and to the population studied for model construction was publicly available. Of the 46 articles, 32 sensibly predicted DDI potentials, but only 23% integrated the genetic aspect to the developed models. Marked differences in concentration time profiles and maximum plasma concentration could be explained by the significant precision of the input parameters such as Tissue: plasma partition coefficients, protein abundance, or Ki values. In conclusion, the models show a good correlation between the predicted and observed plasma concentration values. These correlations are all the more pronounced as the model is rich in data

Abbreviations: DRP, Drug-related problems; DDI, Drug-drug interaction; PK, Pharmacokinetic; ADME, Absorption Distribution Metabolism or Elimination; PD, Pharmacodynamic; CYP450, Cytochrome P-450; UGTs, UDP-glucuronosyltransferases; SULTs, Sulfotransferases; NATs, N-acetyltransferases; GSTs, Glutathione S-transferases; MTs, Methyltransferases; PM, Poor metabolizers; NM, Normal metabolizers; IM, Intermediate metabolizers; UM, Ultra-rapid metabolizers; SLC, Solute carrier; ABC, ATP-binding cassette; QSAR, Quantification of structure-activity relationships; QSP, Quantitative systems pharmacology; PBPK, Physiologically based pharmacokinetic; FDA, Food and Drug Agency; EMA, European medicines agency; PMDA, Japanese Pharmaceuticals and Medical Devices Agency; DGI, Drug-gene interaction; TDM, Therapeutic drug monitoring; CPOE, Computerized physician order entry; HER, Electronic health records.

representative of the population and the molecule in question. PBPK for DDI prediction is a promising approach in clinical, and harmonization of clearance prediction may be helped by a consensus on selecting the best data to use for PBPK model development.

Keywords: physiologically-based pharmacokinetics, drug-drug interaction, clinical setting, metabolism, precision dosing, transporters

INTRODUCTION

In more cases than expected, the therapeutical management process involves a myriad of errors making drug-related problems (DRP) a recurring reviewed subject. In general, a large part of the DRP originates from drug prescribing issues (Perry et al., 2020). Difficulties can range from prescribing an inaccurate dose to inadequate administration frequency on top of a known allergy or a drug-drug interaction (DDIs). Among these risk factors, belonging to extremes of age, renal and liver impairment, or having genetic variations, are likely to increase developing DDI. Combination therapy is becoming increasingly prevalent in managing concurrent or single disease (Bi et al., 2018b), especially in geriatric patients. Previous Swiss studies have shown that polypharmacy prevalence was 11.8% and that it increased with age from 2.9% for age group 40–49 to 25.5% for age group 65–81 (Castioni et al., 2017). Dechanont et al. showed that DDI represents 1.1% of overall hospital admissions in this population and that 22.2% of ADRs are related to DDIs.

Pharmacokinetic and Pharmacodynamic Drug-Drug Interactions

A pharmacokinetic (PK) DDI occurs when a perpetrator drug impacts the absorption, distribution, metabolism, or elimination (ADME) of a victim drug in one or more of the human body compartments. Pharmacodynamic (PD) interactions occur when two medicines directly interact (for example, on the same drug target) without altering the ADME parameters. PK and PD interactions may enhance activity (synergism) or decrease the effects (antagonism), affecting plasma drug levels and effects and having more or less severe consequences depending on the therapeutic margin of a drug (Hanke et al., 2018). The clinical consequences of DDIs can vary significantly in severity, from a simple rash to a life-threatening event or a serotoninergic syndrome (Prieto Garcia et al., 2018; Wang et al., 2019). The absorption of drugs and the ability to metabolize them varies considerably from one individual to another. The intrinsic difference between individual patients is caused by the inheritance of variant alleles, encoding drug-metabolizing enzymes. Genetic variations are estimated to contribute 20–30% of the variability in drug response (Sim et al., 2013).

Drug Metabolism and Transport

Drug metabolism is divided into phase I and phase II reactions (**Figure 1**). Although most phase I metabolic reactions are catalyzed by Cytochrome P-450 (CYP450), the most studied metabolizing enzymes, other enzymes such as oxidoreductase, esterases, and oxidases can also be involved in phase I drug oxidation, reduction, and hydrolysis. Phase II reactions are conjugation reactions in which phase I metabolites or the parental compounds themselves undergo glucuronidation, sulfonation, methylation, acetylation, glutathione, and amino acids conjugation. The main enzymes involved in phase II drug metabolism include UDP-glucuronosyltransferases (UGTs), sulfotransferases (SULTs), N-acetyltransferases (NATs), glutathione S-transferases (GSTs), and various methyltransferases (MTs) (Oda et al., 2015) In parallel to the metabolic enzymes, membrane transporters also play a crucial role in drug absorption, distribution, and elimination. In complement to the metabolic phase I and phase II elimination, the term phase III elimination is sometimes used to describe the excretion of drugs and their metabolites by carrier-mediated uptake of drugs (Döring and Petzinger, 2014). Drug-transporters are membrane-bound proteins expressed in various organs and play an essential role in influencing drug absorption (phase 0) and elimination (phase III) of drugs and their metabolites and hence, therapeutic efficacy.

Genetic Polymorphism of Drug Metabolizing Enzymes and Transporters

During the last decades, genes responsible for drug metabolism and transport and their most common functional variants have been identified based on the sampling of extreme phenotypes. For instance, for the CYP enzymes, four phenotypes with progressively increasing CYP activity can be defined: poor metabolizers (PMs) lacking the functional enzyme, normal metabolizers (NMs) homozygous for normal alleles, intermediate metabolizers (IMs) heterozygous for one deficient allele, or carrying two alleles that result in reduced activity and ultra-rapid metabolizers (UMs). The latter carry multiple gene copies (Ingelman-Sundberg 2005). Based on similarities of the sequence of genes encoding P450 enzymes, 18 CYP450 families are distinguished and branch out into 43 subfamilies (Waring 2020). All genes encoding P450 enzymes in families 1–3 are polymorphic (Zanger and Schwab 2013). Up to now, more than 350 functionally polymorphic CYPs (not counting the subvariants) that affect the function and/or activity of the gene products have been presented on the Human CYP allele nomenclature committee web page (http://www.imm.ki.se/cypalleles) (Zhou et al., 2009). The most important CYP families related to drug metabolism are CYP1A, CYP2C-D-

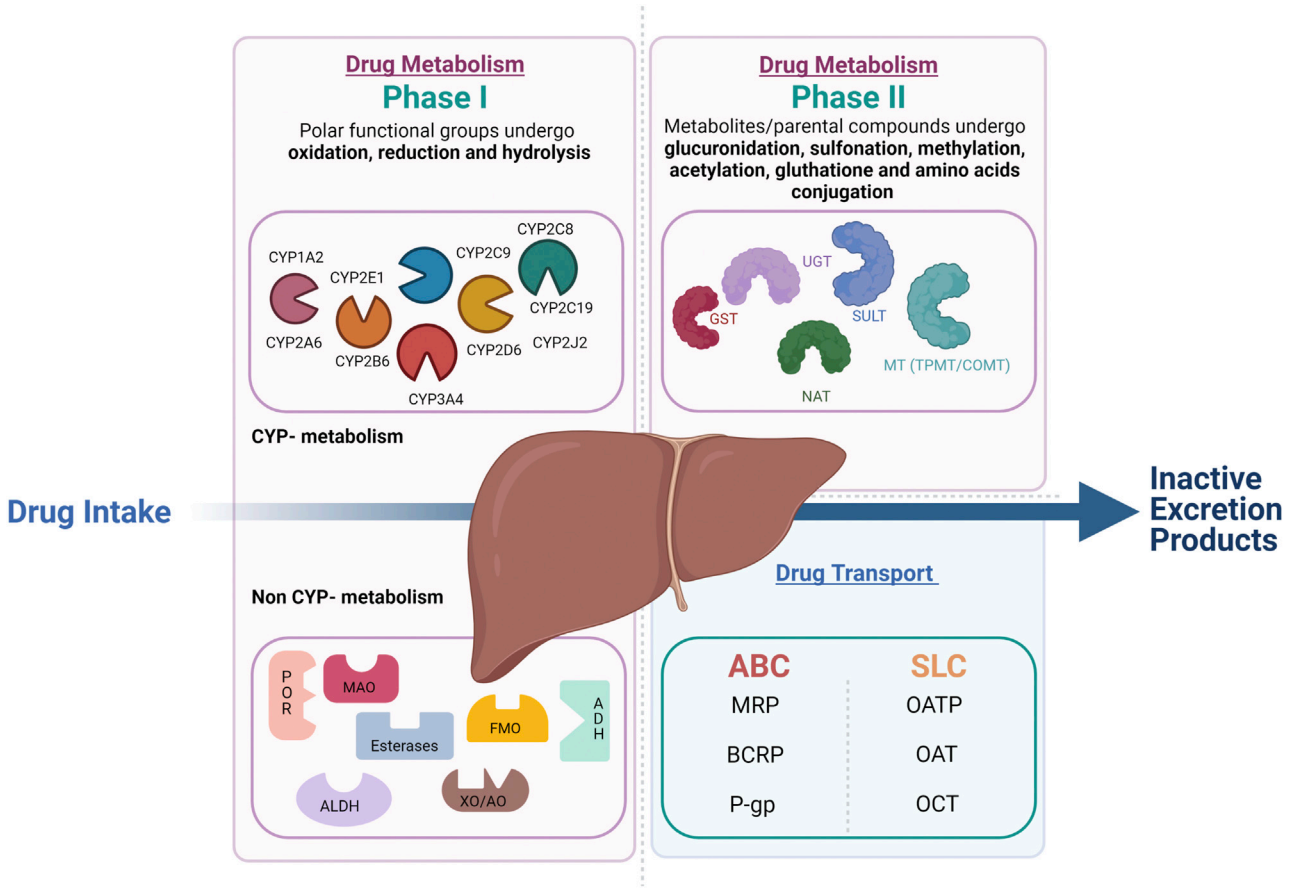

FIGURE 1 | Overview of drug metabolism and transport in the liver. Drug metabolism is divided into phase 1 and phase 2 reactions. In phase 1 reactions, polar functional groups are unmasked or introduced to the molecules through oxidation, reduction and, hydrolysis. The so formed phase 1 metabolites can be readily excreted or can undergo subsequent conjugation reaction with hydrophilic moieties (phase 2 reactions). Transporters play a complemental role to the phase 1 and 2 assuring the phase 0 (uptake) and phase 3 (export) crucial to the drug elimination by metabolism.

E, and CYP3A. Interindividual variability considerably marks CYP1A2. Even though most of the CYP1A2 variability is due to genetic elements, this enzyme's activity and expression are widely influenced by environmental factors. Cigarette smoking and excessive consumption of broccoli, among other things, are well-established CYP1A2 inducers (Anttila et al., 2003; Vanduchova et al., 2016). CYP2C8, CYP2C9, CYP2C18, and CYP2C19 are four highly homologous genes that distinguish CYP2C subfamilies. Of these four genes, CYP2C9 and CYP2C19, with a potential functional impact on the drugs' efficacy and adverse effects, are the most clinically relevant. CYP2C9 is accountable for 15–20% of phase I metabolized drugs (Läpple et al., 2003). CYP2E1 is responsible for the metabolism of 2.5% of clinically relevant xenobiotics, mainly small molecules (Hines 2008). CYP2D6 is the most polymorphic metabolic enzyme, with over 145 different alleles to date (Gaedigk et al., 2017). CYP3A subfamily enzymes include CYP3A4, CYP3A5, CYP3A7, and CYP3A43. The first three shares 85% sequence similarity responsible for 46% of the oxidative metabolism of clinically relevant drugs (Williams et al., 2002). Besides the CYPs mediated–DDI, the DDIs may be related to non-CYP enzymes and transporters, the most important of which, UGTs, uptake transporters (OATPs, OATs, and OCTs), and efflux transporters (P-gp, BCRP). Comparably to CYPs, UGT is principally located in the liver but can also be found in other tissues. UGT1-UGT2 can be divided into 3 subfamilies UGT1A, UGT2A and UGT2B. The UGT1A1, 1A3, 1A4, 1A9, and 2B7 are the hepatic ones responsible for conjugating 80% of common drugs known to be glucuronidated. In addition, many drugs can act as UGT inhibitors or inducers (Uchaipichat et al., 2006; Aceves Baldó et al., 2013). Drug transporters are categorized into two superfamilies: solute carrier (SLC) and ATP-binding cassette (ABC). The SLC transporters are typically involved in the uptake of drugs into the cells across the basolateral membrane through facilitated diffusion or secondary active transport. ABC transporters are efflux transporters that utilize primary active transport. The well-known transporters involved in DDIs are P-gp, BCRP (ABC transporters), OATP1B1/OATP1B3, OAT1/OAT3, OCT2, and MATE1/2K (SLC transporters).

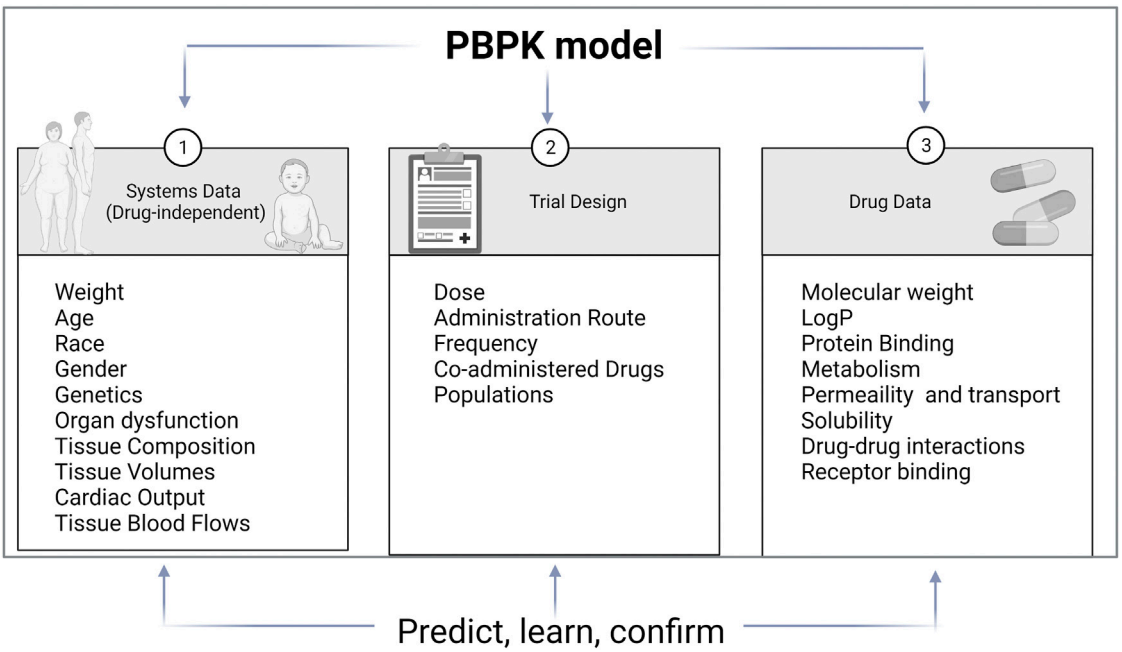

FIGURE 2 | PBPK model components. PBPK models are separated into three main components: the drug, the system and the trial. Drug data include physicochemical and experimental or predicted ADME data. System data include physiological data which relevant for the ADME properties of drugs. Trial data include information on trial design such as administration route, dose regimen or trial duration (adapted from (Jamei 2016))

Predictive Models

Improvement in computational tools led to predictive models used in clinical pharmacology. The main modeling approaches are quantification of structure-activity relationships (QSAR), quantitative systems pharmacology (QSP), and Pharmacokinetic modeling (PK modeling). QSAR is based on physicochemical and structural properties and identifies and explains intra- and inter-individual variability. QSP describes drug activity as a perturbation of a biological system. PK modeling aim to explain all PK characteristics of a drug and describe substrate's and inhibitor's, time-variable concentrations (**Figure 2**). Classical PK models are static mathematical models typically used to describe the relationship between the plasma or relevant tissue concentration of the drug and time. Over time, the classical approach based on a central compartment representing plasma linked to one or two peripheral compartments via rate constant evolved towards multicompartmental models referred to as physiologically based pharmacokinetic (PBPK) (Jones and Rowland-Yeo, 2013). Unlike other approaches, PBPK describe time-variable concentrations of the substrate in the different organs of the body. Comparatively to classical PK, it is a bottom-up, dynamic approach integrating drug-specific data and species physiology (system data, independent from the drug) to assess the impact of single and or combined intrinsic and extrinsic factors such as genetics, physiology, diseases, or co-treatments, on drug PK and PD properties in a population of individuals rather than an average subject. It divides the body into anatomically and physiologically meaningful compartments integrating system specificities and drug properties (Jones and Rowland-Yeo, 2013). PBPK models are built based on the same mathematical framework as classical PK models. PBPK numerous compartments correspond to the different organs or tissues in the body and incorporate biological and physiological components of each. These compartments include the central tissues of the body, namely, adipose, bone, brain, gut, heart, kidney, liver, lung, muscle, skin, and spleen, and are linked by the circulating blood system PBPK model structure is built upon the system properties composed of two parts, the anatomical one, and the drug-specific one. The system-related components consist of an anatomical part that describe the species-specific physiological parameters and a drug-specific part that describes the individual's drug's ADME properties (Jones and Rowland-Yeo, 2013). Therefore, the "system" operates the importance of demographic, anatomical, and physiological variables such as hepatic blood flow, CYP abundance, liver volume, and liver/renal function as a function of disease or age.

Building a "system" property to PBPK models allows for quantitative assessment of the impact of the covariates cited previously. PBPK modeling strategy relies on the iterative "Learn, confirm, and refine" approach (Darwich et al., 2017). The PBPK model is developed and validated in a healthy volunteer population starting from available data on the literature and/or on data collected in preclinical studies or from *in vitro* experiments. Following models building, simulations are run in the target population using relevant "system" and "drug" specific parameters. PBPK is an established tool that is now accepted by the regulatory authorities such as the Food and Drug Agency (FDA), European medicines agency (EMA), and Japanese Pharmaceuticals and Medical Devices Agency (PMDA)

(Shekhani et al., 2020). It provides a mechanistic framework for predicting the time course of systemic and tissue exposure and drug response to various routes of administration and dosing regimens in different populations (age, gender, ethnic groups, healthy volunteers, diseases) (Manolis and Pons, 2009). Open platform initiatives such as PK-Sim and various PBPK platforms such as SimCYP® and GastroPlus® have been developed. They are user-friendly, do not require coding capabilities, and allow easy handling of physiology-based modeling (Rowland et al., 2011).

Despite significant scientific advances over the past 50 years and improved knowledge of enzymes and drug metabolism and disposition, DDI still represents an issue. In addition, many questions and challenges about the interplay between DDI and metabolic enzymes/transporter's genetic variation arise. Therefore, it has become urgent for health to predict vulnerability to DDIs that cause adverse effects. Here we discuss the performance of the PBPK for predicting DDIs and the different sources of information used to build PBPK models to show what needs further investigation. Therefore, we have built a specific knowledge base to document predictions using PBPK, including peer-reviewed publications and models developed in the literature from October 2017 to January 2021. PubMed searches were conducted using "physiologically based pharmacokinetics" and "modeling" in the manuscript's abstract or title. Articles were selected for review if published in English and focused on PBPK modeling applied to human pharmaceutical products. The publication was characterized according to the class of medicines to which it related. In addition, information about the software, type of model, size, and population model was extracted for each article (**Supplementary Material**). The final aim was to build a repository of available PBPK models developed to date to predict DDIs in the different therapeutic areas by integrating intrinsic and extrinsic factors such as genetic polymorphisms of the CYPs/transporters or environmental clues.

DISCUSSION

Related to what has been recently described in the literature (Min and Bae, 2017), a review of recent models suggests that the majority of PBPK models published after October 2017 are designed for the assessment of DDI (68%) followed by dose adaptation for pediatric, and then hepatic and renal failure. Most (73%) of the models were developed using the Simcyp simulator. One of the unique features of this software compared with other PBPK simulation software is that it predicts drug fate in an average population and in "outlier" individuals (Rostami-Hodjegan, 2012). Analysis of the published models also revealed that most of them were built according to a distribution model called full PBPK. This is an entirely mechanistic model where each organ is represented as a compartment instead of the simplified model. In the simplified model, the organs can be grouped into one or two symbolic compartments, called "minimal PBPK" (Kuepfer et al., 2016). An advantage of the full PBPK strategy is that it simulates the exposure of a drug or its metabolites in specific tissues that are not accessible to clinical sampling.

Additionally, depth analysis of the report pointed out the integration of the genetic aspect to the PBPK model in 23% of them. As described above, CYP450 isoenzymes are characterized by significant genetic polymorphism. Since CYP450 isoenzymes functionality is critical to its impact, genetic polymorphism may influence their magnitude (Tod et al., 2013). Although now incorporated in some guidelines, the impact of pharmacogenetic factors on the interaction between a drug and CYP450 isoenzymes (drug-gene interaction [DGI]) does not consider the change in the magnitude of the interaction depending on the genotype in question. This interaction is rarely considered in clinical practice, and systematic evidence of such critical pharmacogenetics effects on DDIs is lacking. Polymorphism also plays a crucial role in the metabolism of drugs with multiple metabolic pathways.

In this optic, Bi et al. (Bi et al., 2018b) assessed the role of previously unrecognized OAT2 transporter-mediated hepatic uptake in the pharmacokinetic of high permeability-low MW acidic and zwitterionic drugs (ECCS 1A) such as tolbutamide and warfarin. To do so, they selected 25 ECCS 1A drugs and tested transport activity using an *in-vitro* transport-transfected cell. The majority of drugs selected showed an active uptake by plated human hepatocytes. The data collected from *in-vitro* experiments were used to power supply the models with additional data related to the transport. The simulation was run considering the uptake transport alone, the metabolism alone, and the interplay between them. The transporter-enzyme interplay approach improved prediction accuracy compared to the other two approaches (average fold error = 1.9 and bias = 0.93). Bi and coauthors underscored the importance of transporters in evaluating and predicting the drug PK and suggest the lack of transporters consideration in the field.

A complementary analysis of the clinical PK-DDI studies by Huth et al. evaluated via a hybrid bottom-up and top-down strategy the effect of DDI inducers or inhibitors of the CYP3A and CYP2C9 enzymes on the systemic exposure of the immunosuppressant Siponimod (Huth et al., 2019). Clinical PK data from single and multiple ascending doses, absolute bioavailability, human ADME, and fluconazole DDI study results were used as bases in the PBPK model building. In addition, the model was verified by fitting the predicted and observed PK profiles. Simulated DDI potential of fluconazole (CYP3A4 and CYP2C9 inhibition effect) and rifampicine (CYP3A4 and CYP2C9 induction effect) on the systemic exposure of Siponimod after oral administration was compared with the respective clinical study. The Final PBPK model was used to assess Siponimod DDI potential as substrate at a steady state in the presence of specific CYP3A4/CYP2C9 inhibitors for six clinically relevant CYP2C9 genotypes. What has been highlighted by these simulations is that when CYP2C9 metabolic activity is decreased (as is the case for CYP2C9 PMs (*3/*3)), CYP3A4 becomes the primary pathway for drug clearance. Thus, the introduction of strong CYP3A4 inhibitors increases DDI risk in CYP2C9*3/*3 subjects compared to other genotypes. This illustrated the impact when both CYP2C9 and CYP3A4 pathways are less functional or inhibited. Moreover, Huth and co-authors with these findings laid the foundation for

DDI drug labeling recommendations, as they established the relevant influence of CYP2C9 polymorphism on the DDI behavior of Siponimod.

Similarly, Gong et al. addressed, with a hybrid bottom-up and top-down full PBPK model, the case of BMS-823778, a potent and selective inhibitor of a microsomal enzyme regulating the tissue concentration of biologically active cortisol (Gong et al., 2018). *In vitro* permeability of BMS-823778 was determined in a Caco-2 cell bi-directional permeability assay and effective permeability was predicted with a Simcyp built-in algorithm based on *in vitro* Caco-2 permeability results. Tissue:plasma partition coefficients (Kp) in various organs including liver, kidney, spleen, adipose, bone, heart, gut, muscle and skin were directly taken from a rat tissue distribution study. Steady state volume of distribution (Vss) was predicted based on the individual input Kp values in the aforementioned tissues with a global Kp scalar of 1, using the Rodgers and Rowland method in Simcyp. All these physicochemical properties and ADME parameters were used to construct the initial model. BMS-823778s major metabolism pathway is CYP2C19, supported by other minor pathways, mainly CYP3A and UGT1A4 (Cheng et al., 2018). Comparative simulations leveraging available pharmacogenetics and PK from clinical studies in healthy subjects, Caucasian, Chinese and Japanese with various CYP2C19 and UGT1A4 functionality were performed to catch the inter-populational PK variability. The verified model was applied to simulate BMS-823778 PK and predict potential DDIs resulting from a CYP3A4 strong inhibitor in subjects with CYP2C19 and UGT1A4 genetic polymorphisms. The *in-vivo* clearance of BMS-823778 and CYP2C19 predicted phenotype were directly correlated. Described clinical pharmacogenetics studies did not demonstrate an impact of UGT1A4 polymorphism on BMS-823778PK. In contrast, the model described the PK profile in subjects with the predicted CYP2C19 PM phenotype and UGT1A4*1/*2 genotypes, who had a 50% increase in exposure BMS-823778 compared to those with normal UGT1A4 activity. With this particular example, the research group has once again demonstrated the place of pharmacogenetics in PBPK models development and the performance of predicting the magnitude of PK and DDI when it is challenging or not feasible in clinical settings.

This is particularly the case for pediatric populations, as illustrated by Zakaria et al. (Zakaria and Badhan, 2018). In African pediatric population groups, the study described an effective PBPK model for predicting the impact of dosage regimen alterations on target seven-day lumefantrine plasma concentrations involving the CYP2B6. A process of five stages was followed for model development, validation, and refinement. The authors started by applying the lumefantrine compound file to healthy, South African, and Ugandan populations and opposing the obtained results to PK data from clinical studies. The second step consisted of modeling lumefantrine-efavirenz interaction and comparison with clinical data of two published studies for validation and refinement. Following this, the model has been applied in the pediatric population and validated against clinical data. Finally, the polymorphic nature of CYP2B6 was also taken into account in the model. Therefore, this study focused on predicting the risk of efavirenz-mediated DDIs on lumefantrine pharmacokinetics in African pediatric population groups considering the polymorphic nature of CYP2B6. After predicting the risk, Zakaria and co-workers proposed adapting of the dosage regimen to avoid the observed phenomenon. Indeed, they demonstrated that an extension of the current artemether-lumefantrine treatment regimen from 3-days to 7-days would counteract the reduction in efavirenz metabolism common with the *6/*6 genotype and hence enhance the attainment of target day-7 lumefantrine concertation in both *1/*1 and *6/*6genotype groups, thereby reducing the risk of recrudescence.

As with CYP2B6, CYP2D6, the most polymorphic metabolic enzyme, is of significant interest when predicting the impact of genetic on the vulnerability and magnitude of DDI. Storelli et al. highlighted the pharmacogenetics testing significance by comparing the magnitude of predicted and observed CYP2D6 mediated DDIs in different CYP2D6 genotypes using PBPK modeling (Storelli et al., 2019). This work consisted of the first study evaluating the usefulness of PBPK in predicting gene-drug-drug interactions with specific CYP2D6 inhibitors and substrates. The group's predictions and observations converged on the following rule of thumb: the higher the CYP2D6 activity, the greater the magnitude of the interaction. Authors faced underpredictions of the DDI when using the bottom-up approach on Simcyp with the experimental KI values in the case of duloxetine and paroxetine models. To solve this, they opted to optimize the models and used, for DDI modeling, KI values obtained from *in vivo* DDI studies, rather than *in-vitro* ones were analyzed and adapted via a sensitivity analysis. The new KI value was then verified with a set of independent DDI data (in-vivo optimized KI values). Through this work, the authors illustrate that *in-vitro* models, although beneficial for collecting information, may not describe specific mechanisms and therefore generate a margin of error in the prediction. Therefore, the comparison of simulated data with clinical data is crucial in the model's validation and refinement. This study illustrates PBPK modeling performance in predicting of CYP2D6 genetic polymorphism effect on DDIs using verified initial models and rich PK from dedicated genetic trials to predict the effect of genotype on drug and substrate exposures.

Similarly, Chen et al. evaluated the systemic exposure of the tyrosine kinase inhibitor gefitinib in CYP2D6 UM and NM (Chen et al., 2018). Itraconazole DDI studies assessed the effect of the CYP2D6 genotype on gefitinib PK. Predictions showed that the gefitinib area under the curve (AUC) in CYP2D6 UM was reduced by 39% compared to NM. However, these changes were considered of limited impact because the reduced exposure was still above gefitinib *in vitro* IC_{90} for the patients of interest. Thus, the authors underline some challenges encountered with drugs identified as highly variable, like gefitinib, when it comes to PK and intersubject drug exposure. The present study demonstrated the unique potential of PBPK in predicting drug-drug interactions in pharmacogenomic subpopulations that could be hard to study due to low allele frequencies in a patient population. Authors suggest PBPK modeling as an alternative to conducting an actual clinical trial in these cases.

In a context of sinogliatin late-stage development and PBPK model development for study design and dose selection, Song

et al. (Song et al., 2018) propose an effective strategy based on mechanistic insight into human drug metabolism and pharmacokinetic properties from preclinical *in vitro* and *in vivo* data using algometric scaling (AS), in vivo-to *in vitro* extrapolation (IVIVE) and steady-state concentration-mean residence time (Css-MRT). As described in the paper, The AS method provided the group for the model development with human clearance and steady-state volume distribution after intravenous administration. The IVIVE strategy allowed the verification of the *in-vitro* metabolic data and confirmed the predominant CYP enzyme involved in in vivo metabolism and corresponding fraction. Concerning the Css-MRT approach, it provided the knowledge on the interspecies difference that enabled selection of the optimal species to construct the preclinical PBPK model in some first in human studies. Instead of basing the model construction of literature research, authors implemented the available models with the collected parameters obtained from human major pharmacokinetic parameters analysis. The developed model successfully predicted human PK and evaluated the effects of extrinsic (e.g., DDI) and intrinsic (e.g., hepatic cirrhosis, CYP genetic) factors on drug exposure supporting the development of the drug candidate.

PBPK modeling is an assessed tool applicable to complex interactions investigation implying multiple drugs and genetic polymorphism, yet some authors propose its application for Physico-chemical DDI detection. The research article by Türk et al. (Türk et al., 2019) described, via whole-body PBPK models, CYP2C8 and organic-anion–transporting polypeptide (OATP) 1B1-based DDGIs involving the perpetrator drug gemfibrozil and the two victim drugs repaglinide and pioglitazone. PK-Sim and Mobi modeling software were used for the model development, and model construction relied on extensive literature research on the physicochemical and ADME processes of the drugs of interest. When available, system-dependent parameters were taken as provided by the simulation software; otherwise, they were collected from the literature. A total of 103 Clinical studies were digitalized from the literature and divided into an internal dataset for model building and parameter optimization and an external dataset for model evaluation. In the process of complex DDI modeling, the group demonstrated that a simultaneous administration of gemfibrozil might decrease the poor solubility of itraconazole, causing a decrease in absorption and thereby a decrease of the plasma concentrations of itraconazole and its metabolite. The same phenomenon was observed with pioglitazone when co-administered with gemfibrozil plus itraconazole. Through these two examples, the group illustrates that PBPK modeling is a valuable tool to develop and test hypotheses for unexpected clinical findings and raise awareness of the possibility of solubility interactions often put aside.

As a narrow therapeutic index drug, warfarin prescription demands a personalized medicine approach to tackle the interindividual variability and balance the therapeutic benefits and bleeding risk. Individualization is made based on genetic variants in CYP2C9 and vitamin K epoxide reductase (VKORC1). As stated by Bi et al. (Bi et al., 2018a), another specificity to this drug is that it is a racemic mixture of R- and S-enantiomers where CYP2C19 and other CYP enzymes metabolize R-warfarin, and S-warfarin is metabolized at 20% by CYP2C9. In this context and following this clinical observation, authors developed a bottom-up full PBPK model to evaluate the potential role of transporter-mediated hepatic uptake in the disposition of both warfarin enantiomers. The authors performed an *in-vitro* -in vivo extrapolation implementing the models with *in-vitro* obtained transporter kinetic data in primary human hepatocytes. Comparatively, to when OAT2-CYPs interplay was considered, when only CYP-mediated metabolism was assumed, authors faced an underprediction of oral clearance of both enantiomers. Despite the lack of clinical data needed to validate the model, the model developed with the OAT2-CYPs interplay recovered clinical pharmacokinetics, drug-drug-interactions, and CYP2C9 pharmacogenetics. Overall, Bi et al. have succeeded in demonstrating the utility of *in-vitro* data-informed- mechanistic modeling and simulations to enable the deconvolution of transporter-enzyme interplay and its role in governing drug pharmacokinetics, especially for untestable scenarios.

Clinical Perspectives

As described above, a considerable part of xenobiotic biotransformation depends on the metabolizing enzymes and transporters. This has an impact not only on drug design but also on drug response. In this context, the regulatory authorities such as the FDA and the EMA have required systematic risk-based methodologies to evaluate drug parameters during the drug development process (Jamei, 2016). PBPK is used for mechanistic studies, aiding clinical development decisions, or drug discovery in the pharmaceutical industry. At the research and drug development, PBPK has already proven itself and is now an integral tool in drug discovery and development. It is a good tool for optimizing clinical trial designs, dose selection, and PK extrapolation from the general population to more specific ones. PBPK modeling can also be applied as an alternative to DDI trials in some special populations where actual DDI trials are hard to conduct due to logistical and ethical reasons (Huang et al., 2013). New drug application approval packages include preclinical and clinical investigation data. The potential effect of a new molecular entity on the metabolism or transport of other drugs, as well as the risk of being affected by other drugs, including recommended clinical index substrates and specific inhibitors or inducers of drug-metabolizing enzymes, are tested prior to the marketing authorization application. In addition to being used as the basis for new drug labels and summaries, the findings of those investigations are made available in the scientific handbooks and databases (Reis-Pardal et al., 2017). This provides healthcare prescribers and providers with the know-how to use the medicine safely and effectively. These data are also a primary source of information for PBPK simulation for treatment adaptation and dose prediction (Kuepfer et al., 2016). PBPK model's part on the drug parameters is built and optimized to obtain the right absorption, distribution, metabolism, and elimination profiles. When physicochemical or ADME parameters are not available, they can either be predicted by

the software according to implemented mathematical formulas or extrapolated from *in-vitro* model measurements (Emoto, Murayama et al., 2009). Different *in-vitro* systems are available to collect data and allow model enrichment to obtain the best predictive results. These systems include microsomes, recombinant enzymes, hepatocytes, and liver cells. Although different from each other, they all have the advantage of reducing the risk associated with potential adverse effects in humans, limiting costs, and having the potential for widespread use (Stillhart et al., 2019). The study population is critical in the prediction process, along with the parameters related to the molecule studied and the galenic formulation of interest. Therefore, different virtual populations have been developed and are available within the PK modeling software. A virtual population is characterized by its demographic parameters such as mean age, the proportion of females and males, but also by physiological and pathophysiological parameters (Hartmanshenn et al., 2016). Organ size, blood flow, and protein abundance parameters, for example, are data that are modified to represent the target population and best predict pharmacokinetics. More recently and with the emergence of knowledge in pharmacogenetics, many simulations have been performed during drug development to predict the vulnerability to DDI in groups of patients with different genotypes (Pastino et al., 2000; Wu et al., 2014; Djebli et al., 2015; Toshimoto et al., 2017). Faced with this advance, many groups are trying to apply the same principle to personalized medicine and are thinking of implementing a pharmacokinetic prediction model based on patient X-specific data within prescription support software. This means individualizing the drugs PK prediction PK by creating a computer model replicating the patients attributes able to affect drug exposure: virtual twin appraoch (Polasek et al., 2018).

Based on the drug and population parameters, PBPK aims to optimize individual drug dosing regimens and ensure therapeutics safety and efficacy. Other methods with the same goal are currently used in the clinic, including therapeutic drug monitoring (TDM). TDM is based on laboratory measurements of a chemical parameter in the patient's biological fluids at crucial times to maintain drug concentrations within a targeted therapeutic window (Ghiculescu, 2008). This clinically implemented drug individualization approach, in contrast to PBPK, is a short-term solution to facilitate dosing and account for DDI. Although it compensates for inter and intra-individual variability in drug response, the measures implemented are only temporary and must be reevaluated for slight changes in intrinsic or extrinsic factors (Ghiculescu, 2008). However, when TDM is available, the generated data can be introduced into a PBPK model to make the prediction more robust. Thus, a multidisciplinary approach combining knowledge of pharmaceutics, pharmacokinetics, and pharmacodynamics is essential to predict the most appropriate drug response in specific individuals.

In a study by Glassman et al., clinical pharmacist's detection of DDI on drug pairs was 44% (Glassman and Balthasar, 2019) and went up to 66% in another study by Weidemann et al. (Weideman et al., 1999). Despite the pharmacological knowledge of pharmacists and physicians, detection tools seem necessary to reduce DDIs, especially for new drugs on the market or complex treatments. Clinical decision support systems are the product of computerized physician order entry (CPOE) implementation combined with the transition from manual order entry to electronic health records (EHR). They have considerably improved the systematic screening and detection of DDIs and decreased prescribing problems and DDIs (Nuckols et al., 2014). Computerized systems implemented with decision-support provide automatic alerts to the prescriber based on analysis of clinical data in CPOE (Riedmann et al., 2011). Alerts can be related to clinical issues such as duplicate therapy, drug allergies, or potential DDIs. Although very advantageous, they have several limitations. First, it provides support only at the step of prescribing, taking into account relevant biochemical parameters in a minimal number of cases. In addition, it has been reported that this type of system generates "alert fatigues," causing them not to consider the recommendations issued by the program at all (Kuperman et al., 2007). For drugs for which polymorphic enzymes/transporters are the main clearance factors, PBPK simulations can be used to give a genotype-specific dose and dose adjustment recommendation. This would be an essential step in precision medicine without performing DDI studies for all the genotypes involved. Accordingly, integrating prediction software with prescribing support software may be of great benefit and a big step forward in personalized medicine (Venkatakrishnan and Rostami-Hodjegan, 2019).

CONCLUSION

Current treatment regimens rely on the anticipated relationship between drug doses, plasma levels, and desired effect. Current perspectives in individualized therapy and personalized medicine aim to quantify anticipated changes in patients, evolving from prediction in general populations to individual patient responses and modeling. This review provides an overview of PBPK model development and its integration into the application for PK predictions and decision-making tools. Forty-six PBPK modeling papers on the prediction of DDI potentials were identified, and the advantages of PBPK modeling, including accounting for time-varying changes and inter-individual variability, were highlighted. In investigating DDI potentials using PBPK modeling, a limited number of drug-metabolizing enzyme-mediated DDIs has been considered by the published studies. Moreover, the simulations were performed mainly on healthy adult populations. Therefore, to broaden the scope of PBPK modeling in predicting DDIs, more information about the physiological properties of the organism and the incorporation of environmental and pathophysiological conditions into disease states is needed (Lenoir et al., 2020; Magliocco et al., 2020). In addition, it must be taken into account that the patient genetic makeup, concerning their drug-metabolizing enzymes and transporters, determines the relationship between drug doses and plasma concentration and thus therapeutic effect. However, many data are required to implement predictive systems in clinics, and genetic knowledge of CYP450 alone is insufficient to predict DDI. Despite its remaining challenges,

PBPK for DDI prediction represents an excellent asset for regulatory authorities and drug development and a promising approach in clinical practice in the frame of model-informed precision dosing and individualized therapy.

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YD supervised the project. KA wrote the manuscript with support of CFS, YD, YG, and JD. All authors provided critical feedback and helped shape the manuscript.

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SYSTEMATIC REVIEW
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Influence of Inflammation on Cytochromes P450 Activity in Adults: A Systematic Review of the Literature

Camille Lenoir[1,2]*, *Victoria Rollason*[1,3], *Jules A. Desmeules*[1,2,3] and *Caroline F. Samer*[1,3]

[1]Division of Clinical Pharmacology and Toxicology, Department of Anesthesiology, Pharmacology, Intensive Care, and Emergency Medicine, Geneva University Hospitals, Geneva, Switzerland, [2]Institute of Pharmaceutical Sciences of Western Switzerland (ISPSO), School of Pharmaceutical Sciences, University of Geneva, Geneva, Switzerland, [3]Faculty of Medicine, University of Geneva, Geneva, Switzerland

Background: Available in-vitro and animal studies indicate that inflammation impacts cytochromes P450 (CYP) activity *via* multiple and complex transcriptional and post-transcriptional mechanisms, depending on the specific CYP isoforms and the nature of inflammation mediators. It is essential to review the current published data on the impact of inflammation on CYP activities in adults to support drug individualization based on comorbidities and diseases in clinical practice.

Methods: This systematic review was conducted in PubMed through 7th January 2021 looking for articles that investigated the consequences of inflammation on CYP activities in adults. Information on the source of inflammation, victim drugs (and CYPs involved), effect of disease-drug interaction, number of subjects, and study design were extracted.

Results: The search strategy identified 218 studies and case reports that met our inclusion criteria. These articles were divided into fourteen different sources of inflammation (such as infection, autoimmune diseases, cancer, therapies with immunomodulator. . .). The impact of inflammation on CYP activities appeared to be isoform-specific and dependent on the nature and severity of the underlying disease causing the inflammation. Some of these drug-disease interactions had a significant influence on drug pharmacokinetic parameters and on clinical management. For example, clozapine levels doubled with signs of toxicity during infections and the concentration ratio between clopidogrel's active metabolite and clopidogrel is 48-fold lower in critically ill patients. Infection and CYP3A were the most cited perpetrator of inflammation and the most studied CYP, respectively. Moreover, some data suggest that resolution of inflammation results in a return to baseline CYP activities.

Conclusion: Convincing evidence shows that inflammation is a major factor to be taken into account in drug development and in clinical practice to avoid any efficacy or safety issues because inflammation modulates CYP activities and thus drug pharmacokinetics. The impact is different depending on the CYP isoform and the inflammatory disease considered. Moreover, resolution of inflammation appears to result in a normalization of CYP activity. However, some results are still equivocal and further investigations are thus needed.

Keywords: inflammation, cytochrome P450, pharmacokinetic, disease-drug interaction, cytokines

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***Correspondence:**
Camille Lenoir
Camille.Lenoir@hcuge.ch

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INTRODUCTION

Cytochromes P450 (CYP) are the major drug-metabolizing enzymes (DME) responsible for 75% of drug metabolism, making them decisive in the efficacy and safety of drugs (Wienkers and Heath, 2005). The interindividual variability in CYP activity is influenced by genetic factors, environmental factors and comorbidities (Lynch and Price, 2007). CYP genetic polymorphisms are well described, resulting in major functional differences (Zhou et al., 2017). CYP are also impacted by drug-drug interactions (DDIs) and several widely used drugs were removed from the market because of serious adverse drug reactions (ADRs) due to DDIs via the CYPs (Wilkinson, 2005). Therefore, the Food and Drug Administration (FDA) requires *in-vitro* evaluation of potential DDIs during the course of drug development (Kato, 2020; Food and Drug Administration).

A less well described but increasingly studied source of modulation of CYP activity and recently reviewed is that of endogenous inflammatory markers (de Jong et al., 2020; Stanke-Labesque et al., 2020). Inflammation is a response to endogenous or exogenous aggression that can be acute or chronic. It is prominent in many diseases, such as infection, trauma, surgery, arthritis, asthma, atherosclerosis, autoimmune disease, various immunologically mediated and crystal-induced inflammatory conditions, diabetes and cancer, to name a few (Gabay and Kushner, 1999; Germolec et al., 2018; Stavropoulou et al., 2018). This universal protective response involves innate and adaptative immunity and is present in virtually all tissues. Acute changes can be associated with variation in the concentrations of several plasma proteins, the acute-phase proteins (APP), and numerous behavioral, physiological, biochemical and nutritional changes (Gabay and Kushner, 1999). Cytokines are the main stimulators of APP production, and interleukin-6 (IL-6) is the key stimulator of APP while other cytokines (IL-1β, Tumor Necrosis Factor α, interferon-γ, transforming growth factor β and possible IL-8) influence APP subgroups (Gabay and Kushner, 1999). Thus, inflammation is a complex and well-orchestrated process involving many cell types and molecules that function as a cascade network, some of which initiate, amplify or sustain the process and others attenuate or resolve it (Gabay and Kushner, 1999; Stanke-Labesque et al., 2020).

Inflammation can impact drug PK through multiple mechanisms which typically occur in the liver, kidney, or intestinal epithelial cells (Stavropoulou et al., 2018; de Jong et al., 2020; Stanke-Labesque et al., 2020). The metabolic activities of CYPs are suppressed by inflammation in most cases, but some CYPs may be induced or remain unaffected (Morgan, 2001; de Jong et al., 2020; Stanke-Labesque et al., 2020). The positive and negative control of gene transcription is generally achieved by the interaction of regulatory proteins with specific DNA sequences on the regulated genes (Morgan, 1997). The impact of inflammation on the metabolic activity of CYPs has been studied in various *in-vitro* and animal models of inflammation, including trauma, infection and administration of endotoxin or cytokines (de Jong et al., 2020; Stanke-Labesque et al., 2020). Information available in the literature suggests that this impact on PK is triggered by cytokines and their intracellular signaling, directly or *via* interaction with the nuclear receptor pathway, on drug transporters and metabolizing enzymes (Liptrott and Owen, 2011; de Jong et al., 2020; Stanke-Labesque et al., 2020). Importantly, no single common pathway has been identified to explain the changes in the entire CYP family and involves different mediators but also different transcription factors (Renton, 2005; de Jong et al., 2020; Stanke-Labesque et al., 2020). Different effects of cytokines are observed in different cell types, which could be explained by a difference in the way intracellular signals from cytokine receptors are generated (Liptrott and Owen, 2011). Different cytokines exhibit a widely different spectrum of activity trough individual CYP isoforms and many different transcription factors (Morgan, 1997; Ruminy et al., 2001; Renton, 2005; Liptrott and Owen, 2011). Their activation by cytokines have been implicated in the downregulation and transcriptional regulation of different CYP isoforms (Morgan, 1997; Ruminy et al., 2001; Renton, 2005; Liptrott and Owen, 2011). Regulation of CYP during inflammation can occur trough pre- and post-transcriptional mechanisms that are cytokine and CYP specific (de Jong et al., 2020; Stanke-Labesque et al., 2020). Pre-transcriptional mechanisms currently described in the literature include transcriptional downregulation of transcription factors, interference with dimerization/translocation of (nuclear) transcription factors, altered liver-enriched C/EBP signaling, and direct regulation by NF-κB (de Jong et al., 2020). Overall, three main mechanisms have been described to explain the downregulation of inflammation in drug metabolizing enzyme and transporters expression and activity, namely inhibition of drug metabolizing enzyme transcription, epigenetic modifications in genes as a result of DNA methylation, modification of histone patterns, release of microRNA and NO-dependent proteasome degradation, which is a post-transcriptional mechanism (Stanke-Labesque et al., 2020).

Therefore, the aim of this systemic review is to evaluate the impact of inflammation on CYP activity in the adult population.

METHODS

The method used to manage the literature search was based on the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) statement (Moher et al., 2009). The detailed PICOS framework (i.e., participants, interventions, comparisons, outcomes, study design) was used as follows: Participants: adults with source of inflammation, -Intervention: victim drugs and CYPs concerned, -Comparison: healthy adults or before the onset of inflammation or receiving treatment for inflammation Outcomes: potential effect of interaction between inflammation and CYP activity, -Study design: clinical trials and case reports/series.

Database and Search Strategy

The literature search was performed in PubMed via MEDLINE, the database of biomedical publications, for studies and case reports/series until January 7, 2021. To expand it, we also

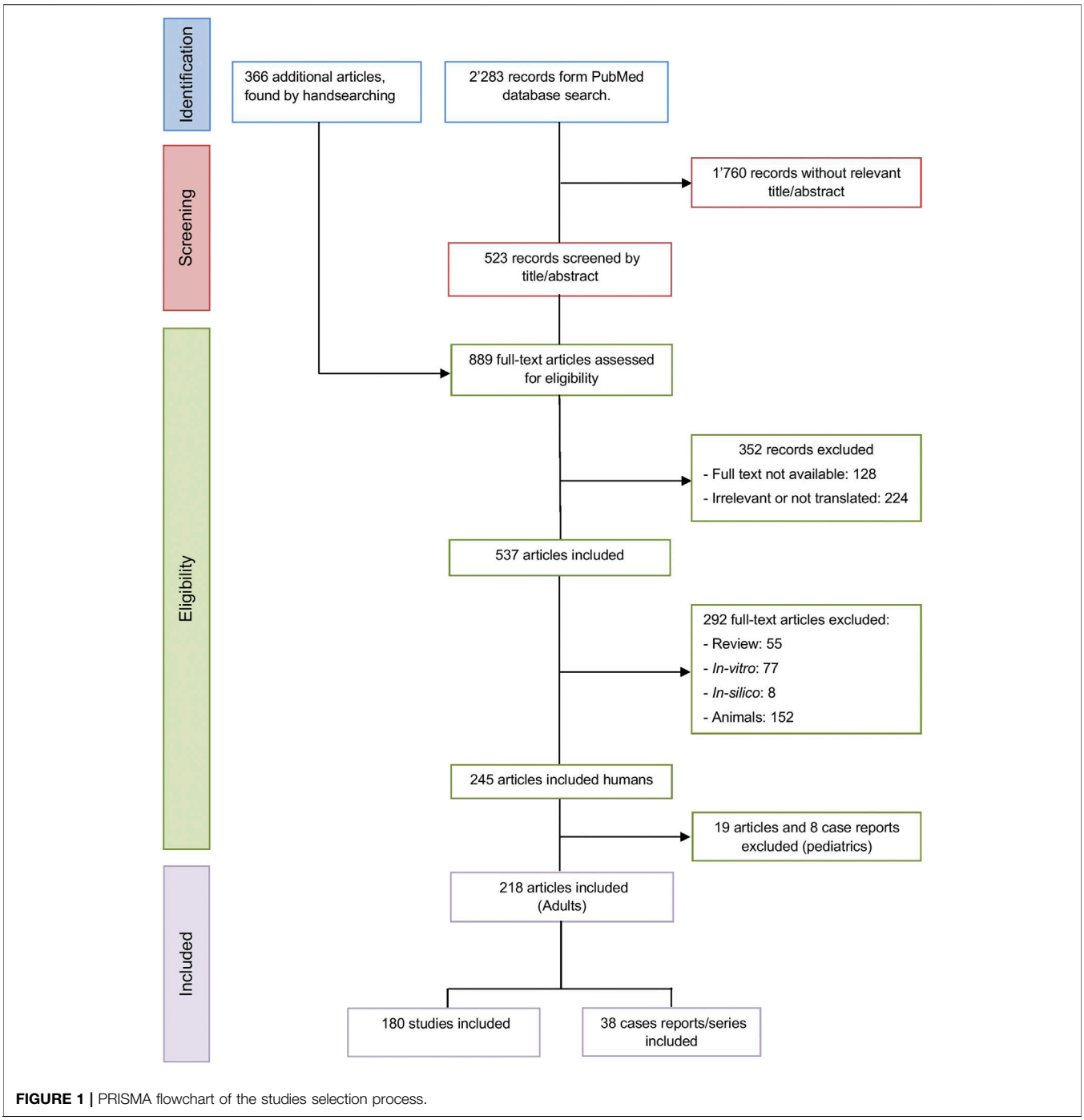

FIGURE 1 | PRISMA flowchart of the studies selection process.

performed a manual search of references for potentially relevant articles. The keywords used were "inflammation", "cytochrome P450", "cytochromes P450" and "CYP450."

Study Selection

We applied the eligibility criteria described below in order to filter relevant publications from the total of results provided by the literature search.

The types of studies included in our literature search were randomized controlled trials, non-randomized studies, and observational studies, including case reports and series, published as full-text articles and congress abstracts in English. The year of publication selected was from database inception until January 7, 2021. Study participants had to be older than 18 years old, including healthy subjects and patients with an inflammatory

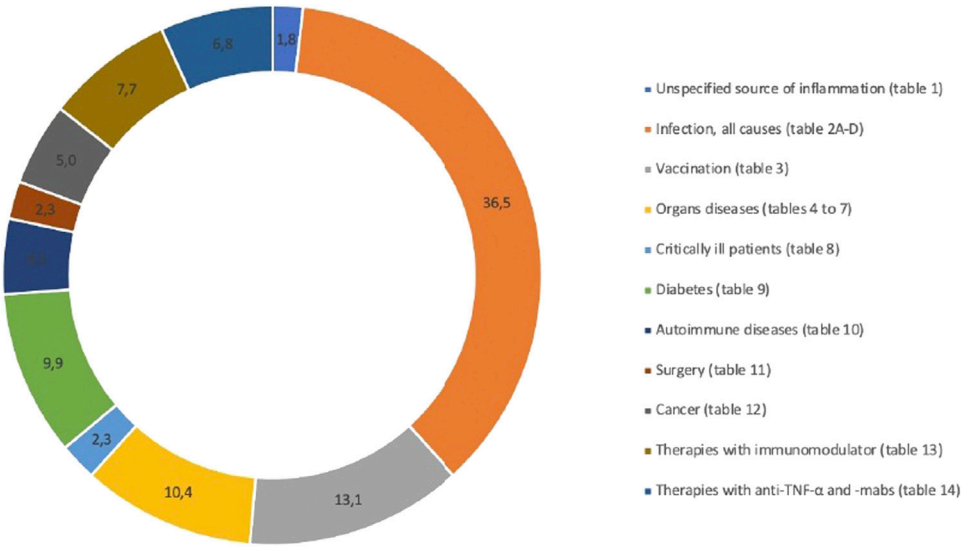

FIGURE 2 | Distribution (%) of included references according to the different sources of inflammation.

condition, caused by disease, treatment or a medical or surgical procedure. The outcomes of interest were the effect of potential inflammation (suggested or provided) on metabolic ratios (MR) of CYP isoforms, the PK/PD and the safety profile of CYP substrates.

Successive steps in article selection included reading the title, abstract and full text according to the predefined eligibility criteria to screen for potentially relevant records. The selected articles were classified into literature reviews and *in-vitro*, animal, *in-silico* and human studies. Then, only studies involving adults (defined as over 18 years old) were kept, classified into studies or case reports/series. The same procedure was applied to assess the inclusion of additional articles identified by the manual search. The study selection process was summarized in a flowchart created according to the PRISMA statement requirements (**Figure 1**) (Moher et al., 2009).

Data Extraction and Management

Articles selected from the search results were collected and exported to the reference management software Zotero (version 5.0.85, © 2006–2018 Contributors) and merged to remove duplicates. Data from the included articles were extracted and synthetized. The authors extracted the following data according to the PICOS framework discussed above. These included study design, sample size, source of inflammation and comparators, victim drugs and CYP involved, and outcomes of interests (potential effect of interaction). When a CYP substrate was used in the article to determine whether or not inflammation or concomitant drugs altered its PK/PD profile, a verification of its metabolic pathway was performed. The verification process was performed using the Summary of Product Characteristics (SmPCs), the Lexi-Interact drug interaction checker and the Geneva table of CYP substrates, inhibitors, and inducers (Uptodate,; Samer et al., 2013).

RESULTS

Identification and Selection of the Studies

The primary search, performed in PubMed, yielded a total of 2′283 articles that were screened according to their title and abstract. Of the remaining 523 articles, an additional 366 articles were identified by cross-referencing and handsearching of the reference list of the relevant articles (n = 889). Of these, 352 records were removed because the full text was not available (n = 128) or because they were considered irrelevant or not translated into English (n = 224). The remaining 537 articles were classified into review articles (n = 55), *in-vitro* (n = 77) or *in-silico* (n = 8) studies, and animal (n = 152) or human (n = 245) studies. The articles and case reports concerning the pediatric population (n = 27) are the subject of another systematic review and were excluded from this work (Lenoir et al., 2021). Finally, 218 articles conducted in adults were included and classified into studies (n = 180) and case reports/series (n = 38) for analysis (**Figure 1**).

Results of the Studies

The 218 eligible publications are summarized in **Table 1** through 14. The drug-disease interactions found in the selected articles were divided into fourteen different sources of inflammation: unspecified source of inflammation (**Table 1**), infection (**Table 2A**), infection-example hepatitis (**Table 2B**), infection-example HIV (**Table 3C**), infection-example SARS-CoV-2 (**Table 2D**), vaccination (**Table 3**), kidney disease (**Table 4**), liver disease (**Table 5**), lung disease (**Table 6**), heart disease (**Table 7**), critically ill patients (**Table 8**), diabetes (**Table 9**), autoimmune diseases (**Table 10**), surgery (**Table 11**), cancer (**Table 12**), therapies with immunomodulator (**Table 13**) and therapies with anti-TNF-α and -mabs (**Table 14**). The most cited inflammation perpetrator was infection and the most studied CYP was CYP3A. CYP3A subfamilies refers to CYP3A4 and

TABLE 1 | Impact of unspecified source inflammation on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
IL-10 injection	tolbutamide (CYP2C9), caffeine (CYP1A2), dextromethorphan (CYP2D6) and midazolam (CYP3A)	12	- significantly but moderately decreased CYP3A4 activity (12 ± 17%, $p < 0.02$) - significantly increased CYP2C9 activity (38 ± 25%, $p < 0.005$), - no significant changes in either CYP1A2 or 2D6 activity	Wienkers and Heath (2005) Double-blind crossover study
Elevated CRP levels (>1.5 mg/dl)	perampanel (CYP3A4)	111 = Total 23 = CRP>1.5 mg/dl 13 = enzyme-inducing AEDs 10 = no enzyme-inducing AEDs	- perampanel C/D increased by 53.5 and 100.8% respectively when CRP >1.5 mg/dl - correlation between serum CRP level and C/D of perampanel ($r = 0.44$, $p < 0.001$)	Lynch and Price (2007) Cohort study
Erythrocyte sedimentation rate (ESR) > 20 mm vs. control	Oxprenolol (CYP2C9, 2D6, 3A4 and 1A2 substrate)	18	- mean oxprenolol AUC 2-fold greater in inflammation group	Zhou et al. (2017) Cohort study
CRP serum levels	tacrolimus (CYP3A4)	31-year-old man	-tacrolimus C/D increased during two inflammation episodes by 54% (cholestasis) and 141% (infection following surgery), and strongly correlated with CRP (r2 = 0.78, $p = 0.079$)	Wilkinson (2005) case report

CYP3A5, because the probe drugs used to assess the activity of CYP3A4 are metabolized by these two isoenzymes and no distinction can be made between them. Distribution in percent of all the references in the different categories are illustrated in **Figure 2**.

Infection

Several studies have assessed the association between infection, represented by elevated levels of CRP, and PK variations of voriconazole. This is of particular interest and voriconazole therapeutic drug monitoring should thus be used to optimize clinical success and safety in these settings (Luong et al., 2016). Increased levels of CRP were correlated with increased voriconazole concentrations or decreased metabolic ratio of voriconazole/N-oxide and this could be explained by CYP2C19 and/or CYP3A downregulation, as voriconazole is mainly metabolized by these two CYPs (van Wanrooy et al., 2014; Encalada Ventura et al., 2015; Dote et al., 2016; Niioka et al., 2017; Vreugdenhil et al., 2018; Schulz et al., 2019). A positive correlation between inflammatory markers and voriconazole concentration was seen in adults, as well as with the severity of infection (van Wanrooy et al., 2014; Dote et al., 2016; Veringa et al., 2017; Gautier-Veyret et al., 2019). Drug metabolism appears to be influenced by the degree of inflammation and standardization of the classification of inflammatory markers elevation seems necessary (van Wanrooy et al., 2014; Niioka et al., 2017; Veringa et al., 2017; Gautier-Veyret et al., 2019). Indeed, voriconazole through concentration increased by 0.015 mg/L every 1 mg/L increase in CRP, and a recent meta-analysis showed that an increase in voriconazole through concentration of 6, 35 and 82% was associated with an increase in the CRP level of 10, 50 and 100 mg/L, respectively (van Wanrooy et al., 2014; Bolcato et al., 2021). As a final evidence to support of a correlation between inflammation and CYP downregulation, inflammation, and its resolution, decreased, and increased voriconazole clearance respectively, suggesting that the improvement of the inflammation allows a return to the baseline (Dote et al., 2016). However, no studies have investigated the duration of the resolution of inflammation-induced metabolic phenoconversion (Stanke-Labesque et al., 2020). This is an important limitation to allow individualization of treatment without therapeutic drug monitoring (TDM), as under-exposure to drug remains a risk (Stanke-Labesque et al., 2020).

CYP downregulation was also demonstrated as a consequence of sufficient inflammation and significant temperature elevation (Elin et al., 1975). Therefore, caution should be exercised in case of infection when administering CYP substrates, as this may result in toxicity and ADRs (Vozeh et al., 1978; Blumenkopf and Lockhart, 1983; Levine and Jones, 1983 1; Raaska et al., 2002; Haack et al., 2003; de Leon and Diaz, 2003; Jecel et al., 2005; Darling and Huthwaite, 2011; Espnes et al., 2012; Kwak et al., 2014; Leung et al., 2014; Takahashi et al., 2015; Clark et al., 2018; Khan and Khan, 2019).

Early works assessed the effect of an infection induced intentionally by lipopolysaccharides (LPS) injection on antipyrine pharmacokinetics, and several studies have assessed the impact of infection on psychotropic agents (clozapine, risperidone). The increase of clozapine levels, a CYP1A2 substrate, due to inflammation has been well studied and demonstrated (Raaska et al., 2002; Haack et al., 2003; de Leon and Diaz, 2003; Jecel et al., 2005; Pfuhlmann et al., 2009; Darling and Huthwaite, 2011; Espnes et al., 2012; Abou Farha et al., 2012; Leung et al., 2014; Kwak et al., 2014; Takahashi et al., 2015; ten

TABLE 2A | Impact of infection on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Lipopolysaccharides (LPS)-induced inflammation	theophylline (CYP1A2), hexobarbital (CYP2C19) and antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	12	- significant repression of CYPs activity (takes several hours to develop)	Kato (2020), Crossover study
Two injections of Gram-negative bacterial endotoxin	theophylline (CYP1A2), hexobarbital (CYP2C19) and antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	9	- significant decrease of clearances of all probes compared with the saline control studies, - endotoxins injections associated with decreased hepatic drug metabolism, mainly CYP1A2 and 2C19	Food and Drug Administration, Cross-over clinical trial
Administration of a single oral dose of 10 mg/kg of etiocholanolone	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	14 = significant fever (fever index >50)	- half-life was significantly prolonged (29.3%, $p < 0.005$) in patients with significant fever	de Jong et al. (2020)
		19 = failed to develop significant fever (fever index <50)	- no significant change of half-life ($p > 0.8$) in patients without significant fever	Cross-over clinical trial
			- no correlation between the magnitude of fever and the extent to which half-life was prolonged	
Acute pneumonia	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	14	- 1.5 fold increased clearance 14 and 28 days after the acute illness - enhancement of clearance in 28 days represented a 36% improvement	Stanke-Labesque et al. (2020) Cohort study
Liver fluke infection (uninfected, infected only and infected with fibrosis)	coumarine (CYP2A6)	- Total = 91	- 26% lower urine levels of 7-hydroxycoumarine (7-HC) after praziquantel ($p < 0.001$) compared to initial assessment	Stavropoulou et al. (2018)
		- 73 completed the two assessments	- infected individuals excreted slightly higher levels of 7-HC in the 0–2 h period	Cohort study
Herpes zoster	warfarin (CYP2C9)	66-year-old woman	- acute spinal subdural hematoma and subarachnoid haemorrhage during the course of a thoracic level infection - 3-fold increased PT times requiring vitamin K administration	Germolec et al. (2018) Case report
Visceral leishmaniasis	midazolam (CYP3A), omeprazole (CYP2C19), losartan (CYP2C9)	24	- significantly increased midazolam CL/F ($p = 0.018$) 2–3 days and 3–6 months after curative chemotherapy - significantly increased omeprazole CL/F ($p = 0.008$) 2–3 days and 3–6 months after curative chemotherapy - CYP2C9 activity not significantly different between	Gabay and Kushner (1999) Cohort study
Influenza A	theophylline (CYP1A2)	50-year-old woman	- toxicity symptoms after infection - increased theophylline levels (1.5x above normal values)	Morgan (2001) Case report
Acute illness	theophylline (CYP1A2)	3	- 2-fold or 3-fold variation in clearance during acute illness - clearance decreased during worsening of airway obstruction in one patient - 2 patients had increased clearance during the improvement of their condition (pneumonia and congestive heart failure)	Morgan (1997) Case series
Elevated CRP levels (>5 mg/L) vs control	citalopram (major CYP2C19, minor CYP3A4) and venlafaxine (major CYP2D6, minor CYP3A4 and 2C19)	15 citalopram	- no statistical differences in citalopram and venlafaxine concentrations or in MR of both drugs in samples with elevated CRP levels	Liptrott and Owen (2011)
		39 venlafaxine		Cohort study
Elevated serum levels of CRP	risperidone (bioactivated by CYP3A4 and CYP2D6)	2 females (56 and 38 years old)	- close temporal association between serum levels of risperidone active moiety (risperidone + 9-hydroxyrisperidone) and CRP - > 3x increase of C/D during elevated CRP serum concentration	Renton (2005) Case report

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TABLE 2A | (*Continued*) Impact of infection on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
			- parallel fluctuation of drug levels and CRP which necessitated dose adjustments, but the MR was unchanged, suggesting that the CYP2D6-catalyzed formation of 9-hydroxyrisperidone was not affected	
Pneumonia	risperidone (bioactivated by CYP3A4 and CYP2D6)	56-year-old man	5-fold higher risperidone dose requirement during pneumonia	Ruminy et al. (2001)
				Case report
Elevated serum levels of CRP (>5 mg/L)	clozapine (CYP1A2), quetiapine (CYP3A4 and CYP2D6) and risperidone (CYP3A4 and CYP2D6)	33 clozapine, 32 quetiapine 40 risperidone	- C/D of clozapine was significantly higher ($p < 0.01$) and CYP1A2 MR (NCLZ/CLZ) significantly lower ($p < 0.05$)	Moher et al. (2009)
			- positive and significant correlation between clozapine and CRP levels ($r = 0.313$, $p < 0.01$) - no difference in C/D or in MR of quetiapine - C/D of risperidone was significantly higher ($p < 0.01$) and MR decreased (NS)	Cohort study
Elevated serum levels of CRP	clozapine (CYP1A2)	27 high drug level	mean CRP value significantly higher ($p = 0.005$) in patients with elevated clozapine level	Uptodate
		36 normal drug level		Case-control study
Elevated serum level of CRP of 130 mg/L	clozapine (CYP1A2)	44-year-old man	- admission to hospital because of symptoms of clozapine toxicity - elevated clozapine levels - condition improved when treatment was discharged	Samer et al. (2013) Case report
Elevated serum level of CRP of 256 mg/L	clozapine (CYP1A2)	50-year-old man	- 5-fold increased plasma levels 4 days after admission	Lenoir et al. (2021)
				Case report
Sepsis	clozapine (CYP1A2)	61-year-old woman	- clozapine toxicity symptoms - increased clozapine serum levels = 4318 ng/ml (References = 350–700 ng/ml)–All patients improved after dose reductions	Luong et al. (2016) Case reports
Suspected infections	clozapine (CYP1A2)	4	- clozapine toxicity symptoms in usually stable patients - patients improved after dose reduction or therapy discontinuation	Dote et al. (2016) Case series
Suspected infections	clozapine (CYP1A2)	62-year-old man	- clozapine levels increased during infection (from 377 ng/ml to 1′628 ng/ml)	Encalada Ventura et al. (2015)
				Case report
Respiratory infection	clozapine (CYP1A2)	34-year-old man	- increased clozapine levels to 1245 ng/ml during infection	Niioka et al. (2017)
				Case report
Lung abscess	clozapine (CYP1A2)	29-year-old man	- increased clozapine levels during infection (from 681 ng/ml to 1′467 ng/ml) - No signs of clozapine toxicity	Encalada Ventura et al. (2015) Case report
Influenza A	clozapine (CYP1A2)	33-year-old woman	- increased clozapine levels during infection (from 661 ng/ml to 1′300 ng/ml) - symptoms of clozapine toxicity	Encalada Ventura et al. (2015) Case report
Pneumonia	clozapine (CYP1A2)	42-year-old man	- increased clozapine levels during infection (from 1′024 ng/ml to 2′494 ng/ml) - symptoms of clozapine toxicity	Encalada Ventura et al. (2015) Case report
Pneumonia	clozapine (CYP1A2)	35-year-old man	- increased median clozapine C/D ratios at the peak of infection	Vreugdenhil et al. (2018)
				Case report
Upper respiratory tract infection	clozapine (CYP1A2)	68-year-old woman	- increased clozapine levels during infection (peaked at 1′096 ng/ml) - toxicity symptoms	van Wanrooy et al. (2014) Case report

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TABLE 2A | (*Continued*) Impact of infection on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Upper respiratory tract infection	clozapine (CYP1A2)	47-year-old man	- On day 24 and 25 (highest level of infection severity), serum concentration levels increased to 881.2 and 663.5 ng/ml, respectively	Schulz et al. (2019) Case report
Urinary tract infection	clozapine (CYP1A2)	51-year-old woman	- increased clozapine levels during infection (peak at 1′066 ng/ml) - patients improved after dose reduction and recovery	Veringa et al. (2017) Case report
Urinary tract infection	clozapine (CYP1A2)	45-year-old woman	- increased clozapine levels during infection (from 705 ng/ml to 2′410 ng/ml) - toxicity symptoms	Encalada Ventura et al. (2015) Case report
Urinary tract infection	clozapine (CYP1A2)	62-year-old man	- increased clozapine levels during infection (from 432 ng/ml to 1′192 ng/ml) - no toxicity symptoms	Encalada Ventura et al. (2015) Case report
Urinary tract infection	clozapine (CYP1A2)	64-year-old woman	- decreased clozapine levels after infection recovery (from 749.4 to 260.0 ng/ml) - toxicity symptoms	Gautier-Veyret et al. (2019) Case report
Infections	clozapine (CYP1A2)	16 patients with 18 episodes	- only 2 episodes did not require any relevant changes of dosage	Bolcato et al. (2021) Case series
Infections	clozapine (CYP1A2)	3	- clozapine toxicity symptoms - 2.5-7-fold increased clozapine serum concentration during infections	Elin et al. (1975) Case series
Diarrheic stools and gastrointestinal bacterial infection	clozapine (CYP1A2)	23 years old man	- at admission, CRP serum concentration = 130 mg/ml and clozapine serum concentration = 9074 nmol/L (References interval 200–2500 nmol/L) - 1 month before, serum concentration = 1919 nmol/L 1 month before admission and fairly constant during the last years	Blumenkopf and Lockhart (1983) Case report
Bacterial pneumonia	clozapine (CYP1A2)	53-year-old woman	- trough concentration = 2074 µg/L at day 0 (before any antibiotics treatments) - previous trough concentrations were three times lower - during the infection, CRP = 152 mg/L and α1-glycoprotein = 2398 mg/L - concentration decreased nearly to the previous levels after 2 weeks (624 ± 214 mg/L)	Khan and Khan (2019) Case report
Increased CRP level	voriconazole (CYP3A4 and CYP2C19)	63	- increased CRP levels associated with significantly increased voriconazole C/D ($p < 0.05$) - CYP3A4 and CYP2C19 downregulated by inflammation	Vozeh et al. (1978) Retrospective study
Increased CRP level	voriconazole (CYP3A4 and CYP2C19)	19	- inflammatory response positively associated with voriconazole concentration ($r = 0.62$, $p < 0.001$) - inflammatory response negatively associated with voriconazole MR (rho = -0.64, $p < 0.001$)	Cohort study Leung et al. (2014) Cohort study
Elevated CRP level	voriconazole (CYP3A4 and CYP2C19)	54	- voriconazole/N-oxide ratio could be predicted by the CRP concentration with a standardized regression coefficient of 0.380 ($p = 0.001$)	Haack et al. (2003) Cohort study
Elevated IL-6, IL-8 and CRP levels	voriconazole (CYP3A4 and CYP2C19)	22	- correlation between IL-6 ($r = 0.46$, $p < 0.0001$), IL-8 ($r = 0.42$, $p < 0.0001$) and CRP ($r = 0.53$, $p < 0.0001$) and trough concentration	de Leon and Diaz (2003) Cohort study

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TABLE 2A | (*Continued*) Impact of infection on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
CRP serum level	voriconazole (CYP3A4 and CYP2C19)	Total = 128	- trough concentration increased by 0.015 mg/L every 1 mg/L increase in CRP	Jecel et al. (2005)
- Elevated (>200 mg/L)			- correlation between trough concentration and CRP levels ($p < 0.001$), and with severity of inflammation	Retrospective study
- Moderate (>41 mg/L, <200 mg/L)				Cohort study
- Control (<40 mg/L)				
Multiple infections along his 5 months hospital stay	voriconazole (CYP2C19 and 3A4), meropenem and their combinations	78-year-old man	- decreased voriconazole dose requirements	Darling and Huthwaite (2011)
				Case report
CRP serum level	voriconazole (CYP3A4 and CYP2C19)	34	- MR significantly decreased with higher CRP concentration after adjustment ($p < 0.001$)	Espnes et al. (2012)
		20 = patients with CYP2C19 genotype performed	- extent of decrease of MR and increase of trough concentration varied between the different genotypes ($p < 0.001$ and $p = 0.04$, respectively)	Prospective study
CYP2C19 genotype				Cohort study
CRP serum levels	voriconazole (CYP3A4 and CYP2C19) and itraconazole (CYP3A4)	41 voriconazole	- C/D of voriconazole and of voriconazole N-oxide positively ($r = 0.61$, $p < 0.01$) and negatively ($r = -0.52$, $p < 0.01$) correlated with CRP levels, respectively	Raaska et al. (2002)
		42 itraconazole	- C/D of itraconazole ($p = 0.33$) and its hydroxide ($p = 0.52$) were not correlated with CRP	Cohort study
CRP serum levels	voriconazole (CYP3A4 and CYP2C19)	31 = with overdose	- mean CRP level significantly higher ($p < 0.0001$) in patients who experienced an overdose (188 mg/L) compared to those who did not (37 mg/L)	Levine and Jones (1983 1)
		31 = without overdose	- patients with CRP levels >96 mg/L (median level) had a 27-fold higher risk of overdose than patients with CRP levels <96 mg/L	Case-control study
Inflammation level	voriconazole CYP2C19 and 3A4)	64-year-old man	- voriconazole C/D associated with inflammation level	Clark et al. (2018)
				Case report
Influenza-like illness	phenytoin (CYP2C9 and CYP2C19 substrates and induces CYP2C9, 2C19 and 3 A)	52-years-old woman	- became increasingly drowsy, moody, complaining of staggering, difficulty to talking and visual disturbance with toxic phenytoin levels (51 μg/ml)	Kwak et al. (2014)
				Case report
Pneumonia	perampanel (CYP3A4)		- 3.5-fold increase perampanel concentrations, - reversible within 7 days after CRP normalization	Lynch and Price (2007)) Case report
Inoculation of Malaria	quinine (CYP3A4)	5	- increase quinine MR during infection ($p < 0.01$)	Takahashi et al. (2015)
				Cross-over study
Infection disease state (pneumonia, endocarditis, wound infection or gastroenteritis) vs healthy state	bisoprolol (CYP2D6 and 3A4) and nitrendipine (CYP3A4)	20	- PK parameters of bisoprolol unchanged ($p > 0.05$)	Hefner et al. (2016)
			- bioavailability of S-enantiomer twice that of R-nitrendipine in infection ($p < 0.01$) - 2-fold increased AUC and Cmax of S-nitrendipine ($p = 0.010$ and $p = 0.012$ respectively) and R-nitrendipine ($p = 0.005$ and $p = 0.029$)	Cohort study
Enteritis with diarrhoea	tacrolimus (CYP3A)	52	- mean tacrolimus trough level 2.3 times higher during enteritis ($p = 0.0175$)	Pfuhlmann et al. (2009)
			- mean trough level returned to their baseline levels 2 weeks after onset	Cohort study

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TABLE 2A | (*Continued*) Impact of infection on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Helicobacter pylori infection in cirrhotic patients	/	21 tested positive and 11 not	Hp-infected cirrhotic patients had a significant lower mean of the monoethylglycinexylide (MEGX) test compared to non-infected patients (p = 0.006), while 13C-galactose breath test (GBT) was not	Abou Farha et al. (2012)
Sepsis	tacrolimus (CYP3)	41-year-old man	151% increased tacrolimus C/D during sepsis	Case-control study Wilkinson (2005)
Dermatitis	clozapine (CYP1A2)	57-year-old woman	- On days 36 and 43 (highest level of dermatitis severity), clozapine serum concentration increased to 889.2 and 1′012 ng/ml, respectively	Case report Schulz et al. (2019) Case report

Bokum et al., 2015; Hefner et al., 2016; Ruan et al., 2017; Clark et al., 2018; Ruan et al., 2018; Ruan et al., 2020). A positive and significant correlation between clozapine and CRP levels (r = 0.313, $p < 0.01$) was found, with a 2- to 6-fold increase in serum levels and the development of toxic symptoms, as well as improvement after dose reduction or infection recovery (Raaska et al., 2002; Haack et al., 2003; de Leon and Diaz, 2003; Jecel et al., 2005; Pfuhlmann et al., 2009; Darling and Huthwaite, 2011; Espnes et al., 2012; Kwak et al., 2014; Leung et al., 2014; Takahashi et al., 2015; ten Bokum et al., 2015; Hefner et al., 2016; Abou Farha et al., 2012; Ruan et al., 2017; Clark et al., 2018; Ruan et al., 2018; Ruan et al., 2020). Further investigations are needed concerning anticoagulant therapy, as only one case of severe bleeding in the context of infection was reported in the literature (Blumenkopf and Lockhart, 1983). First observation of a return to baseline metabolic activity after the end of the disruption that caused inflammation dates from 1985, with the gradual improvement of antipyrine clearance in days after the resolution of pneumonia (Sonne et al., 1985). Later, other authors demonstrated metabolic recovery after improvement of a liver fluke infection following praziquantel treatment (Satarug et al., 1996).

In hepatitis (**Table 2B**), a study suggested an overall downregulation of several hepatic CYPs and transporters with liver fibrosis progression, although the mechanisms of regulation differed and large inter-individual variation existed (Hanada et al., 2012). Indeed, this study assessed that the mRNA level was largely dependent on fibrosis stage and that the role of the different nuclear receptors tested is not the same in the hepatic expression of each CYP isoenzyme (Hanada et al., 2012). CYP3A4 downregulation during HCV infection has been well-described (McHorse et al., 1975; Tuncer et al., 2000; Latorre et al., 2002; Wolffenbüttel et al., 2004). Indeed, numerous studies have described a higher drug exposure of the two most commonly used immunosuppressants, tacrolimus and cyclosporine A, in patients with hepatitis and especially in those with viremia (Tuncer et al., 2000; Latorre et al., 2002; Wolffenbüttel et al., 2004). Moreover, when HCV is treated, CYP activities appear to return to baseline levels in several studies (McHorse et al., 1975; van den Berg et al., 2001; Kugelmas et al., 2003; Ueda et al., 2015; Kawaoka et al., 2016; Saab et al., 2016; Raschzok et al., 2016; Ueda and Uemoto, 2016; Smolders et al., 2017). Indeed, through concentration of tacrolimus decreased after initiation of HCV treatment, such as sofosbuvir, daclatasvir, asunaprevir, simeprivir, ribavirin and interferon, administered alone or in combination, and it required a dosage increase (Kawaoka et al., 2016; Raschzok et al., 2016; Saab et al., 2016; Smolders et al., 2017). Subgroups were identified, such as patients not responding to interferon with higher CYP3A downregulation related to higher levels of circulating cytokines, confirming that CYP modulation is proportional to intensity of inflammation (Morcos et al., 2013). However, conflicting results exist, and clinical recovery from acute liver disease was not accompanied by a corresponding recovery of drug-metabolizing capacity in a study (Breimer et al., 1975). This could be due to a lag between the return to baseline CYP levels and recovery, as clinical recovery from liver disease is not accompanied by a corresponding recovery of drug metabolizing capability (Breimer et al., 1975). Indeed, it is generally recognized that recovery half-lives are approximatively 20–50 h after mechanism-based inhibition and 40–60 h after enzyme induction (Imai et al., 2011).

Several studies have examined the impact of HIV on CYP metabolism (**Table 2C**) and have shown that several concomitant treatments and antiretroviral drugs metabolized by CYP3A have reduced metabolism in HIV-infected individuals, with an increased risk of ADRs. For instance, clindamycin clearance decreased from 0.27 in healthy volunteers to 0.21 L/h/kg in AIDS patients (p = 0.014) and a negative correlation between TNF-α and midazolam clearance was found (Gatti et al., 1993; Jones et al., 2010). Moreover CYP3A inhibitor (ketoconazole or ritonavir) and inducer (rifampicin) effects were less pronounced on antiviral PK in HIV-patients (Gatti et al., 1993; Grub et al., 2001; Jetter et al., 2010; European medicines agency; Packageinserts). It is important to characterize CYP3A modulation in HIV, as many antiviral treatments are metabolized by this pathway, and this could lead to efficacy or safety concerns. However, the AUC of atazanavir was lower in HIV-infected patients than in healthy volunteers and this could

TABLE 2B | Impact of hepatitis on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Chronic hepatitis C	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	12 = chronic hepatitis C 18 = controls	- decreased clearance and greater excretion in urine (about 50%, $p < 0.01$) - no difference in hepatic enzymes levels but Child Pugh Score correlated with clearance ($r = -0.73$, $p = 0.007$)	ten Bokum et al. (2015) Case-control study
Chronic hepatitis C	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	85	- no difference in clearance before and after 6 weeks of interferon treatment - 14% clearance increased ($p < 0.05$) 6 months later among responders but not in those who had failed to respond to interferon	Ruan et al. (2017) Cohort study
Acute viral hepatitis	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	6	- decreased plasma half-life and plasma clearance during the acute phase of hepatitis compared to recovery period ($p < 0.02$)	Ruan et al. (2018) Cohort study
Acute hepatitis	hexobarbital (CYP2C19)	13 = hepatitis 14 = controls	- decreased elimination half-life in patients with hepatitis compared to controls (490 ± 186 min vs. 261 ± 69 min, $p < 0.001$)	Ruan et al. (2020) Case-control study
Hepatitis C infection (IFN)	Cyclosporin A (CyA) and tacrolimus (CYP3A4)	26 = hepatitis C infection 78 = controls	- Lower doses ($p < 0.05$) in hepatitis C as compared to controls, while levels were comparable	Sonne et al. (1985) Case-control study
Acute viral hepatitis C	CyA (CYP3A4)	18 = HCV Ab + 18 = HCV Ab -	- CyA levels significantly higher in HCV Ab + ($p = 0.0001$)	Satarug et al. (1996) Case-control study
Acute viral hepatitis C	CyA (CYP3A4)	11 = anti-HCV + 11 = controls	- altered CyA PK (higher peak levels and drug exposure) in HCV+, especially those with viremia	Hanada et al. (2012) Case-control study
Acute viral hepatitis C	CyA (CYP3A4)	10 = anti-HCV + 14 = controls	- CyA AUC 69% ($p < 0.01$) and 32% ($p < 0.01$) higher in pre- et post-transplant studies in HCV + patients	Hanada et al. (2012) Case-control study
Acute viral hepatitis	meperidine (CYP2B6, 2C19 and 3A4)	14 = acute viral hepatitis 15 = controls	- terminal plasma half-life significantly prolonged in acute viral hepatitis compared to controls ($p < 0.001$) and 2-fold change in total plasma clearance observed ($p < 0.002$)	Latorre et al. (2002) Case-control study
Acute viral hepatitis	meperidine (CYP2B6, 2C19 and 3A4)	5	- total plasma clearance increased from 488 ± 132 ml/min to 1200 ± 555 ml/min and the terminal half-life decreased from 8.24 ± 3.71 to 3.25 ± 0.80 h respectively ($p < 0.005$) - values after recovery were not significantly different from those of the control group	Latorre et al. (2002) RCT
Chronic hepatitis C (CHC)	midazolam (CYP3A4)	107 = controls 35 = CHC naïve to treatment 24 = CHC null responders to IFN	- MR decreased by 37 and 54% ($p < 0.05$) in patients with hepatitis C treatment-naive and interferon null-responders respectively, compared to controls - consistent reductions in CYP3A4 activity between healthy volunteers and patients infected, most substantial difference with interferon null-responders	Tuncer et al. (2000) Case-control study
liver kidney microsome type 1 (LKM-1) antibodies	dextromethrophan (CYP2D6)	10 negative and 10 positive patients for LKM-1	- dextromethorphan-to-dextrorphan (DEM/DOR) ratio was significantly higher in liver kidney microsome type (LKM-1) positive patients ($p = 0.004$), showing that CYP2D6 activity had decrease (antibodies are targeted against CYP2D6)	Wolffenbüttel et al. (2004) Case-control study

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TABLE 2B | (*Continued*) Impact of hepatitis on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Hepatitis A	coumarine (CYP2A6)	9 = hepatitis A	- mean reduction of 37% ($p < 0.05$) of the total urine excretion	McHorse et al. (1975)
		20 = controls	- CYP2A6 lower metabolic activity in hepatitis patients	Case-control study
Hepatitis C virus (HCV) vs control	omeprazole (CYP2C19) and cortisol (CYP3A)	31 = HCV (9 with chronic hepatitis and	- mean omeprazole hydroxylation index in HCV patients were significantly higher compared with healthy subjects, with lower CYP2C19 activity	Smolders et al. (2017)
		22 with cirrhosis)	- mean clearance of cortisol decreased significantly ($p < 0.001$) in CLD patients	Case-control study
		30 = controls		
Chronic HCV treated with sofosbuvir	tacrolimus (CYP3A)	56-year-old male	- through concentration decreased after initiation of HCV treatment that required an increase of dosage	Kawaoka et al. (2016)
		74-year-old male		Case report
HCV treated with daclatasvir/asunaprevir	tacrolimus (CYP3A)	57-year-old man	- case 1: slight increase in trough blood concentration after the start of the combination therapy but no dose adjustment	Saab et al. (2016)
		63-year-old man	- case 2: through blood concentration decreased after the start of the combination therapy and dosage was increased	Case report
HCV before and after treatment	tacrolimus (CYP3A) and cyclosporine (CYP3A)	52	- statistically significant difference in daily dose adjusted per weight or serum levels of tacrolimus after achieving a sustained viral response	Raschzok et al. (2016)
			- no statistically significant difference in daily dose adjusted per weight or serum levels of cyclosporine after achieving a sustained viral response	Cohort study
HCV treated with directly acting antivirals	tacrolimus (CYP3A) and ^{13}C-methacetin (LiMAx test, CYP1A2)	21	- mean LiMAx increased from 344 ± 142 to 458 ± 170 μg/kg/h between the start of treatment and week 12 ($p < 0.001$) (value in healthy volunteers = 430 ± 86 μg/kg/h)	Ueda and Uemoto (2016)
			- tacrolimus C/D decreased over the same period ($p = 0.0017$)	Cohort study
HCV treated with daclatasvir/asunaprevir	tacrolimus (CYP3A)	10	- C/D ratio decreased from 3.95 ng/ml per mg to 2,975 ng/ml per mg after 2 weeks of administration	van den Berg et al. (2001)
				Cohort study
HCV	tacrolimus (CYP3A)	7 = HCV	- dose required to obtain therapeutic levels was comparable in the 2 groups during the first 3 weeks	Kugelmas et al. (2003)
		13 = transplanted for other indications	- dose requirement decreased sharply in HCV patients (20% of the value in controls)	Cohort study
			- dose requirement increased by more than 50% in 2 patients treated with IFN-α/ribavirin	
HCV treated with anti-HCV therapy	tacrolimus (CYP3A) and cyclosporine (CYP3A)	12 (7 cyclosporine and 5 tacrolimus) = responders	- cyclosporine and tacrolimus levels at baseline vs after HCV RNA negativation decreased significantly ($p = 0.018$ for cyclosporine and $p = 0.044$ for tacrolimus)	Ueda et al. (2015)
		18 (7 cyclosporine and 11 tacrolimus) = non-responders	- cyclosporine and tacrolimus levels in non-responders did not change between baseline and the end of anti-HCV therapy ($p = 0.24$ for cyclosporine and $p = 0.32$ for tacrolimus)	Cohort study
HCV treated with simeprevir	tacrolimus (CYP3A) and cyclosporine	2	- C/D ratio of calcineurin inhibitors were elevated in the first 2 weeks in both cases, but decreased thereafter, necessitating an increase in the dose	Morcos et al. (2013)
				Case report

be explained by the absence of correlation between its oral clearance and inflammatory markers in a cohort study, the lack of identical study conditions (doses, sample schedule, meals . . . etc.) between the two groups and the fact that HIV infection was well-controlled (Packageinserts; Le Tiec et al., 2005; Venuto et al., 2018). Indeed, caffeine metabolism was not altered in HIV-infected patient compared with healthy volunteers, but was decreased in AIDS patients (Lee et al., 1993; Jones et al., 2010). Moreover, atazanavir was administered with the booster ritonavir to decrease its clearance, and the effect of inflammation could have been minimized.

More recently, some studies have shown increased plasma concentration of CYPs substrates (mostly CYP3A) during SARS-CoV-2 infection, which may have led to believe that there was a CYPs downregulation due to inflammation (**Table 2D**) (Cojutti et al., 2020; Cranshaw and Harikumar, 2020; Gregoire et al., 2020;

TABLE 3C | Impact of HIV on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
AIDS patients vs control	clindamycin (CYP3A)	16 = AIDS	- clearance values normalized to subject body weight were 0.27 ± 0.06 L/h/kg for the healthy volunteers and 0.21 ± 0.06 L/h/kg for the AIDS patients (p = 0.014)	Breimer et al. (1975)
		16 = healthy volunteers	- ADR following administrations (same dose) were observed in eight patients with AIDS	Case-control study
HIV-infected patients vs control	midazolam (CYP3A), dextromethorphan (CYP2D6) and caffeine (CYP1A2)	17 = HIV-infected	- midazolam clearance was significantly lower in HIV-infected patient compared with healthy volunteers (CI95% = 0.68–0.92) and a significant relationship was found with TNF-α (r = –0.66, p = 0.008)	Imai et al. (2011)
		17 =	- urinary dextrometorphan MR was significantly higher in HIV-infected patients than in healthy volunteers (CI95% = 2.36–42.48) and a trend was observed for an association with the increase in TNF-α concentration (r = 0.49, p = 0.06)	Case-control study
		uninfected	- caffeine metabolism was no significantly different in HIV-infected subjects compared to non-smokers healthy volunteers (controlled for smoking status) (CI95% = 0.83–3.11)	
HIV-infected patients vs control	midazolam (CYP3A) and	30 = HIV-infected	- CYP3A4 activity in HIV infected patients was approximately 50% of the activity in healthy volunteers but it was mainly attributable to a lower intestinal CYP3A4 activity, while hepatic CYP3A was not different	Gatti et al. (1993)
	dextromethorphan (CYP2D6)	12 = healthy volunteers	- CYP2D6 activity was essentially comparable	Case-control study
HIV-positive patients	dextromethorphan (CYP2D6)	61	- 2 of the 59 patients with an NM genotype expressed a PM phenotype and 4 NM genotype patients were less extensive dextrometorphan metabolizers than any of the patients receiving medication known to inhibit CYP2D6	Jones et al. (2010)
				Cohort study
HIV-1 infected patients vs control	darunavir (CYP3A)	Unknown, information obtained from Summary of Product Characteristics (SmPC)	- exposure to darunavir was higher in HIV-1 infected patients	Jetter et al. (2010)
			- explained by the higher concentrations of α1-glycoprotrein in HIV-1 infected patients, resulting in higher darunavir binding to plasma AAG and, therefore, higher plasma concentrations	Case-control study
HIV-infected patients vs healthy volunteers	saquinavir (CYP3A)	33 = HIV-infected	- co-administration of ketoconazole increased saquinavir AUC by 190 and 69% in healthy volunteers and HIV-infected patients, respectively while co-administration of rifampicin decreased saquinavir area under the curve by 70 and 46%	European medicines agency
				Case-control study
		12 and 14 = control		
HIV-infected patients vs healthy controls	atazanavir and atazanavir with ritonavir (CYP3A)	Unknown, information obtained from SmPC	- mean AUC of atazanavir and atazanavir with ritonavir were 29′303 and 61′435 ng*h/mL respectively in healthy volunteers, vs. 22′262 and 53′761 ng*h/ml, respectively in HIV-infected patients	Grub et al. (2001)
				Case-control study
HIV-infected patients vs healthy controls	lopinavir with ritonavir (CYP3A)	Unknown, information obtained from SmPC	- no substantial differences observed between the two groups	Packageinserts
				Case-control study

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TABLE 3C | (*Continued*) Impact of HIV on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
HIV-infected patients vs healthy controls	atazanavir (CYP3A)	10 = HIV-infected	- mean atazanavir AUC in HIV-infected patients was 14′187 ng*h/ml compared with 33′097 ng*h/ml in healthy volunteers	Le Tiec et al. (2005)
		36 = healthy volunteers	- after 14 and 20 days of atazanavir in HIV patients and healthy volunteers, respectively, AUC were 46′073 and 57′039 ng*h/ml	Case-control study
Patients with different stage of HIV infection vs control	caffeine (CYP1A2)	29 = AIDS	- metabolic status was not change in HIV asymptomatic patients but changed in AIDS patients (with acute illnesses or stable)	Venuto et al. (2018)
		29 = AIDS-stable		Case-control study
		18 = HIV-infected 29 = control		
HIV infected patients	atazanavir (CYP3A)	107 = HIV-1 infected	- apparent oral clearance was not significantly correlated with inflammatory biomarkers	Lee et al. (1993)
				Cohort study

Marzolini et al., 2020; Schoergenhofer et al., 2020; Testa et al., 2020). Indeed, the plasma concentrations of some CYP3A substrates (lopinavir, darunavir and direct oral anticoagulants) were significantly increased in patients with SARS-CoV-2 infection (Cojutti et al., 2020; Gregoire et al., 2020; Schoergenhofer et al., 2020; Testa et al., 2020). CRP and IL-6 were also associated with lopinavir concentrations and a trend toward a return to baseline was observed after treatment with tocilizumab (Marzolini et al., 2020; Schoergenhofer et al., 2020). Indeed, lopinavir through level in patients with SARS-CoV-2 infection was twice as high as in HIV patients but concentrations decreased when tocilizumab was administered (Marzolini et al., 2020; Schoergenhofer et al., 2020). However, the impact of inflammation induced by SARS-CoV-2 infection on lopinavir through concentration may be also due to increased orosomucoid levels (Boffito et al., 2021; Stanke-Labesque et al., 2021). Lopinavir is a highly protein-bound drug and the misinterpretation of its overexposure during inflammation could be explained by the fact that total and not unbound concentration was considered (Boffito et al., 2021; Stanke-Labesque et al., 2021). Furthermore, a case report described clozapine toxicity and increased clozapine level from 0.57 to 0.73 mg/L during SARS-CoV-2 infection (Cranshaw and Harikumar, 2020). However, no correlation was found between CRP and hydroxychloroquine plasma concentrations (Marzolini et al., 2020).

Vaccination

Regarding vaccination (**Table 3**), several reports and studies assessed variations of PK/PD parameters of drugs after vaccination, but data remain contradictory. Of the 31 articles included, 28 were exclusively about influenza vaccination while two were about concomitant vaccinations including influenza (pneumococcus, tetanus and hepatitis A). Only one article did not evaluate the influenza vaccination but reported on the impact of *tuberculosis* vaccination (BCG). No significant difference of CYP activity between before or after vaccination was shown in several studies (Britton and Ruben, 1982; Fischer et al., 1982; Goldstein et al., 1982; Patriarca et al., 1983; Stults and Hashisaki, 1983; Stults and Hashisaki, 1983; Hayney and Muller, 2003). In particular, the impact of vaccination on anticoagulants effects has been well-studied but the majority of studies showed no variation of PT time or INR (Farrow and Nicholson, 1984; Kramer et al., 1984; Gomolin, 1986; Raj et al., 1995; Poli et al., 2002; Paliani et al., 2003; Iorio et al., 2006; Jackson et al., 2007; MacCallum et al., 2007; Casajuana et al., 2008). However, the occurrence of bleeding events a few days after vaccination, when the PT time was previously stable, has been described (Kramer et al., 1984; Weibert et al., 1986; Carroll and Carroll, 2009). Moreover, the case of a patient hospitalized because of serum CPK level of 93,000 U/L during treatment with cerivastatin and bezafibrate or the occurrence of tramadol toxicity has been reported (Plotkin et al., 2000; Pellegrino et al., 2013). The patient had been vaccinated 5 days earlier (Plotkin et al., 2000). Other studies, few in number, have found an effect of vaccination on the PK of CYP substrates (Renton et al., 1980; Kramer and McClain, 1981; Gray et al., 1983). However, no study has correlated the data with pro-inflammatory markers.

Organs Diseases

The influence of liver and kidney function on disposition of drugs excreted by the liver and kidney is widely recognized and used to derive dosing adaptations. However, there is now an increasing appreciation that kidney impairment can also reduce non-renal clearance and alter the bioavailability of drugs predominantly metabolized by the liver (Nolin, 2008). Indeed, uremic toxin has been implicated in transcriptional, translational and acute posttranslational modifications of CYP, and it has been recognized that inflammation is a common feature in end-stage renal disease (ESRD) patients (Nolin, 2008; Stenvinkel and Alvestrand, 2002). For example, CYP3A activity increased post-dialysis, meaning that it is the presence of uremic toxin that is responsible for CYP downregulation and not the underlying disease (Nolin et al., 2006). An inverse relationship between hepatic CYP3A activity was found in this study, but it did not prove causality (Nolin et al., 2006). It indicates that uremia can be used as a surrogate for dialyzable toxins that contribute to

TABLE 2D | Impact of SARS-CoV-2 on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
SARS-CoV-2 and treatment with tocilizumab	lopinavir/ritonavir (CYP3A) and hydroxychloroquine (CYP2D6)	41 = without tocilizumab, 51 = tocilizumab (35 before and 16 after)	- lopinavir concentrations positively correlated with CRP (r = 0.37, p < 0.001) and significantly lower after tocilizumab, - no correlation between CRP and hydroxychloroquine plasma concentration	Marzolini et al. (2020), Cohort study
SARS-CoV-2 vs. HIV-patients	lopinavir/ritonavir (CYP3A)	12	- lopinavir trough concentration in patients with SARS-CoV-2 infection were significantly higher than those usually observe in HIV-infected patients (18′000 vs. 5365 ng/ml)	Gregoire et al. (2020), Cohort study
SARS-CoV-2	clozapine (CYP1A2)	38-year-old-man	- symptoms of clozapine toxicity, - clozapine level increased by 0.57–0.73 mg/L and norclozapine increased by 0.22 mg/L to 0.31 mg/L after SARS-CoV-2 infection	Cranshaw and Harikumar (2020), Case report
SARS-CoV-2	lopinavir/ritonavir (CYP3A)	8	- through concentration associated with CRP level (r = 0.81, p = unknown), - through levels were 2-fold higher in patients with SARS-CoV-2 infection than HIV patients	Schoergenhofer et al. (2020), Cohort study
SARS-CoV-2	apixaban (CYP3A), rivaroxaban (CYP3A), edoxaban (CYP3A)	5 = apixaban, 3 = rivaroxaban, 3 = edoxaban	- alarming increase in DOAC plasma levels compared to pre-hospitalization levels, - possible role of concomitant drugs (CYP3A inhibitors) or disease-related organ dysfunctions	Testa et al. (2020), Cohort study
SARS-CoV-2 vs HIV-patients	darunavir (CYP3A)	30 = SARS-CoV-2 25 = HIV	- median CL/F was significantly lower in SARS-CoV-2 patients with IL-6 levels >18 pg/ml than <18 pg/ml or HIV patients (p < 0.0001), - increasing level of IL-6 affected concentration vs time simulated profile	Cojutti et al. (2020), Case-control study

alterations in CYP3A function (Nolin et al., 2006). Indeed, hemodialysis improved CYP3A activity with a 27% increase 2 h post-dialysis in uremic patients, suggesting that potential toxins responsible for this alteration were removed (Nolin et al., 2006). Authors suggested that this improvement occurred independently of transcriptional or translational modifications, contrary to what has been suggested previously (Nolin et al., 2006). However, as shown in **Table 4**, two studies found an association between the modification of CYP activity and inflammation in ESRD patients (Molanaei et al., 2012; Molanaei et al., 2018).

All studies in patients with liver disease described a decrease in CYP activity, compared to controls, as shown in **Table 5**. Indeed, several studies studied antipyrine, an old drug that is metabolized by multiple CYP (Branch et al., 1973; Farrell et al., 1979; Salmela et al., 1980; Teunissen et al., 1984; Schellens et al., 1989; Bauer et al., 1994; Grieco et al., 1998; Frye et al., 2006). They showed that CYP activity and antipyrine metabolism decreased only in severe disease compared to inactive cirrhosis, mild-moderate liver disease or healthy volunteers (Farrell et al., 1979; Bauer et al., 1994; Grieco et al., 1998). Moreover, chronic liver disease appeared to have a higher impact than an acute/reversible pathology (Branch et al., 1973). However, few studies have focused on a specific CYP substrate, and no studies found an association with inflammatory markers. One study demonstrated that CYP2C19, 2E1, 1A2 and 2D6 probe drugs concentrations were inversely correlated to the Child-Pugh score and another one demonstrated that phenacetin clearance decreased by 90% in patients with cirrhosis (Frye et al., 2006; Wang et al., 2010). Concerning CYP2C9, tolbutamide plasma levels increased by 10–20% and irbesartan AUC increased by 20–30% in cirrhotic patients (Ueda et al., 1963; Marino et al., 1998). The same results were found with CYP3A as diazepam clearance decreased in cirrhosis (Klotz et al., 1975). These variations may therefore be attributed to the loss of liver function due to tissue destruction. CYP metabolism appeared to be influenced by other organ's disease, such as clozapine serum levels that increased by 2-fold during chronic obstructive pulmonary disease (COPD) exacerbation and antipyrine clearance that was significantly lower in patient with COPD and antitrypsin deficiency than in healthy volunteers (Laybourn et al., 1986; Leung et al., 2014). In addition, one study showed that inflammatory markers were inversely correlated with CYP1A2 and CYP2C19 activity but not with CYP2D6 and CYP2E1 activity in patients with congestive heart failure (Frye et al., 2002).

Some studies conducted in critically ill patients (**Table 8**), showed that CYP1A2 and 3A metabolic activity were downregulated, and that it may be proportional to the severity and reversibility of the illness (Shelly et al., 1987; Toft et al., 1991; Kruger et al., 2009). For instance, theophylline clearance decreased by 10–66%, atorvastatin AUC increased by 15-fold, and clopidogrel active metabolite decreased by 48-fold, raising concerns about

TABLE 3 | Impact of vaccination on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Influenza vaccination	Erythromycin breath-tests (ERMBT) (CYP3A)	24 = healthy volunteers	- no significant difference between CYP3A4 activity before and 7 days after vaccination but the influenza antigen-specific production of IFN-γ by lymphocytes was highly correlated with the change in ERMBT (r = -0.614, p = 0.020) thus, IFN-γ downregulates the expression/activity of CYP3A4	Boffito et al. (2021) Non-random
Influenza vaccination	ERMBT (CYP3A)	15 = healthy volunteers	- significant inverse correlation between age and change in ERMBT (r = –0.624, p < 0.015) after vaccination	Stanke-Labesque et al. (2021) Non-random
Influenza vaccination	simvastatine (CYP3A)	68-year-old man	- hospitalized because of complaining of extreme weakness and diffuse muscle pain 5 days after influenza vaccine - 24 h after the vaccination, he began to complain of diffuse myalgia and symptoms worsened - serum CPK value at admission was of 93′000 U/L (70 U/L 2 weeks prior to admission)	Hayney and Muller (2003) Case report
Influenza vaccination	chloroxazone (CYP2E1)	10 = healthy volunteers	- no significant difference in the PK parameters before immunization and 7 and 21 days after vaccination	Stults and Hashisaki (1983) Non-random
Influenza vaccination vs controls	^{13}C-aminopyrine breath test (CYP2C19, 1A2 and 3A4)	12 = vaccinated	- significant reduction (22–74%, p < 0.001) in aminopyrine breath test 7 days after vaccination compared to controls	Fischer et al. (1982)
		10 = controls	- metabolic activity depression was not significant 2 days after vaccination but there was still a significant reduction 21 days after vaccination	Non-random
BCG vaccination (*tuberculosis*)	theophylline (CYP1A2)	9 = patients converted to positive Mantoux skin test	- the clearance and half-life were significantly decreased and increased, respectively (p < 0.02), in patients with positive Mantoux skin test, as compared to controls	Stults and Hashisaki (1983)
		3 = controls		Random
Influenza vaccination	theophylline (CYP1A2)	7=3 recovering from an acute exacerbation of COPD and 4 healthy volunteers	- plasmatic concentration before and after influenza vaccination significantly increased	Goldstein et al. (1982) Non-random
Influenza vaccination	theophylline (CYP1A2)	13	- no difference in the mean serum theophylline levels before influenza vaccination and 24h, 72h, 1 week and 2 weeks after vaccination	Britton and Ruben (1982) Non-random
Influenza vaccination	theophylline (CYP1A2)	7 (chronic bronchitis and chronic airflow obstruction thus and 5 men were smokers (CYP1A2 inductor))	- no difference between the clearance rate before and 24 h after vaccination (p = 0.778) - clearance 4–48 h after influenza vaccination was not significantly different (p = 0.789) - serum interferon was not detected in any of the seven subjects before or 8, 16, 24, 46 h and 7–10 days following vaccination	Patriarca et al. (1983) Non-random
Influenza vaccination	theophylline (CYP1A2)	16 (COPD)	- no difference in plasma concentration 24 h before or after vaccine injection	Jackson et al. (2007) Non-random

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TABLE 3 | (*Continued*) Impact of vaccination on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Influenza vaccination	theophylline (CYP1A2)	5	- no significant variations in the serum levels before and 24 h after vaccination	Farrow and Nicholson (1984) Non-random
Influenza vaccination	theophylline (CYP1A2) and chlordiazepoxide (CYP3A)	8 = theophylline	- an effect of vaccination has been shown on theophylline clearance at day 1 after vaccination ($p = 0.016$) but not at day 7	MacCallum et al. (2007)
		5 = chlordiazepoxide	- no effect on chlordiazepoxide metabolism - the effect seems to be greater when initial clearance is higher	Non-random
Influenza vaccination vs controls	theophylline (CYP1A2) and warfarin (CYP2C9)	152 = influenza vaccinated	- no ADR occurred in patients on theophylline in both groups and only one reaction in each group of patients who were taking warfarin	Raj et al. (1995)
		51 = unvaccinated		Case-control study
Influenza, pneumococcal, tetanus and hepatitis A vaccinations	warfarin (CYP2C9)	5′167	- not associated with INR value change	Gomolin (1986)
				Cohort study
Influenza and pneumococcal vaccination vs. controls	warfarin (CYP2C9)	25 = placebo	- no statistically significant increments in mean British Corrected Ratios for prothrombin time 2, 7- or 21-days post injections	Iorio et al. (2006)
		25 = influenza 19 = pneumococcal		Random
Influenza vaccination	warfarin (CYP2C9)	78	- no significant effect on anticoagulant control during the 10 days post-vaccination in the vast majority of individuals	Poli et al. (2002)
				Cohort study
Influenza vaccination	warfarin (CYP2C9)	41	- no significant difference in the mean PT 3, 7 and 14 days after vaccination for the entire group and no patient developed any major or minor bleeding episodes	Paliani et al. (2003)
				Cohort study
Influenza vaccination vs controls	warfarin (CYP2C9)	7	- no difference in the mean PT one, three and 6 weeks after vaccination	Casajuana et al. (2008)
				Cohort study
Influenza vaccination	warfarin (CYP2C9)	104	- no difference in the mean PT-INR values and mean weekly dosage between group 1 (active vaccine at day 0 and placebo at day 42) and group 2 (placebo at day 0 and active vaccine at day 42)	Kramer et al. (1984), Cross-over study
Influenza vaccination	warfarin (CYP2C9)	71 = vaccinated, 72 = controls	- no differences in the anticoagulation levels 3 months before and 3 months after the vaccination, - in the 34 vaccinated patients older than 70 years, a reduction of anticoagulation intensity was achieved in the 3 months after the vaccination and it was not the case in control group	Carroll and Carroll 2009), Case-control study
Influenza vaccination	warfarin (CYP2C9)	49 = patients, 45 = controls	- no difference in INR between patients and control groups before vaccination while 7–10 days after injection, INR significantly increased ($p < 0.00005$), - in patient group, INR increased significantly after vaccination ($p < 0.00001$)	Weibert et al. (1986), Case-control study

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TABLE 3 | (*Continued*) Impact of vaccination on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Influenza vaccination	225 acenocoumarol 4 warfarin (CYP2C9)	100 = intramuscular, 129 = subcutaneous	- INR decreased 24 h after intramuscular vaccination and increased in the subcutaneous group but the difference did not reach statistical significance	Plotkin et al. (2000), RCT
Influenza vaccination	warfarin (CYP2C9)	8	40% prolongation of PT (statistically significance unknown)	Pellegrino et al. (2013), Non-random
Influenza vaccination	warfarin (CYP2C9)	12 (healthy volunteers)	- no significant effect on warfarin metabolism was observed between influenza vaccination or saline injection	Pellegrino et al. (2013), Cross-over study
Influenza vaccination	warfarin (CYP2C9)	81-years-old man	- admitted with hematemesis and a 3-days history of melena and further investigations confirmed a bleeding gastric mucosa but no evidence of oesophagitis, gastritis, duodenitis or ulcer, - monthly PT had been stable and in the therapeutic ranges but the day of admission, PT was 36 s, - 10 days before admission, he received influenza vaccination. Warfarin was withheld and recovered uneventful	Pellegrino et al. (2013), Case report
Influenza vaccination	warfarin (CYP2C9)	64-years-old patient	- death from intracranial haemorrhage (INR = 15 at admission), - INR = 2 4.5 weeks before and all values over the previous 6 months were relatively stable, - vaccine 4.5 weeks before this fatal event	Kramer and McClain (1981), Case report
Influenza vaccination	warfarin (CYP2C9)	12	- small but significant increase in the PT ratio before and after vaccination, - maximal increase occurred on day 14 and represented a 7.6% increase over the baseline value	Gray et al. (1983), Non-random
Influenza vaccination	tramadol (CYP2B6 and 3A, bioactivated by CYP2D6)	85-years-old woman and a and 84-years-old man	- hallucinations and other neurologic symptoms six and 5 days after the administration of two different influenza vaccines	Renton et al. (1980), Case report
Influenza vaccination	carbamazepine (CYP1A2 and 2C9, bioactivated by CYP3A)	15-years-old woman	- vaccination 13 days before admission, but it was well tolerated, and no changes were made in her medication, - serum carbamazepine level was 27.5 μg/ml (ataxia and increasing lethargy) at admission and it decreased to 9.1 μg/ml 4 days after admission	Nolin (2008), Case report
Influenza vaccination	phenytoin (CYP2C9 and CYP2C19 substrates and induces CYP2C9, 2C19 and 3 A)	16	- no significant increase in mean serum concentration were observed on days 7 and 14 following the vaccination, - temporary increases of 46–170% mean serum concentration occurred in four subjects	Stenvinkel and Alvestrand (2002), Cohort study
Influenza vaccination	acetaminophen (CYP2E1), alprazolam (CYP3A), antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	24 (healthy volunteers 9 = acetaminophen, 7 = alprazolam, 8 = antipyrine)	- PK variables were no significantly different ($p > 0.05$) before and 7 and 21 days after vaccination	Nolin et al. (2006), Random

treatment efficacy (Toft et al., 1991; Kruger et al., 2009; Schoergenhofer et al., 2018). However, a systematic review reported that 20–65% of critically patients had an increased renal clearance, defined as a creatinine clearance greater than 130 ml/min/1.73 m^2 (Bilbao-Meseguer et al., 2018). This underscores the fact that inflammation has a different effect on drug clearance through the different mechanisms of drug elimination.

Diabetes

In diabetes (**Table 9**), CYP metabolism has been shown to be downregulated (Salmela et al., 1980; Pirttiaho et al., 1984).

TABLE 4 | Impact of renal diseases on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Severely impaired renal function vs normal	tolbutamide (CYP2C9)	11 = severe kidney impairment , 7 = normal	- Half-life was prolonged in severely impaired renal function patients (n = 11)	Molanaei et al. (2018), Case-control study
Haemodialyzed patients	alprazolam (CYP3A)	26	- ratio of unconjugated alprazolam to 4-hydroxyalprazolam was correlated with CRP levels (r = 0.49, p = 0.01) ADDIN ZOTERO_ITEM CSL_CITATION {"citationID":"Q0Jo8NiX","properties":{"formattedCitation":"(170)","plainCitation":"(170)","dontUpdate":true,"noteIndex":0},"citationItems":[{"id":1099,"uris":["http://zotero.org/users/2161612/items/8PPVMCBX"],"uri":["http://zotero.org/users/2161612/items/8PPVMCBX"],"itemData":{"id":1099,"type":"article-journal","abstract":"OBJECTIVE: To investigate the impact of persistent inflammation in hemodialysis (HD) patients on the pharmacokinetics of alprazolam, a cytochrome P450 (CYP) 3A4 substrate, and its metabolites and the role of HD in the impact of persistent inflammation in this clinical context.\nMETHODS: The study population comprised 26 HD patients (mean age 64 years, range 27-79 years; 19 men, 7 women) who were given 1 mg of alprazolam orally in the evening before the day of HD. Unconjugated and conjugated alprazolam and its 4-hydroxy and α-hydroxy metabolites were measured by liquid chromatography-mass spectrometry at 10, 34 (start of HD) and 38 (end of HD) h after intake. C-reactive protein (CRP) was measured weekly beginning 2 months before study initiation, and alpha 1-acid glycoprotein and 4β-hydroxycholesterol were measured at baseline. CYP3A4 activity was estimated as the ratio of unconjugated alprazolam to 4-hydroxyalprazolam between 10 and 34 h following alprazolam intake.\nRESULTS: After a single dose of alprazolam, plasma concentrations of unconjugated alprazolam and its metabolites decreased gradually, and unconjugated 4-hydroxyalprazolam was eliminated more rapidly than unconjugated alprazolam by HD. In contrast, the plasma concentrations of conjugated alprazolam and its conjugated metabolites increased during the 34 h following drug intake and the subsequent HD decreased their levels by almost 80%. The ratio of unconjugated alprazolam to 4-hydroxyalprazolam was correlated with CRP levels (r(s) = 0.49, P = 0.01). There was no significant correlation between CYP3A4 activity measured by alprazolam (4-hydroxylation) and alpha 1-acid glycoprotein or 4β-hydroxycholesterol. Conjugated alprazolam was also found in the plasma.\nCONCLUSIONS: The correlation between CYP3A4 activity (assessed by alprazolam 4-hydroxylation) and CRP level suggests that inflammation may downregulate CYP3A4 activity. If confirmed, this could have major implications for drug dosing in persistently inflamed patients.","container-title":"European Journal of Clinical Pharmacology","DOI":"10.1007/s00228-011-1163-8","ISSN":"1432-1041","issue":"5","journalAbbreviation":"Eur. J. Clin. Pharmacol.","language":"eng","note":"PMID: 22159869","page":"571-577","source":"PubMed","title":"Metabolism of alprazolam (a marker of CYP3A4) in hemodialysis patients with persistent inflammation","volume":"68","author":[{"family":"Molanaei","given":"Hadi"},{"family":"Stenvinkel","given":"Peter"},{"family":"Qureshi","given":"Abdul Rashid"},{"family":"Carrero","given":"Juan Jesús"},{"family":"Heimbürger","given":"Olof"},{"family":"Lindholm","given":"Bengt"},{"family":"Diczfalusy","given":"Ulf"},{"family":"Odar-Cederlöf","given":"Ingegerd"},{"family":"Bertilsson","given":"Leif"}],"issued":{"date-parts":[["2012",5]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}	Molanaei et al. (2012), Cohort study
Haemodialyzed patients	quinine (CYP3A)	44	- significant correlation between the ratio of quinine/3-OH-quinine and median CRP (r = 0.48, p = 0.001), orosomucoid (r = 0.44, p = 0.003) and IL-6 after 12 h after drug intake (r = 0.43, p = 0.004), - correlation is no longer significant for IL-6 and orosomucoid after adjustment for age, gender, diabetes mellitus, dialysis vintage, PTH, orosomucoid and medications and it remains borderline for CRP (r = 0.05)	Farrell et al. (1979), Cohort study
End stage renal disease (ESRD) vs. control	warfarin (CYP2C9)	7 = ESRD 6 = control	- 50% (p < 0.03) increase plasma warfarin S/R ratio relative to controls	Frye et al. (2006), Case-control study
Moderate and severe kidney impairment vs no/mild kidney impairment	warfarin (CYP2C9)	599 = no/mild 300 = moderate 81 = severe	- patients with moderate kidney impairment required 9.5% lower doses (p < 0.001) compared to controls, - patients with severe kidney impairment required 19.1% lower doses (p < 0.001) compared to controls, - reduced kidney function was associated with lower dose requirements independently of CYP2C9 and VKORC1 genotype and clinical factors	Grieco et al. (1998), Two cohort studies combined, Case-control study

TABLE 5 | Impact of liver diseases on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Mild to moderate hepatocellular changes or inactive cirrhosis and severe liver disease vs control	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	15 = mild-moderate hepatocellular damage, 13 = inactive cirrhosis, 22 = severe liver disease, 21 = controls	- mean value of hepatic CYP concentration did not differ between patients with mild to moderate hepatocellular changes (less than 50% hepatocytes morphologically abnormal) or inactive cirrhosis and controls and antipyrine half-life did not significantly differ between all groups, - CYP concentration was less in patients with severe liver disease (more than 50% hepatocytes morphologically abnormal or active cirrhosis) and, thus, antipyrine half-life was significantly lower ($p < 0.01$) compared to other groups	Bauer et al. (1994), Case-control study
Liver disease vs. control	caffeine (CYP1A2), mephenytoin (2C19), debrisoquin (2D6), and chlorzoxazone (2E1)	20 = liver disease	- significant decrease in metabolite production in patients with liver disease for CYP2C19 ($p < 0.001$), 2E1 ($p = 0.0081$), 1A2 ($p = 0.0054$) and 2D6 ($p = 0.0110$)	Salmela et al. (1980)
		20 = control	- each probe drug was significantly inversely related to the Pugh score	Case-control study
Chronic active hepatitis and cirrhosis vs. control	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	103 = controls, 101 = non-cirrhotic with liver metastases, 102 = chronic active hepatitis, 92 = confirmed cirrhosis, 120 = hepatocellular carcinoma and cirrhosis	- clearance was significantly impaired with respect to healthy volunteers, chronic hepatitis without fibrosis and non-cirrhotic patients with liver metastases, - mean clearance rate of the non-cirrhotic patients with liver metastasis was quite similar to that of patients with healthy livers, - cirrhotic patients with hepatocellular carcinoma also presented significantly impaired clearance compared with that of healthy volunteers and patients with liver metastasis, - elimination of antipyrine may very well be normal in patients with primary or metastatic liver disease, even when there is extensive tumour involvement	Branch et al. (1973), Case-control study
Cirrhotic patient and chronic hepatitis vs. control	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	6 = control, 6 = chronic active hepatitis, 5 = cirrhosis	- half-life and clearance were significantly higher and lower respectively in cirrhotic patients compared with healthy subjects, - no significant differences between hepatitis patients and healthy subjects	Schellens et al. (1989), Case-control study
Diabetics with fatty liver, fatty liver with inflammatory changes and with cirrhosis vs diabetics with normal liver	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	4 = control, 13 = fatty liver, 33 = fatty liver with inflammation, 6 = cirrhosis	- clearances decreased significantly in diabetics with fatty liver ($n = 13$, $p < 0.005$), in diabetics with fatty liver with inflammatory changes ($n = 33$, $p < 0.005$) and in diabetics with cirrhosis ($n = 6$, $p < 0.005$) as compared to diabetics with normal liver	Teunissen et al. (1984), Case-control study
Cirrhosis vs. normal	tolbutamide (2C9)	10 = cirrhotic patients, 7 = normal	- disappearance rate was reduced in five of ten cases, - half-life was prolonged to 7.8–11.2 h (4.4 h in normal group), - plasma levels after 24 h were 11.4–20.8% of the theoretical initial value (5.3% of the theoretical initial value in normal group)	Molanaei et al. (2018) Case-control study
Acute liver and chronic disease	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	14 = control, 38 = liver disease	- half-life was prolonged in patients with liver disease and those with chronic illness had greater increase than those with acute, reversible pathology	Wang et al. (2010), Case-control study
Various liver disease vs. controls	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4), hexobarbital (CYP2C19) and theophylline (CYP1A2)	24 = liver disease, 26 = controls	- clearance of antipyrine, hexobarbital and theophylline are lower than those found in the control subject	Liver disease = Ueda et al. (1963) , Controls = Marino et al. (1998), Case Control
Alcoholic cirrhosis vs. controls	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	23 = alcoholic liver cirrhosis, 17 = control	- clearance was significantly lower in patients with alcoholic cirrhosis as compared with healthy volunteers ($p < 0.001$), - the rates antipyrine formations metabolites were not reduced to the same extent	Klotz et al. (1975) Case-control study
Chronic hepatitis	mephenytoin (CYP2C9 and 2C19 and induces 2C9, 2C19 and 3 A)	35 = chronic hepatitis, 153 = controls	- mean metabolite excretion was significantly lower in patients with liver disease ($p < 0.005$)	Laybourn et al. (1986), Case-control study

(Continued on following page)

TABLE 5 | (*Continued*) Impact of liver diseases on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYP concerned)	Number of subjects	Potential effect of interaction	References and design
Liver disease	mephenytoin (CYP2C9 and 2C19 and induces 2C9, 2C19 and 3 A) and debrisoquin (CYP2D6)	18 = liver disease, 8 = controls	- urinary excretion of mephytoin's metabolite among patients with liver disease was significantly less than among the healthy controls (45% reduction), - the reduction in excretion of mephytoin depended on severity of the disease (28 and 62% decreases for patients with mild and moderate liver disease, respectively), - excretion of debrisoquin's metabolite was comparable between control and disease groups, as groups with mild or moderate disease	Frye et al. (2002), Case-control study
Cirrhotic vs. control	irbesartan (CYP2C9)	10 = hepatic impairment	- trend for moderate (20–30%) increase in AUC and Cmax values in the cirrhotic group compared with control group but the difference did not meet the predetermined criteria for clinical interest	Toft et al. (1991)
Hepatic impairment vs. control		10 = control	- no significant differences of mean half-life, Cmax, clearance and AUC, - patients with hepatic impairment had higher percentage of cumulative urinary extraction of unchanged irbesartan after multiple dose administration ($p < 0.05$)	Case-control study
Cirrhosis vs. control	meperidine (CYP2B6, 3A4 and 2C19)	10 = cirrhosis, 8 = control	- total plasma clearance was of 664 ± 293 ml/min in cirrhotic patients and of 1′316 ± 383 ml/min in healthy volunteers, - clearance was significantly reduced in cirrhosis patients ($p < 0.002$) ADDIN ZOTERO_ITEM CSL_CITATION {"citationID": "a2nlaknkd00","properties":{"formattedCitation": "(168)","plainCitation":"(168)","dontUpdate": true,"noteIndex":0},"citationItems":[{"id": 10553,"uris":["http://zotero.org/users/2161612/items/7HBDUYBB"],"uri":["http://zotero.org/users/2161612/items/7HBDUYBB"],"itemData": {"id":10553,"type":"article-journal","container-title":"Clinical Pharmacology and Therapeutics","DOI":"10.1002/cpt1974164667","ISSN":"0009-9236","issue": "4","journalAbbreviation":"Clin. Pharmacol. Ther.","language":"eng","note":"PMID: 4419525","page":"667-675","source": "PubMed","title":"The effect of cirrhosis on the disposition and elimination of meperidine in man","volume":"16","author":[{"family": "Klotz","given":"U."},{"family":"McHorse","given": "T. S."},{"family":"Wilkinson","given":"G. R."},{"family":"Schenker","given":"S."}],"issued": {"date-parts":[["1974",10]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}	Kruger et al. (2009), Case-control study
Cirrhosis vs. control	diazepam (CYP3A)	21 = liver disease (9 alcoholic liver cirrhosis, 8 acute viral hepatitis and 4 chronic active hepatitis), 33 = control	- half-life showed a more than 2-fold prolongation (105.6 ± 15.2 h vs. 46.4 ± 14.2 h, $p < 0.001$) in patients with cirrhosis compared with age-matched control groups, - a decrease in the total plasma clearance of the drug in cirrhosis ($p < 0.001$)	Shelly et al. (1987), Case-control study
Acute viral and chronic active hepatitis vs control			- patients with acute viral hepatitis had a half-life of 74.5 ± 27.5 h and those with active chronic hepatitis of 59.7 ± 23.0 h, as compared to a normal value in this age group of 32.7 ± 8.9 h ($p < 0.01$)	
Cirrhosis and chronic hepatitis B (CHB)	phenacetin (CYP1A2)	106 = cirrhosis, 41 = CHB, 82 = controls	- clearance decreased by 91.2% ($p < 0.01$) and 67.7% ($p < 0.005$) in the patients with cirrhosis ($n = 106$) and chronic hepatitis B ($n = 41$), respectively	Schoergenhofer et al. (2018), Case-control study

Indeed, antipyrine metabolism was decreased compared with controls in several studies (Salmela et al., 1980; Pirttiaho et al., 1984; Zysset and Wietholtz, 1988). One study using a cocktail approach showed that CYP2B6, CYP2C19 and CYP3A activity decreased, CYP1A2 and CYP2C9 activity increased, and CYP2D6 and CYP2E1 activity was unaffected in type II diabetes (T2D) (Gravel et al., 2019). However, conflicting results exist with tolbutamide and paracetamol half-lifes which were unchanged and increased respectively (Ueda et al., 1963; Adithan et al., 1988). Regarding CYP3A, one study found no impact on amlodipine or immunosuppressant metabolism while nisoldipine clearance was decreased (Wadhawan et al., 2000; Preston et al., 2001; Marques et al., 2002; Akhlaghi et al., 2012). The underlying mechanisms are associated with systemic inflammation and inflammatory

TABLE 6 | Impact of lung diseases on CYP activities.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
COPD exacerbation	clozapine (CYP1A2)	52-year-old woman	- symptoms of clozapine toxicity, - serum levels = 1400 ng/ml (References = 350–700 ng/ml)	Luong et al. (2016), Case reports
Chronic obstructive lung (COLD) and pulmonary disease caused by α1-antitrypsin (AAT) deficiency vs. control	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	35 = AAT, 25 = COLD, 31 = control	- clearance was not different in AAT and COLD patients ($p > 0.2$), - clearance significantly higher in healthy volunteers than in patients with COLD (18%, $p < 0.01$)	Bilbao-Meseguer et al. (2018), Case-control study

TABLE 7 | Impact of cardiac diseases on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Congestive heart failure	caffeine (CYP1A2), mephenytoin (2C19), dextromethorphan (2D6), chlorzoxazone (2E1)	16	- IL-6 levels were inversely correlated to CYP1A2 (r = -0.56, $p = 0.0235$) and CYP2C19 (r = -0.63, $p = 0.0094$) activities, - TNF-α was inversely correlated to CYP2C19 ($r = –0.61$, $p = 0.0118$) activity, - no significant relationship between IL-6 and TNF-α with CYP2D6 and 2E1 activities	Pirttiaho et al. (1984), Cohort study

cytokines. Indeed, it is well-established that chronic inflammation is involved in the pathophysiology of diabetes and the more complex condition of metabolic syndrome (Gravel et al., 2019). TNF-α can lead to the development of diabetes by affecting insulin action, and levels of inflammatory cytokines and markers are reported to be increased in diabetes patients (Darakjian et al., 2021). In a multivariate analysis, IFN-γ, IL-1β, IL-6 and TNF-α were associated with CYP activities, depending on the CYP isoenzyme (Gravel et al., 2019). However, type I (T1D) and type II diabetes did not appear to have the same impact on CYP metabolism (Dyer et al., 1994; Korrapati et al., 1995; Lucas et al., 1998; Zysset and Wietholtz, 1988; Matzke et al., 2000; Sotaniemi et al., 2002; Wang et al., 2003). The impact of inflammation may be different partly because of obesity, which is more common in T2D (Wang et al., 2003). Indeed, obese patients had a 40% increase in CYP2E1 activity (Lucas et al., 1998; Wang et al., 2003). CYP2E1 increased activity could also be attributed to hypo-insulinemia, as administration of insulin reverses this induction at the mRNA level (Lucas et al., 1998). Moreover, moderate controlled T1D had comparable CYP2E1 activity to healthy volunteers (Wang et al., 2003). This was confirmed in other studies that showed an unaffected metabolic clearance rate of antipyrine in well-controlled (by insulin) T1D (Zysset and Wietholtz, 1988; Sotaniemi et al., 2002). This could also be explained by insulin supplementation and the subsequent correction of ketones that leads to a return to baseline level for CYP2E1 expression (Wang et al., 2003). Indeed, ketones have been shown to be an important modulator of CYP2E1 by enhancing its protein expression and mRNA level (Wang et al., 2003). This has been confirmed with CYP1A2, where fluctuations in growth hormone levels, hyperketonemia and variation in glucose metabolic steady state and HbA1C levels may contribute to these changes (Bechtel et al., 1988; Korrapati et al., 1995; Matzke et al., 2000). The difference in classification criteria for T1D and type 2 diabetes may explain the inconsistent findings (Matzke et al., 2000). Further studies to discriminate between these two entities are needed (Zysset and Wietholtz, 1988).

Overall, CYP3A, 2C19 and 2B6 activity appear to be downregulated while CYP1A2 activity was increased and CYP2D6 activity was unchanged in diabetic patients (Bechtel et al., 1988; Urry et al., 2016; Gravel et al., 2019). Conflicting results remain regarding CYP2C9 and CYP2E1 (Ueda et al., 1963; Adithan et al., 1988; Lucas et al., 1998; Gravel et al., 2019).

Auto-Immune Diseases

Few studies observed the impact of auto-immune disease on CYP activities, such as psoriasis, systemic lupus erythematosus (SLE), Behçet's disease, rheumatoid arthritis (RA), Crohn's disease and celiac disease (**Table 10**). In contrast to what has been observed for CYP2D6 in other inflammatory states, two studies observed CYP2D6 downregulation in patient with SLE (Idle et al., 1978; Baer et al., 1986). However, these studies have some limitations, such as the presence of concomitant medications inhibiting the metabolism of CYP2D6 and the absence of adequate randomization (Baer et al., 1986). Even though RA is one of the most prevalent chronic inflammatory disease, only two case-control studies were found in the literature studying the impact of

TABLE 8 | Impact of critically ill patients on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Septicaemia with shock and respiratory failure and multiple organ failure (two or more organ dysfunction)	theophylline (CYP1A2) and ethylene-diamine (CYP3A)	6	- 10–66% reduction of theophylline clearance as compared to healthy volunteers. Half-life was 18.8 h compared to a normal value of 6 h, - 54% reduction of ethylenediamine clearance and half-life was 2.3 h, which is 5 times the normal value of 0.55 h	Zysset and Wietholtz (1988), Cohort study
Critically ill patients (ICU) with sepsis	atorvastatin (CYP3A)	12 = ICU with sepsis	- 18-fold higher Cmax ($p < 0.001$) and 15-fold higher AUC ($p < 0,01$)	Gravel et al. (2019)
vs control		5 = healthy volunteers		Case-control study
Critically ill patients	midazolam (CYP3A)	6	- CYP3A downregulation is proportional to the severity of the patient's illness and reversible, - normal values from other studies ADDIN ZOTERO_ITEM CSL_CITATION {"citationID": "a2lr6jrcbos","properties":{"formattedCitation": "(139)","plainCitation":"(139)","noteIndex": 0},"citationItems":[{"id":10589,"uris":["http://zotero.org/users/2161612/items/8UL6EWVY"],"uri":["http://zotero.org/users/2161612/items/8UL6EWVY"],"itemData":{"id": 10589,"type":"article-journal","container-title": "The Journal of Pharmacy and Pharmacology","DOI":"10.1111/j.2042-7158.1983.tb02960.x","ISSN":"0022-3573","issue":"6","journalAbbreviation":"J. Pharm. Pharmacol.","language":"eng","note":"PMID: 6135777","page":"378-382","source": "PubMed","title":"Comparative plasma pharmacokinetics of theophylline and ethylenediamine after the administration of aminophylline to man","volume":"35","author": [{"family":"Cotgreave","given":"I. A."},{"family": "Caldwell","given":"J."}],"issued":{"date-parts": [["1983",6]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} (139)	Preston et al. (2001), Case-control study
Multiply injured patients vs. healthy volunteers	mephenytoin (CYP2C19), chlorzoxazone (CYP2E1), dapsone (multiple CYP) and flurbiprofen (CYP2C9)	23 = multiple injured patients, 90 = control	- CYP2C19 and 2E1 activity significantly reduced in trauma patients as compared to healthy volunteers, - CYP2C9 and multiple CYP activities (dapsone) higher after injury as compared to healthy volunteers, - CYP2C19 and 2E1 activities correlated with MODS and MOF scores	Marques et al. (2002), Case-control study
Critically ill patients	clopidogrel (bioactivated by CYP2C19), pantoprazole (CYP2C19)	43 = clopidogrel, 16 = pantoprazole	- median ratio of clopidogrel active metabolite to clopidogrel concentration was 0.6 and this ratio was 48-fold higher ($p < 0.001$) in healthy volunteers, - 70% of critically ill patients were insufficiently treated with clopidogrel, - 5-fold increased pantoprazole half-life	Akhlaghi et al. (2012), Cohort study

RA on the PK and PD of verapamil and losartan, respectively (Mayo et al., 2000; Daneshtalab et al., 2006; Smolen et al., 2016). Verapamil is metabolized by CYP3A and 1A2 into norverapamil (Tracy et al., 1999). Verapamil and norverapamil metabolism has been shown to be reduced in patients with RA compared to healthy volunteers (Mayo et al., 2000). Verapamil was not more dromotropic or hypotensive in RA patients (Mayo et al., 2000). Inhibition of CYP2C9 was proportional to RA disease severity in another study, but this was not accompanied by reduced clinical response after losartan administration (Daneshtalab et al., 2006). Same results were found in patients with Behcet's disease. Indeed, one study observed downregulation of CYP2C9 in Behcet's patients (Goktaş et al., 2015). However, losartan's MR in nine patients with Behçet's disease taking colchicine were similar to those not taking colchicine (Goktaş et al., 2015). This may be because the drug had been taken for only 2 weeks (Goktaş et al., 2015).

In Crohn's disease, S-verapamil concentration was higher than R-verapamil while the opposite was found in normal conditions and higher plasma levels of propranolol were

TABLE 9 | Impact of diabetes on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Non-insulin dependent (NID) diabetic subjects with fatty liver vs. healthy subjects	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	21 = diabetes, 11 = control	- NID diabetic subjects with fatty liver have lowered hepatic drug metabolizing enzyme capacity as assessed per unit weight of liver tissue compared with healthy subjects ($p < 0.01$), - the relative clearance rate was significantly slower and the hepatic CYPs concentration lower than in non-diabetic controls ($p < 0.01$)	Wadhawan et al. (2000), Case-control study
Diabetes patients with normal liver	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	4 = diabetes, 13 = controls	clearance decrease significantly ($p < 0.005$) between diabetes patients with normal liver compared to controls	Teunissen et al. (1984), Case-control study
Type I and type II diabetes vs. controls	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	30 = diabetes (15 T1D and 15 T2D), 21 = controls (12 for T1D and 9 for T2D)	- half-life was reduced by 44% compared to the controls ($p = 0.002$), whereas the resulting plasma clearance did not differ between controls and type I diabetics (T1D), - Type II diabetics (T2D) showed a 31% increase in plasma half-life ($p = 0.05$) and they had a significant decrease in corresponding clearance ($p = 0.02$)	Darakjian et al. (2021), Case-control study
Type I and type II diabetes vs. controls	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4), caffeine (CYP1A2) and dextromethorphan (CYP2D6)	15 = T1D, 16 = T2D, 16 = controls	- metabolism was significantly higher in T1D patients than in the patients with T2D and in healthy volunteers, - no change in metabolism between T2D and controls, - CYP1A2 activity was 34 and 42% higher in patients with T1D compared with controls and patients with T2D respectively but these changes did not reach the statistical significance ($p = 0.11$), - no change between groups concerning the CYP2D6 phenotype distribution	Matzke et al. (2000), Case-control study
Type II diabetes vs control	caffeine (CYP1A2) bupropion (CYP2B6), tolbutamide (CYP2C9), omeprazole (CYP2C19), dextrometorphan (CYP2D6), chlorzoxazone (CYP2E1) and CYP3A (midazolam)	38 = T2D, 35 = control	CYP2B6, CYP2C19 and CYP3A activities were decreased by about 45% ($p = 0.01$), 46% ($p = 0.001$) and 38% ($p < 0.0001$) respectively in T2D patients and multivariate models showed that IFN-γ and TNF-α, pro-inflammatory cytokines, partly explain these variations, - CYP1A2 and CYP2C9 metabolic activity were increased in T2D patients ($p = 0.008$ and $p = 0.0008$, respectively) at first sight but this is no longer significant when they have been adjusted for age and gender ($p = 0.07$ and $p = 0.05$, respectively), - CYP2D6 and CYP2E1 activities were not affected by diabetic status ($p = 0.75$ and $p = 0.78$, respectively), - phenotypes were extrapolated from genotypes because patients did not take other co-medications and there is no interaction between genotype/phenotype classification and diabetic status	Lucas et al. (1998), Case-control study
Type II diabetes vs. control	caffeine (CYP1A2)	57 = T2D, 146 = control	- metabolic activity of CYP1A2 was significantly increased in T2D patients compared to control ($p = 0.010$), - but when the 19 diabetic patients who are under insulin injection were removed, the difference was no longer significant ($p = 0.121$)	Dyer et al. (1994), Case-control study
Insulin dependent (ID) diabetes patients vs. control	caffeine (CYP1A2) and debrisoquin (CYP2D6)	28 = ID diabetes patients, 22 = healthy volunteers	- no significant differences for CYP2D6 activity and a significant increase in CYP1A2 activity in diabetes patients ($p < 0.0001$)	Wang et al. (2003), Case-control study
T1D and T2D vs. control	caffeine (CYP1A2)	10 = T1D; 8 = controls, 9 = T2D; 9 = controls	the apparent volume of distribution, apparent clearance, half-life, and peak concentrations of caffeine did not differ between both type of diabetes and controls	Sotaniemi et al. (2002), Case-control study
Diabetic patients vs. controls	tolbutamide (CYP2C9)	10 = diabetic patients, 7 = control	half-life in diabetic patients revealed no significant difference with normal subjects ADDIN ZOTERO_ITEM CSL_CITATION {"citationID": "yU0UBeFO","properties":{"formattedCitation":"(115)","plainCitation": "(115)","noteIndex":0},"citationItems":[{"id":10235,"uris":["http://zotero.org/users/2161612/items/ELGVD5C6"],"uri":["http://zotero.org/users/2161612/items/ELGVD5C6"],"itemData":{"id": 10235,"type":"article-journal","container-title":"Diabetes","DOI": "10.2337/diab.12.5.414","ISSN":"0012-1797","journalAbbreviation": "Diabetes","language":"eng","note":"PMID: 14067739","page":"414-419","source":"PubMed","title":"DISAPPEARANCE RATE OF TOLBUTAMIDE IN NORMAL SUBJECTS AND IN DIABETES MELLITUS, LIVER CIRRHOSIS, AND RENAL DISEASE","volume": "12","author":[{"family":"Ueda","given":"H."},{"family": "Sakurai","given":"T."},{"family":"Ota","given":"M."},{"family": "Nakajima","given":"A."},{"family":"Kamii","given":"K."},{"family": "Maezawa","given":"H."}],"issued":{"date-parts": [["1963",10]]}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"} (115)	Molanaei et al. (2018), Case-control study
Diabetes mellitus vs. controls	paracetamol (CYP2E1)	19 = diabetes mellitus, 10 = healthy volunteers	- half-life was significantly increased ($p < 0.001$) with a corresponding decrease in clearance ($p < 0.001$) when compared with healthy volunteers, - clearance in patients with T2D was significantly decreased compared to T1D patients ($p < 0.01$) but it was not the case for its half-life, - the distribution volume was increased in patients with T1D compared to patients with T2D ($p > 0.05$)	Korrapati et al. (1995), Case-control study
Type II diabetes vs control	amlodipine (CYP3A)	18 = T2D, 20 = control	-no significant difference in AUC in hypertensive patients with and without T2D	Bechtel et al. (1988), Case-control study
Type II diabetes vs control	nisoldipine (CYP3A) and lidocaine (CYP3A)	17 = T2D, 10 = control	- the apparent clearances of both nisoldipine enantiomers in the hypertensive patients with T2D are significantly lower than in hypertensive control patients ($p < 0.05$), - higher ratio of plasma lidocaine/MEGX concentration for diabetic group than in control group ($p < 0.05$), - means that CYP3A4 activities were decreased in the diabetic groups, - significant correlations were found ($p < 0.05$) between the MR of lidocaine and the apparent clearance of nisoldipine enantiomers obtained for both groups	Urry et al. (2016), Case-control study
Diabetes vs. control	CyA (CYP3A)	7 = diabetes, 10 = control	-No difference was found in daily dose needed between both groups ($p = 0.55$) but metabolite-parent concentration ratios for all metabolites except one (AM4N, $p = 0.93$) were significantly lower in diabetic patients ($0.0001 < p$-value < 0.04)	Idle et al. (1978), Case-control study

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TABLE 9 | (*Continued*) Impact of diabetes on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Diabetes vs. control	CyA (CYP3A)	8 = diabetes, 9 = control	AUC adjusted with dosage was significantly lower in diabetic group (p = 0.03) ADDIN ZOTERO_ITEM CSL_CITATION {"citationID": "atdeho0nge","properties":{"formattedCitation": "(194)","plainCitation":"(194)","dontUpdate":true,"noteIndex": 0},"citationItems":[{"id":11162,"uris":["http://zotero.org/users/2161612/items/KYQT5CPG"],"uri":["http://zotero.org/users/2161612/items/KYQT5CPG"],"itemData":{"id":11162,"type":"article-journal","abstract":"BACKGROUND AND OBJECTIVES: Long-term diabetes mellitus may affect the absorption, distribution and metabolism of immunosuppressive agents used after organ transplantation. The aims of this study were to characterize ciclosporin pharmacokinetics in blood and plasma and to compare the ciclosporin unbound concentration and the blood : plasma concentration (B : P) ratio in diabetic kidney transplant recipients.\nPATIENTS AND METHODS: Ciclosporin 12-hour steady-state pharmacokinetics were studied in eight diabetic and nine nondiabetic patients. Ciclosporin concentrations in whole blood and in plasma were measured using liquid chromatography-tandem mass spectrometry, and the ciclosporin fraction unbound (f(u)) was determined by an equilibrium dialysis method utilizing [(3)H]ciclosporin as a tracer. Oral absorption of paracetamol (acetaminophen) was used as a marker for gastric emptying.\nRESULTS: In diabetic patients, the time to the peak blood ciclosporin concentration at steady state (t(max)(,ss)) was prolonged (128 minutes vs 93 minutes in nondiabetic patients, p < 0.01) and, on average, the paracetamol t(max) was prolonged by 30 minutes. The whole-blood dose-normalized area under the concentration-time curve from 0 to 12 hours (AUC(12)) was marginally lower in diabetic patients (p = 0.09) and the plasma AUC(12) was significantly lower (p = 0.03). The ciclosporin f(u) was numerically higher in diabetic patients (1.20 +/- 0.65% vs 0.72 +/- 0.28% in nondiabetic patients, p = 0.066); however, the unbound concentration values were essentially similar in the two groups (0.58 +/- 0.76 microg/L in diabetic patients and 0.52 +/- 0.48 microg/L in nondiabetic patients; p = 0.59). No difference was observed in the ciclosporin B : P ratio between the two groups.\nCONCLUSION: This study indicates that diabetes delays ciclosporin absorption, reduces ciclosporin exposure and increases the ciclosporin f(u) but not the pharmacologically active unbound concentration.","container-title":"Clinical Pharmacokinetics","DOI": "10.2165/00003088-200847110-00004","ISSN":"0312-5963","issue":"11","journalAbbreviation":"Clin Pharmacokinet","language":"eng","note":"PMID: 18840028","page": "733-742","source":"PubMed","title":"Blood and plasma pharmacokinetics of ciclosporin in diabetic kidney transplant recipients","volume":"47","author":[{"family":"Mendonza","given": "Anisha E."},{"family":"Gohh","given":"Reginald Y."},{"family": "Akhlaghi","given":"Fatemeh"}],"issued":{"date-parts": [["2008"]]}}}],"schema":"https://github.com/citation-style-language/schema/raw/master/csl-citation.json"}	Baer et al. (1986), Case-control study
Diabetes vs. control	CyA (CYP3A)	36 = diabetes, 67 = control	- no difference was found concerning dose and through levels	Smolen et al. (2016), Case-control study
Type I and II diabetes vs control	chlorzoxazone (CYP2E1)	7 = T1D, 15 = T2D, 42 = controls	- no difference was found concerning CYP2E1 activity between groups	Mayo et al. (2000), Case-control study
Type II diabetes vs. control	quinine (CYP3A)	12 = T2D, 10 = controls	- PK parameters were comparable in the two groups (p > 0.02)	Daneshtalab et al. (2006), Case control study
Type I and II diabetes vs control	chlorzoxazone (CYP2E1)	14 = T1D, 8 = T2D, 10 = controls	- 2-fold increase in the oral clearance (p < 0.05) in T2D patients compared with T1D and controls, - no difference in oral clearance between T1D and controls	Tracy et al. (1999), Case-control study
Type I and type II diabetes	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	139 = T1D (120 = controls), 99 = T2D (70 = controls)	- clearance decreased in T2D patients as compared to controls, - metabolism is rapid in T1D patients	Goktaş et al. (2015), Case-control study
Type 1 diabetes vs controls	theophylline (CYP1A2)	8 = T1D, 8 = controls	- mean plasma clearance and elimination half-life did not differ significantly between the 2 groups	Sanaee et al. (2011), Case-control study
Gestational diabetes vs. pregnant women	metoprolol (CYP2D6)	10 = diabetes, 13 = control	- PK of the metoprolol isomers in the pregnant women and in gestational diabetes groups did not differ significantly, except for the R-metoprolol half-life (p < 0.05)	Schneider et al. (1976), Case-control study
Gestational diabetes vs. pregnant women	lidocaine (CYP3A)	6 = diabetes, 10 = control	- the ratios of lidocaine and its metabolite MEGX concentrations (lidocaine/MEGX ratio) at 15 and 30 min were significantly higher in the pregnant women with gestational diabetes mellitus compared to the normal pregnant women (58.34 vs. 23.21 at 15 min and 37.52 vs. 15.80 at 30 in, p < 0.05)	Lebwohl et al. (2018), Case-control study

found in Crohn's with reduced metabolic activities of CYP1A2, 2D6 and 2C19 (Schneider et al., 1976; Sanaee et al., 2011). Furthermore, there were no difference between healthy controls and Crohn's disease patients in remission, implying that CYP downregulation is proportional to disease severity and that recovery resulted in a return to baseline metabolic activity (Sanaee et al., 2011). Norverapamil goes through the same process and it is expected that the enantiomers ratio of norverapamil to verapamil remains unchanged (Sanaee et al., 2011).

TABLE 10 | Impact of autoimmune diseases on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Psoriasis vs healthy volunteers	venlafaxine (CYP2D6)	13 = psoriasis, 11 = control	- PK of the enantiomers and of its metabolites were not altered as compared to control	Lang et al. (1996) Case-control study
Systemic lupus erythematosus (SLE) vs. healthy controls	debrisoquin (CYP2D6)	42 = SLE, 147 = control	- In patients with SLE, there is an inhibition in the metabolism of debrisoquin compared to controls because there is significantly more PM patients in patients group ($p < 0.04$)	Tidball (2005), Case-control study
Proctitis vs healthy volunteers	/	11	- patients who suffered from proctitis showed a lower CYP2E1 and 3A4 gene expression in rectal mucosa with severe inflammation compared to normal mucosa ($p < 0.05$), - no significant difference for CYP3A5 ($p = 0.08$)	Baigrie et al. (1992), Cohort study
Behçet's disease vs. healthy subjects	losartan (CYP2C9)	52 = Behçet's disease, 73 = control	- the MR (losartan/E-3174) significantly increase ($p = 0.002$) compare to controls already included who genetic variants and losartan oxidation were already known, - in patients with the wild type CYP2C9 genotype (*1/*1), the MR significantly increased in patients with Behçet's disease compared to controls ($p = 0.006$) but there is no significant differences found for other CYP2C9 genotype	Bergin et al. (2011), Case-control study
Rheumatoid arthritis (RA) vs. healthy volunteers	verapamil (CYP3A4, 1A2, 2C8, 2C9 and 2C18)	8 = RA, 8 = controls	- less metabolized and bound to protein in patients with RA compared to controls, - AUC of verapamil and norverapamil were significantly higher in patients with RA as compared to controls thus, there is no changes in metabolite to parent drug ratio	Haas et al. (2003), Case-control study
Active and controlled rheumatoid arthritis vs healthy subjects	losartan (CYP2C9)	14 = active RA, 12 = controlled RA, 12 = controls	- PK not significantly altered but AUC of its pharmacologically active metabolite was significantly decreased , - MR exhibited a significant correlation with disease severity ($r = -0,35$, $p < 0.05$)	Lenoir et al. (2020), Case-control study
Rheumatoid arthritis	/	49 = RA	- cytokines such as TNF-α, IL-1β and IL-17 increase the CYP7B activity in synovial tissue, - TGF-β down-regulate the CYP7B activity and it results in enhanced formation of 7α-OH-DHEA in the arthritic joint, which may contribute to the maintenance of the inflammation and, thus, the chronicity of the inflammation response	Mostowik et al. (2015), Cohort study
active Crohn's disease (CD), Crohn's disease in remission and healthy subjects	verapamil (CYP3A4, 1A2, 2C8, 2C9 and 2C18)	22 = CD remission, 14 = CD active, 9 = controls	- plasma S-verapamil concentration in patients with active CD was significantly higher than in both healthy controls and patients in CD remission ($p < 0.001$) but not between healthy controls and Crohn's disease remission, - same tendency was seen for R-verapamil but there is no statistical significance, - as in RA patients, the ratio AUC of both S and R norverapamil over their corresponding verapamil enantiomers were not significantly different among the 3 groups of subjects, - there was no higher PD response in patients due to higher verapamil level	Bernlochner et al. (2010), Case-control study
Crohn's disease vs. control	propranolol (CYP2D6)	10 = Crohn's disease, 12 = healthy subjects	- levels were significantly higher in the 10 patients with Crohn's disease than those of the controls ($p < 0.05$)	Harvey and Morgan (2014), Case-control study
Celiac disease	/	9	- reduction in the intestinal content of CYP3A in patients with celiac disease before treatment with a gluten-free diet and increase in intestinal CYP3A protein after the diet	Kacevska et al. (2008), Cohort study

Celiac disease is an autoimmune disease that is triggered by an immune response to gluten and may result in increased morbidity or mortality (Lebwohl et al., 2018). The reduction in intestinal CYP3A content during celiac disease and its increase after a gluten-free diet indicate that local inflammation reduced CYP3A activity but that it returns to baseline with disease improvement (Lang et al., 1996).

TABLE 11 | Impact of surgery on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Surgery	clozapine (CYP1A2)	49-year-old man	- clozapine and norclozapine levels were 1130 ng/dl and 297 ng/dl, respectively (ratio 3.8: 1), 4 days after surgery. On day 2, dosage was reduced due to persistent sedation	Luong et al. (2016), Case reports
(a) Surgery abdominal aortic bypass graft colon resection peripheral vascular bypass graft	/ carbon-14 [^{14}C] ERMBT (CYP3A)	16 (5 a, 6 b and 5 c)	- ERMBT results significantly declined in all groups compared with before surgery - a trend toward difference in ERMBT results between surgery but didn't reach statistical significance (p = 0.06) - the nadir ERMBT result was significantly and negatively correlated (r = -0.541, p = 0.03) with peak IL-6 concentration - test results were significantly different if patients IL-6 peak concentration was IL-6 > 100 pg/ml or <100 pg/ml (35.5 vs. 74.7%, p < 0.001)	Chen et al. (1994) Cohort study
Hip surgery	caffeine (CYP1A2), bupropion (CYP2B6), flurbiprofen (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6) and midazolam (CYP3A)	30	- CYP2C19 and 3A MR decreased by 57% (p = 0.0002) and 61% ($p \leq 0.0001$) respectively with the nadir at D3, - CYP1A2 MR decreased by 53% ($p \leq 0.0001$) with the nadir at D1, - CYP2B6 and 2C9 MR increased by 120% (p < 0.0001) and 79% (p = 0.0018), respectively and peaked at d1, - No change in CYP2D6 MR	Rivory et al. (2002), Cohort study
percutaneous coronary intervention	clopidogrel (bioactivated by CYP2C19)	50	- prolonged post-angioplasty increase is associated with lower platelets' response to clopidogrel	Alexandre et al. (2007), Cohort study
percutaneous coronary intervention	clopidogrel (bioactivated by CYP2C19)	1′223	- platelet aggregation was significantly higher in patients with elevated CRP levels compared to patients with normal CRP levels (p < 0.001)	Charles et al. (2006), Cohort study

Surgery

The impact of surgery on concomitant treatment and analgesia management has been assessed in several studies (**Table 11**). Surgery is associated with an inflammatory response due to muscle or tissue injury to induce repair, regeneration and growth and so inflammatory markers increase after surgery, but not equally (Tidball, 2005; Stavropoulou et al., 2018). IL-1β was only detected during the early perioperative period and for a very short time (Baigrie et al., 1992). IL-6 plasma level peaked 4–48 h after surgery and declined drastically by 48–72 h in all patients without any postoperative complication (Baigrie et al., 1992). CRP level rose more slowly postoperatively compared with the cytokine levels (IL-6, TNF-α and IL-1β) (Bergin et al., 2011). Acute inflammation after elective surgery was associated with a significant decrease in CYP3A metabolic activity (Haas et al., 2003). A recent study with a cocktail approach has concluded that there is an isoform specific impact of inflammation on CYP activities (Lenoir et al., 2020). Indeed, this study showed that CYP1A2, CYP2C19 and CYP3A activities decreased significantly by 53, 57 and 61%, whereas CYP2B6 and CYP2C9 activities increased significantly by 120 and 79% (Lenoir et al., 2020). However, surgery did not significantly impact CYP2D6 activity (Lenoir et al., 2020). These findings were confirmed by a case report that showed a toxic increase in clozapine levels 4 days after surgery and by authors who further showed that clopidogrel efficacy was reduced in patients undergoing percutaneous coronary intervention, because clopidogrel must be bioactivated by CYP2C19 to be effective (Bernlochner et al., 2010; Leung et al., 2014; Mostowik et al., 2015).

Cancer

Inflammation is linked to all stages of cancer (risk of development, initiation, invasion, metastasis and mortality) as highlighted in **Table 12** (Harvey and Morgan, 2014). Certain immune-mediated diseases have been associated with cancer such as inflammatory bowel disease (IBD), chronic infection by Helicobacter pylori and chronic psoriasis associated with an increased risk of colorectal, gastric and skin cancer, respectively (Harvey and Morgan, 2014). The first pro-cancer immune signals are via tumor cells that successively produce cytokines and act to increase transcription factors, induce epigenetic changes and initiate angiogenesis (Harvey and Morgan, 2014). Cytokines are involved from neoplastic transformation of cells to tumor progression and metastasis, and are thus involved in several cellular events leading to cancer (Kacevska et al., 2008). These signals and others induced to respond to cancer are opposed by antigen-presentating cell-mediated anticancer immune responses (Harvey and Morgan, 2014). Moreover, the greater the antitumoral response is, the more the cancer outcome is improved whereas some T-cells subsets are associated with tumor promotion (Harvey and Morgan, 2014). Some cytokines have tumor-promoting, antitumor effects or both (Kacevska et al., 2008). Some cytokines could be produced by the tumor itself

TABLE 12 | Impact of cancer on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Liver metastasis before cytostatic treatment vs. healthy controls	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A4)	12 = liver metastasis, 12 = controls	- no significant difference between patients with liver metastases before cytostatic treatment and controls	Williams et al. (2000), Case-control study
Bone marrow transplantation for haematological malignancies (radiation and chemotherapy)	CyA (CYP3A)	6	- concentration peak value occurred 15.8 days after bone marrow transplantation and it's corresponded to a 3- or 4-fold increase relative to the steady state day ($p > 0.015$), - CyA concentration peak and IL-6 peak levels are interdependent because there was a correlation between these two parameters ($r = 0.794$, $p = 0.03$)	Burns et al. (2014), Cohort study
Cancer	ERMBT (CYP3A)	40	- patients with CRP >10 mg/L had an average 30% reduction in CYP3A4 metabolic activity ($p = 0.0062$), - 1/Tmax values were negatively correlated with both CRP ($r = –0.64$, $p < 0.00001$) and α-glycoprotein ($r = -0.45$, $p < 0.005$), - 3 patients were treated by a CYP3A4 inhibitor while 4 patients were on long-term treatment with dexamethasone (inducer) but correlation with CRP remained significant ($r = –0.55$, $p = 0.002$) after removal of these patients	Helsby et al. (2008), Cohort study
Advanced cancer patients with normal liver function	midazolam and docetaxel (CYP3A)	56	- high midazolam concentration and free docetaxel AUC were associated with sever neutropenia (and conversion to febrile neutropenia), - high midazolam concentration was correlated with elevated ferritin level ($r = 0.32$, $p = 0.02$) (indicator of an inflammatory state), - according to authors, inflammation favors a reduction in CYP3A activity and thus, could lead to an overexposure to its substrates	Yasu et al. (2017), Cohort study
Advanced cancer patients who were suitable for palliative chemotherapy	docetaxel (CYP3A)	68	- occurrence of grade 3/4 non-haematological toxicities were not associated with high docetaxel exposure but with baseline concentrations of AAGP ($p = 0.03$) and CRP ($p = 0.05$), - results from correlation analysis between inflammation markers and docetaxel clearance were not given, as the results from EBT	Mafuru et al. (2019), Non-randomized clinical trial
Cancer patients vs healthy subjects	omeprazole (CYP2C19)	16 = cancer, 77 = controls	CYP2C19 activity differed significantly ($p < 0.0001$) in the EM cancer patients compared of the References population with EM genotype	Piscitelli et al. (1998), Case-control study
Multiple myeloma	proguanil (CYP2C19)	25	- significant discordance between the CYP2C19 activity predicted by genotype and the measured phenotype ($p < 0.0001$), - no significant difference in CRP and IL-6 concentrations between discordant and concordant subjects ($p = 0.072$ and $p = 0.694$, respectively)	Elkahwaji et al. (1999), Cohort study
Advanced cancer	omeprazole (CYP2C19)	31	- comparison of the predicted phenotype from genotype and the measured MR of CYP2C19 found a statistically discordance ($p < 0.0005$), - of the 30 cancer patients with genotypic EM status, 11 were CYP2C19 PM, - no significant correlation between the levels of any individual cytokine (CRP, IL-1β, Il-1α, IL-6, TNF-α, TGF-β and CRP) and CYP2C19 metabolic activity	Israel et al. (1993), Cohort study
Hematopoietic cell transplantation	voriconazole (CYP3A4 and CYP2C19)	67	- CRP levels were significantly correlated ($r = 0.22$, $p < 0.001$), - higher voriconazole trough concentration >1.0 ug/ml was observed in higher CRP level >4 mg/dl	Jonkman et al. (1989), Cohort study

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TABLE 12 | (*Continued*) Impact of cancer on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Hematologic patients	voriconazole (CYP3A4 and CYP2C19)	113	- concentration was significantly correlated with IL-18 in acute myeloid ($r = 0.456$, $p < 0.0001$), acute lymphoblastic ($r = 0.317$, $p = 0.019$), and chronic myeloid leukaemia ($r = 0.737$, $p = 0.04$), - concentration and TGF-β1 were correlated ($r = 0.436$, $p < 0.001$) in acute myeloid leukaemia patients only, - according to authors, IL-6 level could partially predict the voriconazole trough concentration because these two factors were weakly inversely correlated in hematologic patients regardless of underlying disease	Williams et al. (1987), Cohort study
Hepatocellular carcinoma	phenacetin (CYP1A2)	148 = carcinoma, 82 = controls	- clearance did not significantly differ between the healthy participants and patients with hepatocellular carcinoma	Schoergenhofer et al. (2018), Case-control study

(Kacevska et al., 2008). Inflammation has therefore a pivotal role in cancer and the proliferation of malignant cells by a dynamic equilibrium in the tumor environment (Harvey and Morgan, 2014). Cytokines present in the tumor environment are also launched in the systemic circulation and have general effects on the function of distant organs such as the liver (Kacevska et al., 2008). Inflammatory markers levels are dependent on tumor types, but high level of CRP, IL-6, IL-1β have been associated with poor prognosis (Kacevska et al., 2008). Some results suggest that high IL-6 is associated with decreased CYP3A metabolic activity but can also nonspecifically downregulate CYP-dependent drug metabolism (Chen et al., 1994). CRP and α-glycoprotein were also negatively correlated with CYP3A activity and cancer patients with significant acute-phase response may have reduced CYP3A drug metabolism, which may have implications for the safety and efficacy of chemotherapy (Rivory et al., 2002; Charles et al., 2006; Alexandre et al., 2007). Inflammatory status and lymphocyte count should thus be included in the evaluation of the benefit/risk ratio before the initiation of a cytotoxic chemotherapy (Alexandre et al., 2007). Concerning CYP2C19, studies showed that CYP2C19 activity was not solely predicted by the genotype in cancer patients (Williams et al., 2000; Helsby et al., 2008; Burns et al., 2014). Indeed, CYP2C19 activity was reduced in cancer patients, with a discordance between the measured phenotype and the predicted phenotype from the genotype. However, no significant correlation was found between CYP2C19 activity and the levels of cytokine, whereas this was the case for voriconazole through concentration (Helsby et al., 2008; Burns et al., 2014; Yasu et al., 2017; Mafuru et al., 2019). The mechanism behind the decrease of CYP2C19 activity observed in cancer patients may be related to the inflammatory response even though it remains debated (Helsby et al., 2008; Burns et al., 2014; Yasu et al., 2017; Mafuru et al., 2019). Other authors showed that cancer has no impact on CYP1A2 metabolic activity as compared to liver disease or infection (Wang et al., 2010).

Therapies With Immunomodulator, anti-TNF-α and -Mabs

As biological therapies aim to decrease the underlying inflammation of the disease, interleukins (IL) injections are expected to have an impact on CYP activity, as underlined in **Table 13**. As an example, IL-2 doses of $9–12 \times 10^6$ units daily may downregulate CYP activities in patients with HIV infection and cancer in whom this treatment is administered to boost the immune system (Piscitelli et al., 1998; Elkahwaji et al., 1999). Conflicting results exist regarding IFN administration, with a discrepancy between acute and chronic treatment (Williams and Farrell, 1986; Williams et al., 1987; Jonkman et al., 1989; Israel et al., 1993; Hellman et al., 2003; Sulkowski et al., 2005; Gupta et al., 2007; Furlanut et al., 2010; Brennan et al., 2013). However, case reports and more specific studies assessing CYP metabolic activity lean toward CYP downregulation and care must be taken to avoid interactions and ADRs (Craig et al., 1993; Adachi et al., 1995; Serratrice et al., 1998; Hassan et al., 1999; Becquemont et al., 2002). The level of anticoagulation should be closely monitored when interferon is given together with warfarin, as it appears that CYP are downregulated (Adachi et al., 1995; Serratrice et al., 1998). Additionally, the timing of IFN-α administration relative to concomitant chemotherapy should be considered to avoid a decrease in CYP3A4 and 2B6 activity and thus to achieve better efficacy (Hassan et al., 1999). For example, interferon-α-2b inhibits CYP1A2, 2D6 and 2C19 and these findings pose new challenges for patients on these therapies with respect to PK interaction with concomitant drugs commonly used (Islam et al., 2002). Further studies are needed to measure the impact of IFN and new cytokine therapies coming on the market on CYP activities. Cytokines act on CYP in an isoform-specific manner, and it is likely that IFN or IL modulate different CYP while they have no impact on others. Moreover, it is crucial to understand whether the modulation of CYP activity is due to this kind of therapy, to the underlying disease which may be inflammatory,

TABLE 13 | Impact of therapies with immunomodulator on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Treatment with IL-2	indinavir (CYP3A)	8 = HIV seropositive patients (observational), 9 = HIV seropositive patients (prospective)	- in the HIV seropositive-patients, the mean concentration of indinavir was significantly increased on day 5 of IL-2 therapy, - in the nine HIV seropositive-patients, the mean indinavir AUC increased significantly by 88% between day 1 and day 5 of IL-2, - mean IL-6 concentrations during IL-2 therapy increased between day1 and day5 from 4- to 86-fold, - study combines observations made in one observational and one prospective (as part of a phase II trial) studies	Williams and Farrell (1986), Cohort study and non-randomized
Treatment with IL-2	/	5 = 3 or $6x10^6/m^2$ units of IL-2, 6 = 9 or $12x10^6/m^2$ units of IL-2, 7 = 0 units of IL-2, Patients with cancer	- in non-tumorous liver fragment removed with the tumor in each patients, authors observed that CYPs proteins (CYP1A2, 2C, 2E1 and 3A), monooxygenase activities of methoxyresorufin and erythromycin and total CYPs were significantly decreased only in the group of patients treated with highest doses of IL-2, compared to control	Furlanut et al. (2010), Randomized clinical trial
Treatment with IFN-α	theophylline (CYP1A2), antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A), hexobarbitone (CYP2C19)	7	- no significant difference in TNF-α, IL-1β, IL-6 and CRP activities after both acute (initiation) and chronic (2 weeks) IFN-α injections compared to baseline, except for TNF-α activity that significantly decreased after chronic therapy, - significant effects of acute IFN-α administration on the oral clearance of the three probe drugs were not detected, - chronic exposure to IFN-α was associated with a significant lowering clearance (33% compared with baseline, $p < 0.05$) but no significant correlations were observed between the changes in theophylline clearance and changes in serum cytokines or acute phase proteins, - chronic IFN-α therapy decreased antipyrine oral clearances by 20% but this did not reach statistical significance and it appeared to have no effect on the metabolism of racemic hexobarbitone	Sulkowski et al. (2005), Cohort study
Treatment with IFN-α	aminophylline (CYP1A2)	12 = healthy volunteers	- after IFN-α treatment in healthy volunteers, there were significant 10–15% increases ($p < 0.05$) in the terminal elimination half-life and AUC of aminophylline administered intravenously, - the total clearance showed a comparable decrease ($p < 0.05$)	Gupta et al. (2007), Non-randomized

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TABLE 13 | (*Continued*) Impact of therapies with immunomodulator on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Treatment with IFN	theophylline (CYP1A2)	5 = hepatitis B, 4 = healthy subjects	- a reduction of theophylline elimination was observed in 8 subjects (remaining subject was a healthy control) and was ranged from 33 to 81%, compared to initial theophylline clearance study, - no impact of the hepatitis on these results because there was no clinical or biochemical change in the liver disease, - a second theophylline clearance study was done 4 weeks after the interferon's injection and it was back to initial value	Hellman et al. (2003), Non-randomized
Treatment with IFN-α	antipyrine (CYP1A2, 2B6, 2C8, 2C9, 2C18 and 3A)	5 = hepatitis B, 4 = healthy subjects	recombinant leukocyte α-interferon reduced the antipyrine clearance by 16% ($p < 0.01$) and the half-life increased but this was not significant	Brennan et al. (2013), Non-randomized
Treatment with IFN-α	warfarin (CYP2C9)	52 year-old-woman	- her prothrombin time increased to 16.7–20.4 s with a rise in serum warfarin concentration from <0.8 μg/ml to 5.2 μg/ml 10 days after the onset of IFN-α therapy, - dose was reduced and both anticoagulation and serum warfarin concentration had returned to nearly baseline values	Adachi et al. (1995), Case report
Treatment with IFN-α-2b	acenocoumarol (CYP2C9)	46-year-old-woman	- at the beginning of the treatment, anticoagulant effect of acenocoumarol increased (thrombotest decreased from 30–35–19%), - when IFN-α-2b dosage decreased because of infection remission, anticoagulant effect decreased (thrombotest increased from 25–40–69%), - it led to the adaptation of the dosage of acenocoumarol to be on thrombotest range, - anticoagulation level decreased from 1 day after injection to 2 or 3 days later	Serratrice et al. (1998), Case report
Treatment with IFN-α-2b	ERMBT (CYP3A)	6 = chronic hepatitis C, 4 = healthy controls	- ERMBT before and 20–26 h after IFN-α-2b injection, - IFN-α-2b induced a small significant decrease in ERMBT ($p < 0.05$), - at baseline CYP3A4 activity was lower in patients with hepatitis C but the effect of IFN appeared to be not different	Craig et al. (1993), Non-randomized
Treatment with IFN-α	cyclophosphamide (CP) (CYP2B6 active metabolite and CYP2C9, 2C19 and 3A substrate)	10	- administration of IFN-α before CP caused a 63% decrease in its clearance ($p = 0.004$) compared to an administration of IFN-α 24 h after CP, - there is a 45% decrease in exposure of CP active metabolite's (4-OHCP) when IFN-α was administered before CP, expressed as AUC ($p = 0.002$), compared with that observed when IFN-α was administered 24H after CP, - this resulting in a greater decrease in leukocyte count (45%, $p = 0.02$) when IFN-α was given after CP in the 10 patients with multiple myeloma	Hassan et al. (1999), RCT

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TABLE 13 | (*Continued*) Impact of therapies with immunomodulator on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Treatment with IFN-α-ribavirin	dextromethorphan (CYP3A4 and CYP2D6, by measuring different metabolite) and caffeine (CYP1A2)	14	- mean CYP3A4 activity increased from 0.18 ± 0.06 in patient with HCV before beginning of IFN-α-ribavirin treatment to 0.48 ± 0.53 1 month after but this did not reach statistical significance (p = 0.19) - a similar evolution of CYP2D6 activity could be observed during the first month of treatment (148 ± 0139 to 421 ± 641, p = 0.08), - CYP1A2 activity did not changed, going from 0.39 ± 0.11 before treatment to 0.32 ± 0.13 after 1 month, - pretreatment CYP3A4 and CYP2D6 activities of the 14 studied patients were significantly lower than those observed in 35 healthy volunteers (p = 0.0006 and p = 0.0008 respectively), - after 1 month of antiviral treatment, CYP3A4 and 2D6 did not differ significantly from those in healthy volunteers, probably because of the recovery of HCV patients	Becquemont et al. (2002), Non-randomized
Treatment with IFN-α-2b	caffeine (CYP1A2), mephenytoin (CYP2C19), debrisoquin (CYP2D6), chlorzoxazone (CYP2E1) and dapsone (CYP2C8 and CYP2C9)	17 = patients with high-risk resected melanoma	- IFN-α-2b inhibits immediately the activity of CYP1A2 (p = 0.001) and 2D6 (p < 0.001) in patients with high-risk resected melanoma, - inhibition of CYP2C19 was detected for the first time at day 26 (p < 0.001) after the initiation of high-dose IFNα-2b treatment (20 MU/m2/day i.v for 5 days/weeks during 4 weeks and 10 U/m2/day s.c for 3 days/week x 48 weeks), - no significant inhibition was seen for CYP2E1	Islam et al. (2002), Cohort study
Treatment with peginterferon-α-2b	dextromethorphan (CYP2D6) and, fluoxetine (CYP2D6 active metabolite)	20	- MR before and after initiation of peginterferon-α-2b and ribavirin therapy go from 0.10 ± 0.40 to 0.04 ± 0.09 and that's mean that metabolite production of dextromethorphan increased after hepatitis C, but it is not significant (p = 0.087), - mean serum concentrations of fluoxetine and its metabolite (norfluoxetine) at baseline and 2 months later during combined antiviral treatment didn't change significantly, - only the half-life of fluoxetine showed a significant reduction during combined antiviral therapy (p = 0.014)	National Center for Biotechnology Information (2012), Cohort study
Treatment with peginterferon-α-2a	methadone (CYP3A, 2C8 and 2D6)	24 with hepatitis C	- treatment did not alter the pharmacokinetic of methadone in patients, - increase exposure of total methadone by 10–15% was not statistically significant	Wu and Fleming (2011), Non-randomized
Treatment with peginterferon-α-2b	methadone (CYP3A, 2C8 and 2D6)	20 with hepatitis C	- a barely significant increase in total methadone exposure of 15–16% was observed after 4 weekly injection of peginterferon-α-2b - this increase was not clinically significant because there were no symptoms of methadone overdose	Ling et al. (2009), Non-randomized

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TABLE 13 | (*Continued*) Impact of therapies with immunomodulator on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Treatment with peginterferon-α-2a	theophylline (CYP1A2), tolbutamide (CYP2C9), mephenytoin (CYP2C19), debrisoquin (CYP2D6) and dapsone (CYP3A)	14	- theophylline AUC increased significantly but Cl/F difference was not significant, - no effect on the PK of any other probe drug	Schmitt et al. (2011), Cohort study
Treatment with INF-β	mephenytoin (CYP2C9 and 2C19 and induces 2C9, 2C19 and 3 A) and debrisoquin (CYP2D6)	10 with multiple sclerosis in the first stage	(S)/(R) mephenytoin ratio (p = 0.5) and debrisoquine MR (p = 0.4) were not statistically significant different before and during regular INF-β treatment	Zhuang et al. (2015), Non-randomized

or to its resolution by these same therapies (reduction of inflammation caused by the disease).

The impact of–mabs therapies are summarized in **Table 14**. Monoclonal antibodies have a high degree of specificity against an antigen or an epitope (National Center for Biotechnology Information, 2012). In 2018, more than sixty therapeutic monoclonal antibodies were approved and used in the United States for their action against specific immune cells such as lymphocytes and cytokines or against specific enzymes, cell surface transporters or signaling molecules (National Center for Biotechnology Information, 2012). Consequently, a number of studies have examined the impact of monoclonal antibodies on CYP metabolic activity, assuming that these drugs, by reducing inflammation, return CYP metabolic activity to baseline (Ling et al., 2009; Schmitt et al., 2011; Wu and Fleming, 2011; Zhuang et al., 2015; Tran et al., 2016; Lee et al., 2017; Wen et al., 2020) (**Table 14**).

A return to baseline level after treatment of inflammation was not always observed (Wollmann et al., 2017; Davis et al., 2018). A lag was observed in some cases, such as basiliximab coadministration, which increased tacrolimus through concentration on day 3 but decreased on day 30 (Sifontis et al., 2002). Moreover, OKT3 (also known as muromonab, a CD3 receptor antibody) treatment transiently increased CyA through concentration, and authors suggested that OKT3 inhibits CYP3A4 metabolic activity by inducing transient cytokine release (Vasquez and Pollak, 1997). No changes were observed in drugs PK parameters before and after monoclonal antibodies administration, possibly because CYP metabolic activity was similar in psoriasis disease and in healthy volunteers (Bruin et al., 2019; Khatri et al., 2019). However, these therapies are used for a variety of diseases, with different levels of proinflammatory markers. In addition, a recently published study assessed the impact of clazakizumab, an anti-IL-6 antibody, in kidney transplant recipients with antibody-mediated rejection (ABMR) on CYP3A and CYP2C19 activity by pantoprazole and on tacrolimus and CyA concentrations (Mühlbacher et al., 2021). In contrast to earlier observations, prolonged blockade of IL-6 did not enhance CYP metabolism (Mühlbacher et al., 2021). This could be because the included patients did not have systemic inflammation before initiation of clazakizumab, with IL-6 and CRP levels in the normal range (Mühlbacher et al., 2021). Thus, clazakizumab did not increase CYP metabolism because the included patients had unaltered CYP expression, as ABMR may be different from other disease states, such as infection or autoimmune disease, where systemic inflammation is present (Mühlbacher et al., 2021).

DISCUSSION AND PERSPECTIVES

Our systematic review identified 218 publications that evaluated the impact of inflammation on CYP activities which we divided into 17 sources of inflammation. Indeed, current literature suggests that cytokine signalling pathways differ according to the trigger of inflammation, leading to heterogeneous effects on CYP activity, with different magnitude, potency and time-course (de Jong et al., 2020; Stanke-Labesque et al., 2020). This analysis allowed us to identify areas where the literature is abundant, such as infections like pulmonary infection, hepatitis or HIV and for some therapeutic agents like immunosuppressants or clozapine, and others where further research is needed, such as for auto-immune diseases, and other specific diseases such as diabetes or the anti-inflammation treatments.

Our analysis also identified that studies should be more specifically conducted to assess whether resolution of inflammatory episodes allows a return to baseline of CYP activities. Indeed, inflammatory diseases are chronic, but with a possibility of remission, and acute inflammatory events can punctuate life (infection, surgery, cancer…). A better understanding of the mechanisms of modulation and return to the initial state would make it possible to anticipate changes in the PK of concomitant treatments at different phases of the disease or of the patient's life. This could be done through the impact of anti-inflammatory treatments as well as monoclonal antibody therapies. These therapies are relatively new and much remains to be discovered, but they are highly targeted, and the impact of these different molecules could be isoform specific.

Our literature review highlighted the different effect of inflammation according to the CYP considered. Several studies have investigated the impact of infection on drugs of the nervous systems, mainly CYP2D6 substrates without always showing a significant impact. It now appears that CYP2D6 activity is not modulated by inflammation and this is confirmed in chronic hepatitis C patients where downregulation is linked to the presence of liver kidney microsomal type 1 (LKM-1) antibodies (Girardin et al., 2012). LKM-1 antibodies are often produced during chronic HCV infection and appear to be

TABLE 14 | Impact of therapies with anti-TNF-α and -mabs on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
Basiliximab	tacrolimus (CYP3A)	12 = treatment, 8 = control	- 63% increased tacrolimus trough concentration in basiliximab group at day 3 vs controls ($p < 0.05$), - tacrolimus through concentration decreased in basiliximab group 30 days after transplantation, - Authors suggest that basiliximab induced alteration in drug metabolism because its binding to IL-2R on activated T cells allows circulating IL-2 to bind to IL-2R on hepatic and intestinal cells resulting in a down-regulation of CYP3A4	Wen et al. (2020), Non-randomized
OKT3 (muromonab)	CyA (CYP3A)	17 = OKT3, 16 = controls	- on days 1 and 3, CyA through concentration did not differ but it was significantly higher in OKT3-group at day 5 as compared to control ($p < 0.0001$), - on days 7 and 10, CyA through level did not differ again	Tran et al. (2016), Case-control study
Adalimumab	duloxetine (CYP1A2 and 2D6)	22 years-old woman	- adalimumab was initiated for a refractory psoriasis but the peripheral neuropathy became unbearable leading to double the duloxetine's dosage while she had a long-standing treatment by duloxetine and pregabalin, - authors did not suggest any interaction's mechanism but it could be possible that the decrease of TNF-α by adalimumab led to a lift of the inhibition of CYPs, - no apparent interaction with pregabalin, which is eliminate by renal way	Lee et al. (2017), Case report
Infliximab	verapamil (CYP3A4, 1A2, 2C8, 2C9 and 2C18)	12 = RA with infliximab, 8 = RA controls, 12 = healthy controls	- serum CRP and IL-6 concentrations were significantly greater in RA patients who were on nonbiologic antirheumatic therapy compared with controls ($p < 0.05$ and $p < 0.001$, respectively), - CRP and IL-6 concentrations were not significantly different between RA patients taking infliximab and control subjects, - difference in RA patients who were on nonbiologic treatment in all PK parameters of verapamil, but it did not reach statistical significance but no difference between controls and RA patients who were taking infliximab, - infliximab did not show overall superiority to placebo on depressive symptom outcome	Davis et al. (2018), Case-control study
Infliximab	antidepressants	30 = infliximab, 30 = placebo		Wollmann et al. (2017), RCT
Secukinumab	midazolam (CYP3A)	24 = Psoriasis Area Severity Index (PASI) score >12 taking secukinumab	- secukinumab treat the immune-mediated disease by neutralizing the underlying inflammation and tissue destruction, - patients with PASI score >12 taking secukinumab, a decreased in IL-6 and CRP levels were observed after the start of treatment, - any change was seen in the PK parameters of midazolam before and after the administration of secukinumab, - PK parameters of midazolam in patients with psoriasis (study subjects) were close to those in found in healthy subjects in a previous study	Sifontis et al. (2002), Non-randomized

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TABLE 14 | (*Continued*) Impact of therapies with anti-TNF-α and -mabs on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
risankizumab	caffeine (CYP1A2), warfarin (CYP2C9), omeprazole (CYP2C19) and metoprolol (CYP2D6)	21	- risankizumab is an antibody that acts against IL-23 and it is involved in immune and inflammatory response thus, risankizumab inhibits its cells signalling pathway and the release of pro-inflammatory cytokines, - metabolic activity of CYP1A2, 2C9, 2C19, 2D6 and 3A4 were assessed before and 12 weeks after onset of treatment and any differences were observed, - authors conclude that treatment with risankizumab is not expected to cause CYP-mediated drug interactions	Vasquez and Pollak (1997), Non-randomized
tocilizumab	simvastatin (CYP3A)	12	- exposure to simvastatin was significantly reduced by approximately half at 1 and 5 weeks after tocilizumab infusion	Bruin et al. (2019), Randomized
sirukumab	midazolam (CYP3A), omeprazole (CYP2C19), warfarin (CYP2C9), caffeine (CYP1A2)	12	- administration of probe drugs 1 week before and 1, 3 and 6 weeks after sirukumab administration, - AUC of midazolam, omeprazole and S-warfarin decreased and those of caffeine increased as compared with those before sirukumab administration, - it was not because it is a CYP inducers, but because the inhibition by inflammation may be reversed by its IL-6 antagonism, - for CYP1A2, this result suggests that inflammation induce its metabolic activity, - authors suggest that, according to literature, IL-6 may have a biphasic impact on CYP1A2 activity depending on the IL-6 concentration, with an induction observed with low level of IL-6	Khatri et al. (2019), Non-randomized
dupilimumab	midazolam (CYP3A), omeprazole (CYP2C19), warfarin (CYP2C9), caffeine (CYP1A2) and metoprolol (CYP2D6)	13	- no impact of blockade of IL-4 and IL-13 signalling on the metabolic activity of CYP3A, 2C19, 2C9, 1A2 and 2D6	Mühlbacher et al. (2021), Non-randomized
biological disease-modifying antirheumatic drugs	4β-hydroxycholesterol (4βOHC) (CYP3A)	31 = TNF-α inhibitor, 5 = IL-6 inhibitor, 5 = B-cells inhibitors, 52 = controls	- levels did not change after the onset of any of the three treatments, - a trend was observed that lowest baseline 4βOHC levels (higher inhibition of CYP3A4 metabolic activity) showed highest relative increase in at follow-up and thus a highest regain in metabolic activity of CYP3A4 after initiation of treatment, - authors suggest that the absence of variation in 4βOHC levels in this study could be explained by the low level of inflammation in these patients because 4βOHC level in the study population at baseline was only 30% lower than in control groups	Girardin et al. (2012), Cohort study and case-control study
TNF-α inhibitor	4βOHC (CYP3A)	31	- CRP values were lower than before 3 months treatment, but the difference was not statistically significant ($p > 0.2$) and 4βOHC levels were not significantly affected ($p > 0.9$) by the initiation of treatment, - significant negative correlations were observed between 4βOHC and IL-1ra and IL-6 ($r = -0.410$, $p = 0.022$) and CXCL8 ($r = –0.403$, $p = 0.025$)	Chládek et al. (1999), Cohort study Same subject as in Girardin et al. (2012)

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TABLE 14 | (*Continued*) Impact of therapies with anti-TNF-α and -mabs on CYP substrates, explained totally or partially by modulation of CYP activity.

Inflammation characterized by	Victim drugs (CYPs concerned)	Number of subjects	Potential effect of interaction	References and design
etanercept	CyA (CYP3A)	42-year-old male	-2.5-fold increase of clearance after initiation of etanercept	Yang et al. (2003), Case-report
daclizumab	caffeine (CYP1A2), warfarin (CYP2C9), omeprazole (CYP2C19), dextromethorphan (CYP2D6) and midazolam (CYP3A)	30 = multiple sclerosis	- daclizumab treatment had no effect on CYP1A2, 3C9, 2C19, 2D6 and 3 A activity in patients with multiple sclerosis as compared to before treatment	Hefner et al. (2015), Cohort study
sarilumab	Simvastatin (CYP3A)	19	- plasma exposure decreased by 45% in RA patients 1 week after sarilumab injection, as compared to baseline, - one dose led to decreased of CRP level and IL-6 inhibition and, thus, restauration of CYP3A enzyme activity	Harbrecht et al. (2005), Cohort study

proportional to liver disease severity (Girardin et al., 2012). Moreover, it is well-known that CYP2D6 has an important inter- and intra-individual variability, in accordance with the available literature (Chládek et al., 1999). All sources of inflammation combined, the most studied CYP was CYP3A, which is in fact the CYP that metabolizes nearly 50% of the drugs on the market. Patients with inflammation/infection are, however, prone to receiving multiple drugs, and the impact on other CYPs should be carefully evaluated, in particular in critically ill patients or patients at different stages of HIV, where data is scarce. Studies should also be careful to exclude the impact of co-medications (CYP inhibitor and inducer) as a confounding factor.

In organ diseases, current studies in liver diseases have not been able to determine whether CYP downregulation is caused by a decrease of CYP content or not, and in renal diseases it was not possible to identify whether the modulation of CYP activity was rather due to elimination issues (Farrell et al., 1979; Yang et al., 2003). Therefore, it is challenging to study inflammation as an independent factor in PK variability and not as a consequences of organ damage.

Our literature review also found that inflammation is a complex process, which is expressed differently depending on the disease and conditions and therefore, extrapolation between different types of inflammation should be avoided. Indeed, the hepatic expression of CYP2C19 could for example be regulated by other tumor-associated inflammatory factors than those regulating CYP3A (Burns et al., 2014). Moreover, different levels of inflammation led to different magnitudes of voriconazole through concentration increases for instance in association with CRP levels (van Wanrooy et al., 2014; Bolcato et al., 2021). In most studies, significant changes in CYP activities occurred in the presence of severe inflammation, characterized by elevated levels of inflammatory markers or a severe disease state, such as AIDS, advanced cancer or polytrauma patients (Gatti et al., 1993; Lee et al., 1993; Farrell et al., 1979; Grieco et al., 1998; Bauer et al., 1994; Harbrecht et al., 2005; Charles et al., 2006; Alexandre et al., 2007; Helsby et al., 2008; Abou Farha et al., 2012; ten Bokum et al., 2015; Hefner et al., 2015; Yasu et al., 2017; Gautier-Veyret et al., 2019). A minority of studies have evaluated the impact of inflammation on drugs PK and metabolism as an independent factor of variability, as only a few have included inflammation factors as covariates, such as biomarkers of renal or liver function (Stanke-Labesque et al., 2020).

Additionally, inflammation may have a different impact on CYPs activities depending on their baseline activity and on genotypic and environmental factors, such has concomitant treatments. Indeed, inflammation further increased the perampanel concentration/dose (C/D) ratio in patients not treated with drug inducers (Yamamoto et al., 2018). Voriconazole is also metabolized by highly polymorphic CYPs and inflammatory marker levels have a differential impact on voriconazole trough concentration whether patients are extensive, intermediate or ultra-rapid metabolized for CYP2C19 (Veringa et al., 2017). Moreover, a recent meta-analysis showed that voriconazole trough concentrations were independently influenced by both CYP2C19 and CYP3A4 genotype, considered individually or by a combined genetic score, in addition to CRP levels (Bolcato et al., 2021). In contrast, another cohort study showed that voriconazole overdoses were significantly associated with elevated CRP levels (>96 mg/L) but that CYP2C19 and CYP3A4 genotype, considered alone or combined in a genetic score, were not significantly different between overdose and non-overdose patients (Gautier-Veyret et al., 2019). Therefore, inflammation and pharmacogenomics may mutually minimize their reciprocal influence on CYP phenotype. Indeed, genotype did not predict correctly the phenotype in patients with inflammatory disease and the effect of inflammation was not as important as expected in CYP variants carriers (Helsby et al., 2008; Goktaş et al., 2015; Burns et al., 2014; O'Neil et al., 2000; Williams et al., 2000;). Consequently, inflammation could induce dynamic phenoconversion, characterized by dynamic phenotype-genotype mismatch, and studies examining the impact of inflammation on CYPs should assess CYP genotypes and phenotypes as covariates. It should however be pointed out that most of the included studies did not take into account routine treatment given to treat the diseases themselves.

Predictive models based on known interactions between molecular, environmental and lifestyle data by computational algorithm are increasingly developed to support the decision to individualize treatment (Iriart, 2019). Simulation of the

concentration-time profiles of a drug and its metabolite(s) and concomitant estimation of PK parameters using dynamic physiologically based pharmacokinetic (PBPK) models allow prediction of plasma concentration curves (Sager et al., 2015). There are increasing developments in regulatory guidances (Sager et al., 2015). Inflammatory disease is an example of a special population and numerous PBPK models have been developed and validated to predict IL-6 mediated drug-disease (Machavaram et al., 2013; Xu et al., 2015; Jiang et al., 2016; Radke et al., 2017; Xu et al., 2018; Machavaram et al., 2019). While IL-6 appears to be the key element in modulating CYP activities during inflammation, a recent study developed a model that predicted the impact of systemic CRP levels on CYP3A4 and CYP2C19 activities (Simon et al., 2021). Optimal drug use leads to takes into account the contribution of covariates to predict the dose needed to achieve a target concentration and thus reduce the inter- and intra-individual variability in drug response (Darwich et al., 2021).

This review focuses on CYP regulation, but other mechanisms, such as enzymes and transporters, involved in drug absorption, distribution, metabolism and elimination may be involved in changes in drugs PK during inflammatory states, although they are less studied. Studies described changes in plasma protein binding and renal excretion during inflammation that could affect CYP substrates metabolism (Gorski et al., 2000; Hefner et al., 2015; Helland et al., 2018). Plasma protein binding may influence total clearance for low-extraction drugs but not unbound clearance and may or may not influence half-life, depending on clearance and volume of distribution (Boffito et al., 2021). The unbound concentration and not the total concentration must be considered when assessing drug exposure to a highly protein-bound drug, otherwise there is a risk of misinterpretation of lopinavir overexposure (Boffito et al., 2021; Stanke-Labesque et al., 2021). For example, by taking into account plasma protein concentration, the authors concluded that CyA biotransformation by CYP3A may be downregulated by diabetes (Akhlaghi et al., 2012). Decreased albumin concentration may increase the unbound concentration in diabetics, which should theoretically increase CyA metabolic clearance (Akhlaghi et al., 2012). But the lower production of almost all metabolites has shown that the correct hypothesis is rather a reduced CYP activity (Akhlaghi et al., 2012). In fact, CyA metabolites that involved amino acid 1 showed significantly lower dose-normalized AUC values in diabetic patients compared with nondiabetics suggesting that CYP3A4 metabolic activity was not decreased (Mendonza et al., 2008). Its dose-adjusted metabolite-parent concentration ratio was decreased in the diabetic groups, but no difference was found concerning doses and trough levels of CyA in a retrospective study (Wadhawan et al., 2000; Akhlaghi et al., 2012).

Phase 2 drug metabolic enzymes appear to be affected in a cytokine-specific manner, as infection resulted in a significant downregulation of several genes encoding hepatic uridine 5′-diphospho-glucuronosyltransferases (UGT) (Stanke-Labesque et al., 2020). Pregnane X receptor (PXR) and constitutive androstane receptor (CAR), two nuclear receptors, are also cytokine dependent and mediate the expression of glutathione S-transferases (GST), UGTs and sulfo-transferases (SULT) in humans (Wu and Lin, 2019). However, unlike voriconazole, posaconazole's PK did not appear to be influenced by inflammation. This could be explained by a metabolism by phase 2 enzymes mainly (Märtson et al., 2019). Literature reviews on physiological changes related to drug PK and PD during inflammation may be useful to determine what investigations are needed to complement the data in the literature, such as the impact of inflammation on P-gp and other drug transporters, as one study showed that an increase in bioavailability due to downregulation of P-gp could not be ruled out (Sanaee et al., 2011).

Moreover, hepatic transporters that belong to ATP-binding cassette (ABC) and solute carrier (SLC) transporters have been shown to be significantly reduced during inflammatory states in animal and in-vitro studies (Stanke-Labesque et al., 2020). For instance, animals studies have shown that mRNA levels of MRP, OATP or BSEP were decreased in mice during inflammation (Wu and Lin, 2019). NF-κB, a transcription factors involved in the mechanism of action of cytokines on metabolizing enzyme gene expression, is also known to regulate the expression of numerous ABC and SLC transporters, including ABCB1 in humans and MDR1, MRP, BCRP, OATP, NTCP in rats and mice (Wu and Lin, 2019).

Given all of the above, it should be acknowledged that our literature search has some limitations. First, the completeness of the search cannot be guaranteed as we only searched one database and only published articles. Second, there is inevitably heterogeneity between the studies selected due to the different methodologies employed and low comparability between the studies identified. In addition, the diversity of the sources of inflammation studied and assessment of the clinical impact severity limits the robustness and generalizability of the results. Interpretations should therefore be addressed with particular caution.

CONCLUSION

This systematic literature review shows that inflammation is a major contributing factor to CYP metabolic activity variations. The proportion of the drug cleared by CYP metabolism, the patient's genotype and concomitant medications should also be taken into account.

Compelling evidence suggests that inflammation has a differential impact on the various CYP isoforms with a different magnitude. CYP3A and CYP2C19 are downregulated and inflammation has no impact on CYP2D6 activity. Regarding other main CYPs, the impact remains unclear and requires further investigation. Moreover, the effect of inflammation depends on its severity and the inflammatory markers released, even if this remains debated. Indeed, the origin of the inflammation may differ as well as the inflammatory mediators involved, possibly leading to different impact on CYP activities. The reason why some CYP metabolic activities were modulated in some diseases and not in others may be partly explained by this heterogeneity in inflammatory markers.

Nonetheless, some results are still debated such as the impact of vaccination and infection, and further investigations are required to well characterize the impact of inflammation on CYP activity.

CYP is a major source of interindividual variability, and it appears crucial to be able to predict their activity to individualize drug dosing and take into account the patient's underlying pathophysiological conditions and the PK characteristics of the drug concerned. Measurement of inflammation induced CYP phenoconversion and the development of endogenous markers of CYP metabolism should enable the measurement of CYP activity variation due to disease progression and could have implications for personalized medicine and provide new opportunities.

To conclude, inflammatory conditions in patients are a major factor to be considered to predict variability in drug response and avoid efficacy or safety issue in clinical practice.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

CL participated in the manuscript conceptualization, experimental design, writing and data analysis. CFS, JAD and VR participated in the manuscript conceptualization, supervision, overall manuscript review and English review.

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Pharmacogenetics Based Dose Prediction Model for Initial Tacrolimus Dosing in Renal Transplant Recipients

Lekshmy Srinivas[1]*, *Noble Gracious*[2] and *Radhakrishnan R. Nair*[1]*

[1]Laboratory Medicine and Molecular Diagnostics, Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India, [2]Department of Nephrology, Government Medical College, Thiruvananthapuram, India

Tacrolimus, an immunosuppressant used in solid organ transplantation, has a narrow therapeutic index and exhibits inter-individual pharmacokinetic variability. Achieving and maintaining a therapeutic level of the drug by giving appropriate doses is crucial for successful immunosuppression, especially during the initial post-transplant period. We studied the effect of *CYP3A5*, *CYP3A4*, and *ABCB1* gene polymorphisms on tacrolimus trough concentrations in South Indian renal transplant recipients from Kerala to formulate a genotype-based dosing equation to calculate the required starting daily dose of tacrolimus to be given to each patient to attain optimal initial post-transplant period drug level. We also investigated the effect of these genes on drug-induced adverse effects and rejection episodes and looked into the global distribution of allele frequencies of these polymorphisms. One hundred forty-five renal transplant recipients on a triple immunosuppressive regimen of tacrolimus, mycophenolate mofetil, and steroid were included in this study. Clinical data including tacrolimus daily doses, trough levels (C_0) and dose-adjusted tacrolimus trough concentration (C_0/D) in blood at three time points (day 6, 6 months, and 1-year post-transplantation), adverse drug effects, rejection episodes, serum creatinine levels, etc., were recorded. The patients were genotyped for *CYP3A5**3, *CYP3A4**1B, *CYP3A4**1G, *ABCB1* G2677T, and *ABCB1* C3435T polymorphisms by the PCR-RFLP method. We found that *CYP3A5**3 polymorphism was the single most strongly associated factor determining the tacrolimus C_0/D in blood at all three time points ($p < 0.001$). Using multiple linear regression, we formulated a simple and easy to compute equation that will help the clinician calculate the starting tacrolimus dose per kg body weight to be administered to a patient to attain optimal initial post-transplant period tacrolimus level. *CYP3A5* expressors had an increased chance of rejection than non-expressors ($p = 0.028$), while non-expressors had an increased risk for new-onset diabetes mellitus after transplantation (NODAT) than expressors ($p = 0.018$). Genotype-guided initial tacrolimus dosing would help transplant recipients achieve optimal initial post-transplant period tacrolimus levels and thus prevent the adverse effects due to overdose and rejection due to inadequate dose. We observed inter-population differences in allele frequencies of drug metabolizer and transporter genes, emphasizing the importance of formulating population-specific dose prediction models to draw results of clinical relevance.

Keywords: pharmacogenetics, tacrolimus, dose prediction model, *CYP3A5*, renal transplantation, NODAT, rejection

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***Correspondence:**
Lekshmy Srinivas
lekshmys@rgcb.res.in
Radhakrishnan R. Nair
radhakrishnan@rgcb.res.in

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INTRODUCTION

Tacrolimus, a calcineurin inhibitor immunosuppressant used in solid organ transplant recipients, exhibits interindividual pharmacokinetic variability that affects the dose required to reach the target concentration in blood (Coto et al., 2011). Attaining and sustaining a therapeutic level of the immunosuppressants by administering appropriate doses is crucial, particularly during the initial post-transplant period. The success of a transplant depends on a fragile balance between immunosuppression and rejection (Provenzani et al., 2013). Due to the narrow therapeutic window of tacrolimus, therapeutic drug monitoring (TDM) is essential to maintain adequate blood concentrations to prevent graft rejection due to inadequate immunosuppression and toxicity due to higher drug levels (Brunet et al., 2019). Despite the advances in medicine, attaining and maintaining the optimal therapeutic range of tacrolimus specific to different post-transplant time points remains a challenge (Herrero et al., 2013; Ben-Fredj et al., 2020).

Tacrolimus is metabolized by the Cytochrome P450 3A5 and 3A4 enzymes (CYP3A5 and CYP3A4) in the gut and liver, and transported in the gut by an efflux pump, P-glycoprotein (P-gp) (Sakaeda et al., 2003; De Jonge et al., 2012). *CYP3A5* and *CYP3A4* genes are part of a cluster of Cytochrome P450 genes on the long arm of chromosome 7 (7q21.1) (Li et al., 2014). The ATP Binding Cassette Subfamily B Member 1 (*ABCB1*) or Multi-Drug Resistance 1 (*MDR1*) gene, which codes for P-gp, is also located nearby (7q21.12) (Chen et al., 1990). Polymorphisms in *CYP3A5*, *CYP3A4*, and *ABCB1* genes could have important roles in tacrolimus blood concentration and dose requirement (Haufroid et al., 2006; Mourad et al., 2006; Tamashiro et al., 2017). The gene expression and enzyme activity of CYP3A5 depends mainly on the *CYP3A5**3 polymorphism (6986G > A, rs776746) located in the intron 3. A nucleotide change from A to G creates a cryptic splice site, which causes altered mRNA splicing resulting in a premature termination codon and hence a non-functional protein (Kuehl et al., 2001). Individuals with the *CYP3A5**3/*3 genotype are considered to be *CYP3A5* non-expressors. *CYP3A4* gene expression is regulated by *CYP3A4**1B and *CYP3A4**1G polymorphisms. The promoter polymorphism *CYP3A4**1B (-392A > G, rs2740574) may be associated with enhanced CYP3A4 expression owing to reduced binding of a transcriptional repressor (Amirimani et al., 2003). In the case of *CYP3A4**1G, the G to A substitution at IVS10 + 12 is correlated with an increased transcription of the *CYP3A4* gene (He et al., 2011). A missense mutation in exon 21 of *ABCB1* gene, G2677T, results in an Ala to Ser amino acid change at position 893 of the protein, and has been associated with altered P-gp expression (Seven et al., 2014). The *ABCB1* C3435T (I1145I) is a synonymous polymorphism which has been shown to correlate with the expression levels and function of P-gp. In *ABCB1* C3435T polymorphism, there is a C to T substitution at nucleotide position 3435 in exon 26. Although it does not change its encoded amino acid with Ile at position 114522, it can affect the post-transcriptional processing of mRNA or affect the process of alternative transcript splicing (Tamura et al., 2012).

Though many studies have been carried out concentrating on the role of *CYP3A5* on tacrolimus blood levels, the pharmacogenetic factors identified so far were of insufficient predictive value and not much of clinical use (Coto et al., 2011; Boughton et al., 2013; Chen and Prasad, 2018).

There is a lack of data from South Indian patients on the effect of multiple genes on tacrolimus trough concentrations in the early post-transplantation period. This study was carried out to investigate the effect of *CYP3A5*, *CYP3A4*, and *ABCB1* genes on dose-adjusted tacrolimus trough concentrations in South Indian renal transplant recipients from Kerala. The study aimed to build a pharmacogenetics-based dosing equation to calculate the required starting daily dose to be administered to each patient to attain optimal initial post-transplant period tacrolimus level based on his genotype. Genotype-guided dosing, rather than dosing based solely on the patient's body weight, maybe a preferred strategy to determine the initial dose of tacrolimus in patients undergoing solid organ transplantation. This would help prevent the adverse effects of overdose and transplant rejection due to inadequate dose. We also looked into the association of the selected gene polymorphisms with drug-induced adverse effects and rejection episodes. In addition, we analyzed the global variation in distribution of these polymorphisms by comparing their allelic frequencies in our population with the other world populations allele frequencies data.

MATERIALS AND METHODS

Study Subjects

For this prospective study, 156 renal transplant recipients belonging to an ethnically matched Malayalam-speaking population of Kerala, South India, receiving tacrolimus as an immunosuppressant were recruited from the Department of Nephrology, Government Medical College, Thiruvananthapuram. All 156 patients were genotyped. Since follow-up data were available only for 145 patients, further analyses were performed only using these patients. Patients who were more than 15 years of age and less than 60 were included in the study. Patients receiving mTOR inhibitors (sirolimus, everolimus) along with tacrolimus, or medications known to influence drug levels (diltiazem, fluconazole) were excluded from the study. Patients with delayed graft function and early graft dysfunction within 1-week post-transplantation were excluded from the association analysis of genetic polymorphisms with dose-adjusted tacrolimus trough concentration (C_0/D) since the treatment modalities for these might affect tacrolimus levels and thus, the study results.

The study was approved by the Human Ethics Committees of Rajiv Gandhi Centre for Biotechnology and Government Medical College, Thiruvananthapuram. Informed, written consent in a standard consent form was obtained from all the study subjects to participate in the study after being provided with a full explanation of study protocols, objectives, benefits, and risks.

All patients were on a triple immunosuppressive regime [tacrolimus, mycophenolate mofetil (MMF), and steroid]. All

study participants received an initial tacrolimus starting dose of 0.075–0.1 mg/kg body weight per day in 2 divided doses from day minus 2 of transplantation as per the institutional protocol. The dose was then adjusted to achieve a target tacrolimus trough concentration (C_0) of 7–10 ng/ml for first 3 months of transplantation.

Sample Size Estimation

Sample size and statistical power were calculated by one-sample *t*-test using nQuery Sample Size Software (Statistical Solutions, Cork, Ireland) by comparing mean tacrolimus drug levels in the whole population vs. patients with specific genotypes in *CYP3A5*, *ABCB1* (Vattam et al., 2013) and *CYP3A4* (Li et al., 2014). The required sample size to study the effects of *CYP3A5*, *CYP3A4*, and *ABCB1* polymorphisms on the dose-adjusted tacrolimus level (with 80% power and test significance level, $\alpha = 0.05$) was 137.

Clinical Data Collection

Patient demographic characteristics like age of the patient during transplant, gender, body weight, etc., were recorded. Data including tacrolimus daily doses (mg) and trough levels C_0 (ng/ml) in blood on day 6, 6 months, and 1-year post-transplantation, concomitant medications and events including rejection and adverse drug effects like tacrolimus toxicity, NODAT and post-transplant erythrocytosis, serum creatinine levels and all lab investigation results were collected from the medical record during the follow up visits. Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) method was used to determine tacrolimus concentration in blood. The daily tacrolimus dose was noted, and the weight-adjusted tacrolimus dose was calculated using daily tacrolimus dose/weight (mg/kg per day). The dose-adjusted tacrolimus trough concentration (C_0/D) was calculated by dividing the measured C_0 by the corresponding daily weight-adjusted tacrolimus dose (ng/ml per mg/kg).

DNA Isolation and Genotyping

We collected 5 ml of peripheral blood from the patients in K_2-EDTA coated Vacutainer® for DNA isolation. Genomic DNA was isolated using the DNA Isolation Kit for Mammalian Blood (Roche Life Science, United States).

The patients were genotyped for 5 SNPs from *CYP3A5* (*3/rs776746), *CYP3A4* (*1B/rs2740574 and *1G/rs2242480) and *MDR1* or *ABCB1* genes (Ex22 G2677T/rs2032582 and Ex27 C3435T/rs1045642). Genes and SNPs were selected based on their functional significance and previous reports on association with tacrolimus concentrations.

Genotyping was performed by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. PCR amplification was carried out using specific primers in the Applied Biosystems® Veriti® or Eppendorf® Mastercycler® thermal cyclers. PCR was done in a final volume of 10 µL. PCR reaction mix consisted of 100 ng of genomic DNA, 5 pmol each of forward and reverse primers, and 1X EmeraldAmp® PCR Master Mix (Clontech Laboratories, Inc.). Cycling parameters were initial denaturation at 94°C for 1 min, annealing for 30 s, extension at 72°C for 20 s, and a final extension step at 72°C for 1 min; the number of cycles was 30. The amplified PCR products was digested with 5 Units of the respective restriction enzyme (NEB Inc., United States and ThermoFisher Scientific, United States) and a specific 1X buffer in a final volume of 15 µL. PCR primers, annealing temperature, restriction enzymes, amplicon and allele sizes for each polymorphism are summarized in **Supplementary Table S1**.

TABLE 1 | Baseline characteristics of the study population.

Number of patients (N)	**145**
Age (years) (mean ± SD)	36.61 ± 10.58
Gender [male (%)/female (%)]	118(81.4)/27 (18.6)
Type of donor (*n* = 141) n (%)	
Live	110(78)
Cadaver	31(22)
Native kidney disease n (%)	
Chronic glomerulonephritis	82(56.6)
Reflux nephropathy	11(7.6)
Diabetic nephropathy	7(4.8)
Others	22(15.2)
Unknown	23(15.9)
Induction n (%)	
Basiliximab	20(13.8)
ATG	20(13.8)
Rituximab	7(4.8)
None	98(67.6)

Statistical Analysis

Quantitative variables were summarized using mean and standard deviation. Categorical variables were represented using frequency and percentage. Independent sample *t*-test and ANOVA were used for comparing continuous variables between groups. Pearson Chi-square test was used for comparing categorical variables between groups. Linear regression was used to find out independent predictors of tacrolimus C_0/D ratio. All statistical analyses were performed using the SPSS® statistical software package (version 22.0, IBM Inc., Armonk, NY, United States). A *p*-value of <0.05 was considered statistically significant. Pair-wise Linkage disequilibrium (LD) between SNPs was calculated using UNPHASED software for genetic association analysis, version 3.1.7. Deviations from the Hardy-Weinberg equilibrium (HWE) were tested for all polymorphisms by comparing observed and expected genotype frequencies using the miniPCR Hardy-Weinberg calculator spreadsheet (https://www.minipcr.com/wp-content/uploads/miniPCR-Hardy-Weinberg-Calculator.xlsx). The global variation in distribution of the polymorphisms was analyzed by comparing their allelic frequencies in our population with the 1000 genomes browser Phase 3 populations allele frequencies data (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) downloaded from the Ensembl database (https://asia.ensembl.org/index.html). A description of 1000 Genomes Project Phase 3 populations used for comparison in the present study is given in **Supplementary Table S2**.

TABLE 2 | Tacrolimus dosing in the study population at 3 time points.

	6th day	6 months	1 year
	(*n* = 139)	(*n* = 89)	(*n* = 66)
Bodyweight (kg) (mean ± SD)	59.55 ± 12.82	62.98 ± 12.58	63.63 ± 12.81
Tacrolimus dose (mg/day)	3.88 ± 1.17	3.58 ± 1.16	3.38 ± 1.21
Tacrolimus concentration (ng/ml)	7.11 ± 3.99	6.79 ± 2.5	6.68 ± 2.64
Weight adjusted tacrolimus dose (mg/kg/day)	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.02
Concentration/Dose ratio (C_0/D) (ng/ml)/(mg/kg)	113.87 ± 60.42	132.47 ± 69.65	139.44 ± 70.2

TABLE 3 | Genotype and allele frequencies of SNPs in the study population.

Polymorphism	Genotype	N (%)	Allele	N (%)
*CYP3A5**3 rs776746 (N = 145)	*3/*3	69(47.6)	*3	199(68.6)
	*1/*3	61(42.1)	*1	91(31.4)
	*1/*1	15(10.3)		
*CYP3A4**1G rs2242480 (N = 142)	*1/*1	54(38)	*1	176(62)
	*1/*1G	68(47.9)	*1G	108(38)
	*1G/*1G	20(14.1)		
CYP3A4*1B rs2740574 (N = 141)	AA	126(89.4)	A	267(94.7)
	AG	15(10.6)	G	15(5.3)
ABCB1 C3435T rs1045642 (N = 144)	CC	21(14.6)	C	102(35.4)
	CT	60(41.7)	T	186(64.6)
	TT	63(43.8)		
ABCB1 G2677T rs2032582 (N = 142)	GG	19(13.4)	G	102(35.9)
	GT	64(45.1)	T	182(64.1)
	TT	59(41.5)		

TABLE 4 | Association of SNP genotypes with tacrolimus C_0/D.

	6th day			6 months			1 year		
Gene/genotype	**n**	**C_0/D**	***p* value**	**n**	**C_0/D**	***p* value**	**n**	**C_0/D**	***p* value**
CYP3A5**3									
*3/*3	50	145.45 ± 54.99	<0.001[a]	43	163.06 ± 74.29	<0.001[a]	32	176.1 ± 64.65	<0.001[a]
*1/*3	33	77.1 ± 38.43		38	107.38 ± 50.61		28	114.07 ± 57.21	
*1/*1	9	66.46 ± 36.09		8	87.18 ± 53.18		6	62.38 ± 33.75	
CYP3A5 Non-expressor	50	145.45 ± 54.99	<0.001[a]	43	163.06 ± 74.29	<0.001[a]	32	176.1 ± 64.65	<0.001[a]
CYP3A5 Expressor	42	74.82 ± 37.77		46	103.87 ± 51.04		28	104.95 ± 57.01	
CYP3A4**1G									
*1/*1	39	146.11 ± 58.02	<0.001[a]	33	168.89 ± 72.7	<0.001[a]	26	180.48 ± 63.39	<0.001[a]
*1/*1G	38	92.65 ± 49.31		41	109.5 ± 58.99		31	121.37 ± 60.75	
*1G/*1G	13	72.02 ± 38.72		13	108.25 ± 55.88		8	66.21 ± 29.39	
CYP3A4**1B									
AA	80	117.36 ± 60.12	0.048[a]	81	132.12 ± 71.19	0.972	63	137.28 ± 70.85	0.472
AG	9	75.87 ± 44.56		5	133.26 ± 55.7		1	189	
***ABCB1* C3435T**									
CC	10	142.57 ± 71.69	0.193	12	125.08 ± 79.03	0.73	11	106.66 ± 56.38	0.216
CT	38	115.5 ± 54.5		33	140.33 ± 72.36		23	151.03 ± 69.46	
TT	43	104.97 ± 60.14		43	129.2 ± 66.65		32	142.39 ± 73.43	
***ABCB1* G2677T**									
GG	8	147.86 ± 71.44	0.219	10	149.08 ± 75.37	0.706	8	133.32 ± 60.61	0.727
GT	42	107.75 ± 55.52		35	127.95 ± 74.06		28	146.16 ± 72.68	
TT	39	111.87 ± 61.19		41	131.69 ± 66.48		28	131.38 ± 72.58	

[a]*Statistically significant.*

TABLE 5 | Linear regression analysis to find independent predictors of 6th day tacrolimus C_0/D ratio.

Variable	Coefficient (95% CI)	*p* Value
Constant	158.74(103.01–214.46)	<0.001[a]
Age	−0.36(−1.41–0.69)	0.495
Male gender	3.95(−22.19–30.1)	0.764
CYP3A5[a]3	−40.48[−68.12–(−12.83)]	0.005[a]
ABCB1 C3435T	−14.48(−40.56–11.59)	0.272
ABCB1 G2677T	12.66(−14–39.34)	0.348
CYP3A4[a]1G	−11.54(−37.41–14.31)	0.377
CYP3A4[a]1B	−14.66(−51.97–22.64)	0.436

R square = 0.353; p *value <0.001.*
[a]*Statistically significant.*

RESULTS

The baseline characteristics of the study population are given in **Table 1**. Tacrolimus dosing and trough concentrations of the patients at three time points (6th day, 6 months, and 1 year after transplantation) are listed in **Table 2**. The genotype frequencies of all the SNPs were similar to those expected under Hardy-Weinberg equilibrium ($p > 0.05$). The observed genotype and allele frequencies of the polymorphisms are presented in **Table 3**.

We observed no statistically significant differences in the tacrolimus C_0/D ratio between men and women ($p > 0.5$ at all timepoints). Similarly, we observed no significant association between patients' age and tacrolimus C_0/D ratio at any timepoint.

Association of SNP Genotypes With Tacrolimus C_0/D

We tested the association of the five SNPs with tacrolimus C_0/D at different time points after transplantation. Of these, *CYP3A5**3 and *CYP3A4**1G showed strong associations with tacrolimus C_0/D at all three time points after transplantation (**Table 4**). Tacrolimus C_0/D was highest in patients with homozygous *CYP3A5* *3/*3 genotype (non-expressors) compared to *CYP3A5**1/*3 and *CYP3A5* *1/*1 genotypes (expressors) ($p < 0.001$). Among the *CYP3A4**1G genotypes, C_0/D of the patients with *CYP3A4* *1/*1 was highest ($p < 0.001$). *CYP3A4**1B AA genotype showed a marginal association with higher C_0/D on post-operative day 6 ($p = 0.048$). The *ABCB1* variants did not demonstrate a significant association with C_0/D at any of the post-transplant time points.

CYP3A4 and *CYP3A5* genes are both located in 7q21.1. We found a moderate degree of linkage disequilibrium between *CYP3A5**3 (rs776746) and *CYP3A4**1G (rs2242480) polymorphisms (D' = 0.922, $r^2 = 0.64$).

Personalized Initial Dosing Equation Based on Genotype, Age, and Gender

We performed a linear regression analysis to find the association of 6th-day tacrolimus C_0/D ratio with multiple factors, including *CYP3A5**3, *CYP3A4**1G, *CYP3A4**1B, *ABCB1* C3435T, *ABCB1* G2677T genotypes, age, and gender (**Table 5**). A dosing equation to calculate the required tacrolimus dose/kg to attain the desired target tacrolimus level during the initial post-transplant period was built using this linear regression (**Eq. 1**). For uniformity, tacrolimus trough levels of all the patients on day 6 post transplantation were used for tacrolimus initial dose calculation.

Equation 1 Dosing equation to calculate starting tacrolimus dose/kg to attain optimal initial post-transplant period tacrolimus level

$$\textit{Required tacrolimus dose/kg} = \frac{\textit{Desired tacrolimus level on Day } 6}{159 - \left(40 \times \textit{CYP3A5 genotype}\right)} \quad (1)$$

CYP3A5 genotype = 0 for *3/*3, 1 for *1/*3, and 2 for *1/*1.

Association of *CYP3A5* Expressor Status With Drug-Induced Adverse Effects and Rejection Episodes

The mean nadir serum creatinine was 1.2 ± 0.37 mg/dl. The mean serum creatinine levels at the end of 6 months and 1 year were 1.41 ± 0.58 mg/dl and 1.44 ± 0.74 mg/dl respectively. We did not find any association of any of the SNPs with renal allograft function at the end of 1 year. In our study population, 22 (15.2%) patients developed post-transplant erythrocytosis within a year. Sixty-seven patients (46.2%) had new onset of diabetes mellitus (NODAT) within the 1st year of transplantation. Biopsy proven tacrolimus toxicity was observed in 40 (28%) patients. 24.1% of patients developed graft rejection within a year. A total of 19 (13.1%) patients had biopsy-proven acute graft rejection within first week of transplant. Sixteen patients (11%) had delayed graft function.

The genetic associations of NODAT, renal allograft rejection, and tacrolimus toxicity are summarized in **Table 6**. *CYP3A5* non-expressors (*3/*3 genotype) had a 2.22-fold higher risk of developing NODAT compared to expressors (*1/*1 + *1/*3 genotypes) ($p = 0.018$, 95% CI = 1.14–4.33). *CYP3A5* expressors had a 2.43 times higher chance of developing rejection within a year of transplantation ($p = 0.028$, 95% CI = 1.08–5.44). *CYP3A5* expressor status showed a trend towards association with biopsy-proven acute graft rejection within first week of transplant ($p = 0.052$, 95% CI = 0.11–1.01). We did not observe a significant association of *CYP3A5* expressor status with the development of tacrolimus toxicity.

Global Variation in Allele Frequency Distribution of the Polymorphisms

Allele frequency distribution of the selected *CYP3A5*, *CYP3A4*, and *ABCB1* polymorphisms in our Kerala population and other world populations is presented as bar diagrams in **Figure 1** and **Supplementary Figure S1**. Allele frequencies of drug metabolizer and drug transporter gene polymorphisms in our study population were found to be considerably different compared to other populations worldwide. The *CYP3A5**3 (G) allele, which is the major allele in the South Asian (including our study population), Ad Mixed American, East Asian, and European

TABLE 6 | Association of CYP3A5 expressor status with post-transplant complications.

Complication	Occurrence	CYP3A5 non-expressor	CYP3A5 expressor	OR (95%CI)	*p* value
NODAT	YES	39	28	2.22 (1.14–4.33)	0.018[a]
	NO	30	48		
Rejection	NO	58	52	2.43 (1.08–5.44)	0.028[a]
	YES	11	24		
Tacrolimus toxicity	YES	22	18	1.51 (0.72–3.15)	0.266
	NO	46	57		

[a]*Statistically significant.*

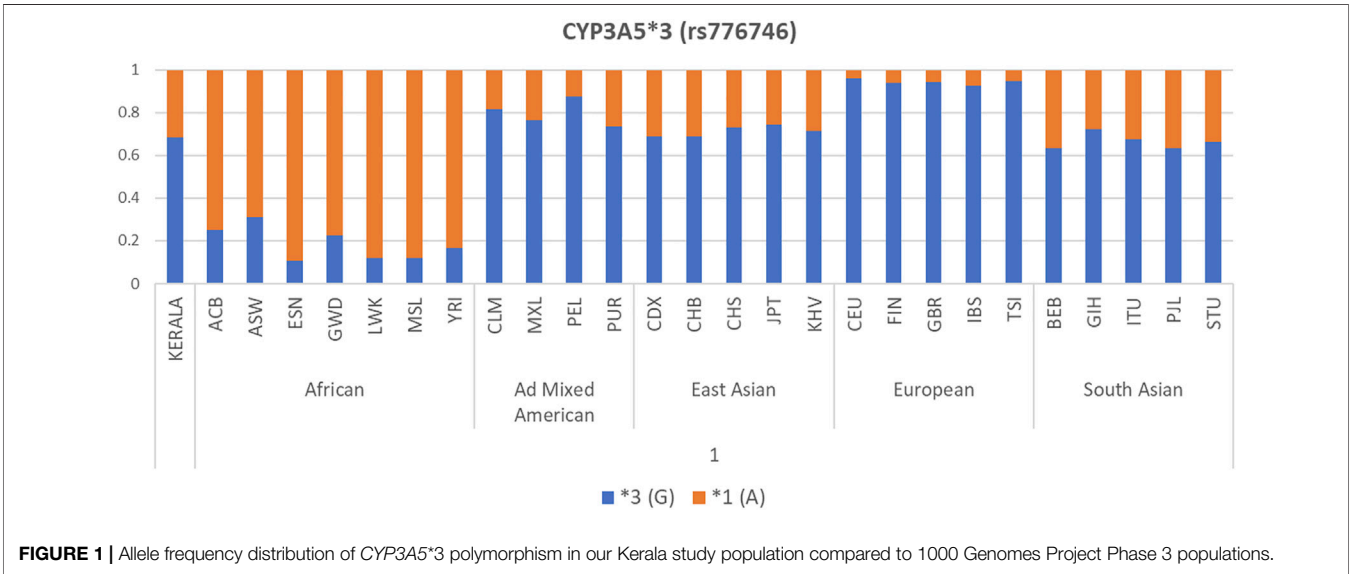

FIGURE 1 | Allele frequency distribution of *CYP3A5**3* polymorphism in our Kerala study population compared to 1000 Genomes Project Phase 3 populations.

populations was found in very low frequency in African populations.

DISCUSSION

Therapeutic drug monitoring (TDM) has been an essential and indispensable tool for tacrolimus dosing to control the drug blood concentrations, owing to its narrow therapeutic index and significant inter and intra individual variations in levels. However, since dose modification based on TDM can be done only after the patient is exposed to the drug, the development of a pre-transplant drug level predictive marker is the need of the hour. In our study, we attempted to develop pharmacogenetics-based dose prediction model for initial dosing of tacrolimus in the South Indian population from Kerala.

We investigated the contribution of five polymorphisms in three genes involved in the metabolism and transport of tacrolimus to the dose-adjusted tacrolimus concentration at three different time points. Consistent with the results of earlier studies (Li et al., 2014; Khan et al., 2020; Mendrinou et al., 2020; Hannachi et al., 2021), we found a strong association of *CYP3A5**3 polymorphism with tacrolimus C_0/D at different time points after transplantation ($p < 0.001$). Differential expression of *CYP3A5* is known to influence the tacrolimus bioavailability in individuals. The A > G substitution at nucleotide *6936* in intron 3 of the *CYP3A5* gene, referred to as the *3 allele, results in a splicing defect and formation of a truncated protein that is, not functional, unlike the A nucleotide or *1 allele which is correlated with a high expression of the *CYP3A5* protein. Carriers of one or more copies of the active or wild allele (*1) are *CYP3A5* expressors and have increased tacrolimus clearance. Individuals with homozygous *3/*3 genotype are *CYP3A5* non-expressors (Chen and Prasad, 2018). Apart from *CYP3A5*, the functional SNPs of *CYP3A4* gene may also influence tacrolimus pharmacokinetics. *CYP3A4**1G (rs2242480) and *CYP3A4**1B polymorphisms are known to have an effect on CYP3A4 enzymatic activity (He et al., 2011; Pączek et al., 2012). Our finding of association of *CYP3A4* polymorphisms with tacrolimus trough levels was also in line with previous reports (Hesselink et al., 2003; Tamashiro et al., 2017). The *CYP3A4* and *CYP3A5* genes are both located in 7q21.1 and the moderate degree of LD between *CYP3A4**1G (rs2242480) and the functional variant *CYP3A5**3 (rs776746) found in our study might also have an influence on the effect of *CYP3A4**1G on tacrolimus C_0/D. We found no association of *CYP3A4* polymorphisms with tacrolimus C_0/D in multivariate regression analysis which suggests that the

strong association found in univariate analysis could be due to this linkage disequilibrium.

We found no association of polymorphisms in the *ABCB1* gene with tacrolimus level in our population. Prasad et al. (2020), along with an association of *CYP3A5* with tacrolimus level and dose requirement, reported association of *ABCB1* G2677T/A polymorphism with tacrolimus level, dose requirement and P-gp expression in North Indians. They also observed a combined effect of these polymorphisms on tacrolimus dose requirement. Studies on the association of *ABCB1* polymorphisms with tacrolimus pharmacokinetics have yielded inconsistent results across different populations. Positive associations have been reported in Turkish (Ciftci et al., 2013), Caucasian (Kravljaca et al., 2016), Chinese (Wei-lin et al., 2006) and Egyptian (Helal et al., 2017) populations. Haufroid et al. (2006) reported a significant effect of *CYP3A5*, but not *ABCB1* polymorphisms on tacrolimus pharmacokinetic parameters in renal transplant recipients from different ethnic groups. The discrepancies observed in these studies may be due to the ethnic differences in ABCB1 genotype and allele frequencies between populations, which might affect the results of genetic association studies (Seven et al., 2014).

In our study, *CYP3A5**3 polymorphism emerged as the single most strongly associated factor determining the dose-adjusted tacrolimus concentration in blood. Using this information, we formulated a simple and easy to compute equation that will help the clinician to calculate, the starting tacrolimus dose per kg body weight to be administered to a patient. The equation was developed using multiple linear regression which also took into account the *CYP3A4* and *ABCB1* polymorphisms, age and gender which may have a minor, but vital role in a patient's tacrolimus concentration. This genotype-based tacrolimus dose calculation may be beneficial in determining the first tacrolimus dose to be given prior to transplantation. This may help in choosing the individualized dose for each patient thereby prevent rejections due to drug under-dosing and adverse effects due to over-dosing.

Several attempts have been made to optimize tacrolimus dosing based on the transplant recipient's genotype. Haufroid et al. (2006) observed no association of tacrolimus pharmacokinetic parameters with *ABCB1* polymorphisms, but found a very significant effect of *CYP3A5* polymorphism early after the first administration of tacrolimus in a group of patients belonging to different ethnic groups. They provided a strong argument for a doubling of initial dose in patients carrying at least one *CYP3A5**1 allele identified by genotyping patients before transplantation. The French Tactique trial (Thervet et al., 2010) found that the initial tacrolimus dosing based on *CYP3A5* genotype led to significantly more patients reaching the target drug range 3 days after the start of treatment compared to typical, bodyweight-based tacrolimus dosing. The Clinical Pharmacogenetics Implementation Consortium (CPIC) (Birdwell et al., 2015) recommended increasing the starting dose by 1.5–2 times the recommended starting dose in patients *CYP3A5* intermediate (*1/*3 genotype) or extensive metabolizers (homozygous *1/*1 genotype), though total starting dose should not exceed 0.3 mg/kg/day. They recommended that TDM should also be used to guide dose adjustments. A new classification and regression tree model was developed by Wang et al., in 2020 to establish the starting dose of tacrolimus based on the *CYP3A5* genotype and hemoglobin values in Chinese renal transplant recipients. Our results do not contradict these previous attempts to formulate guidelines for genotype-based tacrolimus dosing.

We found that *CYP3A5* expressors (*1/*1 + *1/*3) had an increased chance of rejection than non-expressors (*3/*3). *CYP3A5* expressors, due to their high tacrolimus clearance, have low drug trough concentrations, which may lead to inadequate immunosuppression resulting in rejection. Achieving target blood tacrolimus concentrations during the early post-transplantation period is critical in preventing rejection and improving graft survival. Our findings will help in early identification of patients at a higher risk of developing rejection and possibly prevent rejection by strengthening their immunosuppression.

NODAT, defined as the development of diabetes for the first time after transplantation is a common undesired consequence following solid organ transplantation. It is associated with reduced patient and graft survival and an increased cardiovascular risk (Kasiske et al., 2003; Cosio et al., 2005; Pham et al., 2011). The prevalence of NODAT in solid transplant recipients has been reported to vary from 2 to 53% (Choudhury et al., 2019). We observed a high prevalence of NODAT (46.2%) in our study population, which was not quite unexpected, given Kerala's high incidence of type 2 diabetes mellitus (21.9%) (Vijayakumar et al., 2019). We found that *CYP3A5* non-expressors had an increased risk of developing NODAT than expressors (p = 0.018), owing to their higher tacrolimus bioavailability. The calcineurin inhibitors, tacrolimus and cyclosporine are known to have diabetogenic effects (Heisel et al., 2004). Tacrolimus have been reported to be associated with a higher risk for impaired glucose tolerance (IGT) and NODAT compared to cyclosporine (Reisæter and Hartmann, 2001; Gourishankar et al., 2004). Early identification of patients at a higher risk of developing NODAT may help mitigate NODAT by lifestyle and pharmacological interventions.

Our observation of strong inter-population variations on comparing allele frequencies of *CYP3A5, CYP3A4*, and *ABCB1* gene polymorphisms in our South Indian study population with 1000 genomes Phase 3 populations belonging to different ethnic groups shows that our population is unique with respect to the allele frequency distribution. These inter-ethnic differences in allele frequencies of drug metabolizer and transporter genes emphasize the importance of formulating population-specific dose prediction algorithms based on these gene polymorphisms to draw results of clinical relevance. Population-specific genetic backgrounds should also be taken into account while carrying out pharmacogenetic analyses and clinical trials.

To summarize, we developed a multiple linear regression model-based equation specific to the South Indian population from Kerala to calculate the initial tacrolimus dose/kg to attain optimal initial post-transplant period tacrolimus level. Genotype-guided initial tacrolimus dosing would help transplant recipients achieve optimal tacrolimus levels and thus prevent the adverse

effects due to overdose and rejection due to inadequate dose. We envision to carry out further randomized control trial based on this genotype-dependent dosing for tacrolimus efficacy and toxicity minimization. We also found that *CYP3A5* expressors had an increased chance of rejection than non-expressors and non-expressors had an increased risk of developing NODAT than expressors. Our findings will help the clinicians to identify patients at a higher risk of developing rejection and NODAT at an early stage and possibly prevent these by pharmacological interventions and lifestyle modifications.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Human Ethics Committees of Rajiv Gandhi Centre for Biotechnology and Government Medical College, Thiruvananthapuram. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LS and RN contributed to conception and design of the study. LS carried out sample collection, genotyping and statistical analysis. NG coordinated the clinical part of the study. LS wrote the manuscript and all authors contributed to manuscript revision, read, and approved the final manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2021.726784/full#supplementary-material

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