

# Phenoconversion and *in vivo* phenotyping of hepatic cytochrome P450: Implications in predictive precision medicine and personalized therapy

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## Abstract

Drug dose efficacy/toxicity depends on a number of factors including genetic and nongenetic factors, a pre-existing disease, and coadministration of other substances and drugs. Cytochrome P450 (CYP) proteins play a crucial role in drug metabolism where they catalyse a number of Phase I oxidation reactions. Concurrently administered drugs and substances, besides the CYP genotype are crucial and can induce/inhibit the CYP activity, thus affecting drug biotransformation and its bioavailability, compromising with drug efficacy, or even causing toxicity due to slow metabolism. Hepatic CYP is particularly important as it metabolizes about ¾ of all drugs. Determining the metabolite/drug ratio (*in vivo* CYP phenotyping) can be an important tool that can help in drug dose optimization for the drugs metabolized by specific CYPs as the genotype may not always reflect the true enzyme activity. Clinically important CYP isoforms commonly reported in drug oxidation reactions and which mainly include CYP3A4/5, CYP2C19, CYP2C9 and CYP2D6 need to be analysed for their activity *in vivo*, in at least the cases of unpredictable treatment outcomes. The activity levels of other less commonly reported but no less important CYPs, such as CYP2B6, one of the most polymorphic human CYP involved in the metabolism of artemisinin, bupropion, cyclophosphamide, efavirenz, ketamine and methadone, and reported for its high inter-individuals and within-individual variability may also be determined on a case-to-case basis. This review highlights the variations in CYP activity due to various reasons and the importance of *in vivo* phenotyping over genotype in ascertaining drug bioavailability and dose optimization, implicating metabolite/drug ratio determination for personalized treatment of especially chronic liver disease patients.

**Keywords:** CYP phenotyping; drug metabolism; personalized therapy; precision medicine.

## Introduction

The efficacy or toxicity of a therapeutic dose is affected by a host of factors, which include drug absorption and its binding to plasma proteins, hepatic extraction ratio, portal-systemic shunting, biliary excretion, enterohepatic circulation, renal clearance, and genetic variations in proteins involved in drug metabolism, including gene polymorphisms and epigenetic mechanisms.<sup>[1,2]</sup> Cytochrome P450 (CYP) is a system of proteins/isoenzymes expressed as membrane-bound proteins, mostly in the endoplasmic reticulum of the liver cells. The group of proteins primarily catalyses oxidative reactions of Phase I metabolism and drug elimination reactions in humans.<sup>[3]</sup> The system is responsible for the biotransformation of a number of drugs and xenobiotics in humans and has emerged as an important determinant of the pharmacological properties of drugs and their adverse interactions and reactions.<sup>[4]</sup>

In humans, the liver is the primary organ for drug/xenobiotics metabolism. CYPs are the most well-known drug-metabolizing enzymes expressed in the liver.<sup>[3]</sup> Hepatic CYP (hCYP) is particularly important as, in their lifetimes, humans are exposed to 1–3 million foreign substances, including drugs.<sup>[5]</sup> It can biotransform most of these substances, including approximately ¾ of all drugs in clinical use.<sup>[6]</sup> An understanding of the qualitative and quantitative aspects of CYP metabolism is important for a better insight into drug metabolism and its pharmacokinetics in normal and disease conditions, especially in patients in advanced stages of liver diseases. CYP is also crucial for drug development. The anticancer stilboestrol diphosphate and cyclophosphamide were designed to produce active drug moiety upon activation by the CYP.<sup>[7,8]</sup> This review highlights the role of *in vivo* CYP phenotyping in clinical practice, especially in situations of mismatch between the genotype-based prediction of an individual and the true capacity of the enzyme to metabolize a drug under the influence of extrinsic or nongenetic factors.

## CYP/P450

CYP or P450 is a superfamily of heme-containing redox proteins or monooxygenases in humans, animals, plants, fungi, protists, and bacteria.<sup>[3,9–11]</sup> Members of the CYP family catalyse the oxidative biotransformation of a range of substances, converting their lipophilic centres to hydrophilic centres to increase water solubility and facilitate the elimination of the converted metabolite in urine or bile.<sup>[12]</sup> Poor activity of CYP for its substrate/drug slows down the biotransformation, causing an accumulation of the drug and drug toxicity. Rapid biotransformation,

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on the other hand, may render the normal dose ineffective. In general, biotransformation of a drug decreases its therapeutic efficacy.<sup>[13]</sup> However, prodrugs are more active upon biotransformation.<sup>[14]</sup>

CYP isozymes, in addition to their role in Phase 1 oxidation, catalyze C-hydroxylation, heteroatom oxygenation, heteroatom release (dealkylation), epoxide formation, and a number of more complex reactions and contribute to the synthesis of cholesterol, steroids, prostacyclins, thromboxane A<sub>2</sub>, and the degradation of vitamin D.<sup>[3,15–18]</sup> In humans, CYP is primarily associated with the endoplasmic reticulum membrane but also resides in mitochondrial and plasma membranes. It may have specific roles such as protecting brain areas from bilirubin neurotoxicity.<sup>[3,19–22]</sup> A CYP isoform can metabolize only one or a very limited number of substrates (e.g., CYP19), or may act on multiple substrates.<sup>[23]</sup> In humans, three families of CYP (CYP1, 2, and 3) are responsible for about 75% of all Phase I drug reactions and metabolism of a huge number of dietary constituents and endogenous chemicals.<sup>[24]</sup> Families like CYP51 may not be present in some species.<sup>[11]</sup> The Human Genome Project has listed 57 CYP-encoding genes divided among 18 families and 43 subfamilies.<sup>[25,26]</sup> An analysis of the human genome sequence identified >59 CYP gene-like sequences that lacked regulatory sequences for RNA/protein synthesis. In total, over 300,000 CYP sequences have been mined and preserved in databases, including >16,000 plant CYPs.<sup>[27]</sup> Well over 41,000 CYP sequences have been assigned nomenclature, and a majority of the remainder has been sorted by BLAST searches into clans, families, and subfamilies.<sup>[27]</sup> The University of Tennessee Health Science Centre CYP Homepage is a comprehensive resource on CYP nomenclature and sequence information.<sup>[26]</sup> CYP is called P450 because it absorbs 450 nm when bound to CO. CYP families are identified by a number (e.g., CYP1) followed by a subfamily letter (e.g., CYP1A). An individual protein or isoform is differentiated from another by placing a number after the subfamily (e.g., CYP1A1). Among different forms of CYP, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, and CYP3A5 are involved in the metabolism of most drugs. CYP3A4 and CYP2D6 alone contribute to >50% of all CYP-related drug metabolism.<sup>[3]</sup>

### Clinically Relevant P450 Proteins

Functional genomics/pharmacogenomics of P450 report the role of CYP1, CYP2, and CYP3 in about 78% of all hepatically-cleared drugs.<sup>[28]</sup> CYP3A4/5 contributes to the clearance of a majority of these drugs (37%), followed by CYP2C9 (17%), CYP2D6 (15%), CYP2C19 (10%), CYP1A2 (9%), CYP2C8 (6%), and CYP2B6 (4%).<sup>[28]</sup> In humans, an estimated 90% of drugs involve 6 CYPs (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5).<sup>[29]</sup> CYP3A4/5, CYP2C9, CYP2D6, and CYP2C19 account for about 79% of all drug oxidation reactions in humans.<sup>[28]</sup> CYP3A4 and CYP2D6 are the most significant.<sup>[30]</sup> Another CYP, CYP2B6, is important for its role in the metabolism of anticancer drugs cyclophosphamide and ifosfamide. CYP2B6 is one of the most polymorphic CYPs in humans. It has been reported in the metabolism of common drugs like artemisinin, bupropion, cyclophosphamide, efavirenz, ketamine, and methadone.<sup>[31]</sup> CYP2B6 is induced by phenobarbital and cyclophosphamide, and inhibited by secobarbital.<sup>[32,33]</sup>

### Variations in CYP Activity and Drug-Drug Interactions

CYP gene polymorphisms, its epigenetic regulation, and environmental cues, including the CYP inhibitors and inducers, can significantly alter an individual's response to a drug.<sup>[18,34]</sup> Variations in CYP activity due

to genetic variability in CYP genes arising as a result of mutation and alleles can significantly impact drug therapy in individuals and people of different ethnicities.<sup>[35,36]</sup> At the genetic level, the ability to metabolize a drug is determined by the pairing of CYP alleles. An allele can be functional (Normal or Wild-type, predominant in a population), or defective (Variant, diminished or no activity). Two Wild-type generally confer a 'normal' rate of metabolism (Extensive metabolizers, EM phenotype), as opposed to variants. Individuals with two variant alleles have little/no activity (Poor metabolizers, PM phenotype), while those inheriting one of each allele show intermediate activity (Intermediate metabolizer, IM phenotype). Gene amplification/duplication (>2 copies of Wild-type) confers a faster metabolism (Ultrarapid metabolizers, UM phenotype).<sup>[37,38]</sup> In short, the PM phenotype shows little or no activity, IM demonstrates decreased activity, and UM exhibits increased activity relative to the normal metabolizer (NM) phenotype.<sup>[39]</sup> PM and UM can significantly alter a drug dose response. PM type usually suffers more adverse reactions at normal dose levels, either due to gene deletion or due to being homozygous for functionally variant alleles.<sup>[40]</sup> On the other hand, UM often fails to respond to a normal drug dose. IM, as discussed, are heterozygous for variant alleles, and a normal or extensive metabolizer (EM) has two functionally competent alleles.<sup>[6]</sup>

Interesting case scenarios have been reported due to genetic variations in CYP. In a case of fatal morphine toxicity in a breast-fed baby, an extra copy of Wild-type CYP2D6 in the mother on codeine reportedly caused a 50% increase in morphine, hence morphine toxicity in the breastfed baby.<sup>[41,42]</sup> CYP2D6 converts codeine to morphine. Variable levels of endoxifen, the active metabolite of tamoxifen, have been reported in extensive, intermediate, and poor metabolizers due to variations in CYP2D6.<sup>[43]</sup> Carriers of CYP2C9\*2/\*3 alleles require a lower dose of warfarin to avoid supratherapeutic anticoagulation. Similarly, the CYP2D6 loss-of-function genotype is at risk of ventricular arrhythmia when treated with thioridazine. Acute dystonic reactions in patients with homozygous CYP2D6 on metoclopramide, and even deaths have been reported due to CYP variants (CYP2B6—methadone metabolism, and CYP2D6—fluoxetine metabolism).<sup>[44,45]</sup> An estimated 5–10% of Caucasians have a genetically determined decreased capacity for CYP2D6.<sup>[46]</sup> Similarly, the efficacy of prodrugs is significantly altered by a CYP variant. The US FDA has listed a number of pharmacogenomic biomarkers of clinical importance, including 72 CYP2D6, 25 CYP2C19, 17 CYP2C9, 3 CYP2B6, and one each of CYP1A2 and CYP3A5 (<https://www.fda.gov/drugs/science-and-research-drugs/table-pharmacogenomic-biomarkers-drug-labeling>).

In addition to genetics (genotype/diplotype), significant differences in the metabolic capacities of individuals may arise from a combination of non-genetic extrinsic factors such as lifestyle, smoking, alcohol, and diet, resulting in phenoconversion.<sup>[30,47,48]</sup> Age, hormonal status, drugs, co-administration of drugs in combination therapy, and the presence of a specific clinical condition, particularly a chronic disease of the liver, have also been reported as determinants of CYP phenoconversion.<sup>[49]</sup> In a small study in a local cohort in India, 20% of the population was PM type for the drugs metabolized by CYP2B6.<sup>[31]</sup> Diet, drugs, and xenobiotics, including pesticides, are particularly important as these substances not only act as substrates for CYP but may also modulate its activity.<sup>[50,51]</sup> Imatinib (used for the treatment of leukaemia) is both a substrate and an inhibitor of CYP3A4.<sup>[52]</sup> Co-administration of imatinib with another CYP3A inhibitor, simvastatin, can markedly increase the plasma concentrations of CYP3A4 substrates.<sup>[53]</sup> Similarly, the concurrent use of tacrolimus and omeprazole (substrates for CYP2C19 and

Table 1. P450 in common drug-drug interactions

CYP	Drug/Drugs	Interacting substance/drugs	Category
CYP3A4	Ethinylestradiol contraceptives <sup>[62]</sup>	Carbamazepine, phenytoin, phenobarbital <sup>[63,64]</sup>	Inducers
	Simvastatin <sup>[65]</sup>	Erythromycin, clarithromycin, telithromycin <sup>[66]</sup>	Inhibitors
	Prednisone <sup>[67]</sup>	Diltiazem, <sup>[68,69]</sup> verapamil <sup>[70,71]</sup>	Inhibitors
	Buspirone, tandospirone <sup>[72,73]</sup>	Ketoconazole, <sup>[73]</sup> Grape fruit juice <sup>[74]</sup>	Inhibitors
CYP2D6	Risperidone <sup>[75]</sup>	Fluoxetine <sup>[76]</sup>	Inhibitor
	Tramadol <sup>[77,78]</sup>	Paroxetine <sup>[79]</sup>	Inhibitor
CYP2C9	Warfarin <sup>[80,81]</sup>	Amiodarone, desethylamiodarone <sup>[82]</sup>	Inhibitors*
		Metronidazole <sup>[83]</sup>	Inhibitor

\*: Also inhibit CYP3A4

CYP3A4) increases supratherapeutic toxicity risk.<sup>[18]</sup> On the other hand, CYP inducers result in rapid biotransformation and drug elimination, as reported in the case of temsirolimus (metabolized by CYP3A4). The dose of temsirolimus may require an increase when concurrently administered with a CYP3A4 inducer (fosphenytoin or phenytoin). Rifampin is another CYP3A inducer which decreases the availability of imatinib. Treatment failure in poor metabolizers on prodrugs is also a matter of concern.<sup>[54,55]</sup>

CYP-mediated metabolism can be highly specific for certain drugs, as in the case of metoprolol, which can be used as a substrate only by a specific CYP (CYP2D6). In other instances, drugs like warfarin can be metabolized by multiple isoforms (CYP1A2, CYP2D6, and CYP3A4).<sup>[56,57]</sup> The antifungal drug terbinafine is metabolized by one isoform but inhibits another.<sup>[58]</sup> These heterogeneities in CYP function may lead to complications in therapy. In an example, an elderly stable patient on warfarin reported difficulty with coagulation when fluconazole was added to her prescription to contain recurrent vulvovaginal candidiasis.<sup>[30]</sup> In the literature, a number of common drug-drug interactions have been reported, involving mostly CYP3A4, CYP2D6, and CYP2C9 (Table 1). Comprehensive information on drug interactions involving CYP can be found in Drug Interactions Flockhart Table™. The table lists eight CYPs which metabolize 382 drugs. These include CYP3A4 (166), CYP2D6 (66), CYP2C19 (41), CYP2C9 (36), CYP1A2 (34), CYP2B6 (15), CYP2C8 (12), and CYP2E1 (12) (numbers in parentheses indicate the number of listed drugs in that category). It is, therefore, important to understand CYP-mediated drug metabolism for the drugs that behave as CYP substrates and also modulate (inhibit/induce) its activity, for example, CYP3A4.<sup>[51,59–61]</sup>

Hepatic CYP and Liver Injury

CYP is present in all tissues, including the liver, lung, plasma, kidney, intestinal wall, and other body organs. However, the liver is the major organ and site for most CYP activity. CYP inhibition usually results in more adverse reactions at a normal dose level due to slower drug biotransformation and, therefore, increased drug accumulation and toxicity, inflicting damage to the liver. The condition worsens in chronic liver disease patients. Hepatic injury has further been reported to selectively modulate CYP activity.<sup>[84]</sup> In the case of compounds with little or no pharmacological activity (prodrugs), P450 may convert an inactive compound (prodrug) into a pharmacologically-active metabolite (drug). In other instances, CYP-mediated activation of substances like acetaminophen and halothane is a common cause of drug-induced

liver injury (DILI). Aflatoxin biotransformation is also mediated by the CYP.<sup>[85]</sup> Aflatoxins have been reported to cause a 30-fold increase in cancer risk in hepatitis B-positive persons. Hepatotoxic chemicals like thioacetamide affect CYP activity directly, independently of the cirrhosis induced by thioacetamide.<sup>[86]</sup> In the liver, CYP constitutes a major component of the hepatic microsomal ethanol-oxidizing system (MEOS). CYP2E1 metabolizes ethanol, endogenous acetone, acetol, steroids, and PUFA, leading to an increased generation of reactive oxygen species (ROS) and ROS stress—a risk factor for non-alcoholic, and also alcoholic, liver diseases.<sup>[87]</sup> CYP2E1 is also known to be induced by lipids and to mediate autophagy inhibition by ethanol. It can activate several pre-carcinogens, and hence has been postulated as a cofactor in hepatocellular carcinoma (HCC).<sup>[88]</sup> However, few reports suggest a suppressive role of CYP2E1 in HCC, possibly due to its ability to manipulate the Wnt/Dvl2/β-Catenin pathway via ROS.<sup>[89]</sup>

CYP in Liver Cirrhosis and Other Chronic Conditions

Chronic liver diseases have been reported to impair CYP function.<sup>[90,91]</sup> Alcoholic and viral liver diseases, cholestasis, and cirrhosis of the liver can significantly alter hCYP function, including up to a 50% inhibition of hCYP1A2 activity in cirrhotic patients.<sup>[91–93]</sup> In another study, a 20–40% reduction in CYP/mg liver tissue has been reported in liver cirrhosis.<sup>[94]</sup> CYP1A2, CYP2C19, and CYP3A4 in particular are sensitive to liver diseases.<sup>[90]</sup> Chronic hepatitis, liver cirrhosis, and HCC cause a significant change in CYP2C19, affecting the biotransformation of drugs metabolized by CYP2C19.<sup>[84,95]</sup> Investigations on hCYP are important as liver disease patients, especially cirrhosis and cancer patients, receive multiple medications for treatment and associated comorbidities.<sup>[96]</sup> Drugs like midazolam, chlorzoxazone, and debrisoquine cause a significant diminution of CYP3A4, CYP2E1, and CYP2D6 activity, respectively. Liver cirrhosis particularly affects the pharmacokinetics of drugs with high hepatic extraction rates (omeprazole, metoprolol, midazolam), while drugs with low-to-moderate extraction rates (caffeine, efavirenz, flurbiprofen) are less affected. The effect of disease on CYP is CYP-specific. CYPs like CYP1A2, 2B6, 2C19, 2D6, and 3A decrease in chronic disease/Child C cirrhosis, but CYP2C9 does not, precluding the empirical use of liver-function parameters as surrogate CYP markers.<sup>[97,98]</sup>

Immune-Mediated Hepatotoxicity

A range of antidrug and autoantibodies causing DILI are immune-mediated.<sup>[99]</sup> Tienilic acid or ticrynafen and isoniazid (INH) bind to CYP, initiating immune hepatotoxicity via anti-CYP antibodies.<sup>[100]</sup> CY-



P2D6 is a major autoantigen in type 2 autoimmune hepatitis.<sup>[101]</sup> In INH-induced hepatotoxicity, anti-INH antibodies can be detected in patients' sera along with anti-CYP2E1, anti-CYP3A4, and anti-CYP2C9.<sup>[99]</sup> INH forms covalent adducts with CYP2E1, CYP3A4, and CYP2C9. However, no antibody is seen in INH-treated controls without significant liver injury.

### Gut Microbiota and CYP

Microbiome is a functional modifier of CYP metabolism.<sup>[102]</sup> Several studies in the literature indicate this relationship between gut microbiota and host drug metabolism.<sup>[103–105]</sup> The metabolic activity of hCYP3A is reported to be altered by gut microbiota, causing a significant accumulation of the substrate/drug metabolized by it (midazolam).<sup>[106–111]</sup> Drugs can be directly metabolized by the gut microbiota.<sup>[112]</sup> Antibiotics can also lower hCYP3A and 2B.<sup>[103,113]</sup> CYP3A4 (CYP3A11 in mice) has been implicated in the first-pass metabolism of many antibiotics and chemotherapeutics (irinotecan).<sup>[111,114–116]</sup> CYP3A constitutes approximately 30% of all hCYPs and is responsible for about half of all hepatic and intestinal xenobiotics metabolism. Clinical conditions like gastrectomy can increase hCYP3A by increasing lithocholic acid-producing enteric bacteria in mice.<sup>[107]</sup> Studies on germ-free mice show a significant alteration in CYP, such as CYP3A11.<sup>[117,118]</sup> Gut-associated microbiome-derived dietary polyphenol ellagic acid and its colonic metabolites, urolithin-A and B, can modulate CYP1A1 and 1B1, and CYP3A5. More similar findings suggest an important role of 'microbe-drug' interactions on CYP and drug metabolism.

### CYP and Drug Resistance

Drugs absorbed by the small intestine often take the CYP3A4 route. CYP3A4 inhibitors (grapefruit juice) may act locally on the small intestine and inhibit enterocyte CYP3A4.<sup>[119]</sup> In *Drosophila melanogaster*, resistance against  $\alpha$ -amanitin is attributed to CYP.<sup>[120–122]</sup> Whole-genome microarray of  $\alpha$ -amanitin-resistant fly stock showed a constitutive up-regulation of CYP.<sup>[122]</sup> Such a property needs to be studied in humans.

### Phenoconversion, Therapeutic Dose Efficacy and *in vivo* Phenotyping

Mismatch between genotype-based prediction of an individual and her/his true capacity to metabolize a drug under the influence of extrinsic or non-genetic factors (phenoconversion) is not an uncommon phenomenon.<sup>[123]</sup> Phenoconversion has been found to convert a genotypically EM into a PM phenotype, significantly impacting the genotype-based clinical presumption and influencing any potential for advancing the prospects of precision or personalized therapy and predictive precision medicine.<sup>[124]</sup> Population-based studies in phenoconverted individuals with a genotype-phenotype mismatch have been reported in literature, such as CYP2B6 and CYP2D6.<sup>[31,125]</sup> In routine clinical practice, phenoconversion involving CYP can be a major issue, compelling the inclusion of lifestyle, living environment, hormonal balance, diseases, and drug-drug interactions in therapeutic dose decisions and disease management. It is important to ensure that the right patient receives the right treatment at the right time, in the right dose, and via the right mode for better clinical outcomes.

Age and sex-linked or hormonal variations in CYP have also been reported in the literature as determinants of interindividual differences in drug pharmacology, pharmacokinetics, and pharmacody-

namics, particularly in the elderly population (slow metabolism), neonates (where several enzyme systems including the CYP system are not fully developed), and diseased individuals.<sup>[16,126–131]</sup> In a typical case of CYP1A2 polymorphism, a low inducibility CYP1A2 genotype has been reported associated with an increased risk of myocardial infarction, independent of smoking status, indicating the possibility of a CYP1A2 substrate that is detoxified rather than activated.<sup>[132]</sup> The effect of age and hormonal status of an individual on CYP has been extensively reviewed in the literature for anticholinergic drugs, which show a strong association with these variables.<sup>[133]</sup> General prescribing guidelines caution against the use of anticholinergic medications in older individuals. More recently, these drugs have been identified as a potential risk for developing dementia.<sup>[134,135]</sup> Intriguingly, women often experience increased drug exposure, a likely contributor to more adverse reactions in women than in men.<sup>[136–138]</sup> The age-related changes which may increase anticholinergic drug exposure include pseudocapillarization of the liver sinusoidal endothelial cells and a roughly 3.5% decline in CYP content for each decade of life.<sup>[133]</sup> CYP2D6 and CYP2C19 PM phenotypes are crucial in modifying anticholinergic drug exposure in a significant proportion of the population, explaining higher plasma levels of anticholinergic drugs and an increased drug dose exposure at a normal dose level in poor metabolizers.<sup>[128,133,139]</sup> Susceptibility to drug-induced liver injury (DILI) also increases in old age.<sup>[140]</sup> However, data is scanty on the susceptibility of the older population to DILI due to limited participation of older individuals in clinical trials, warranting proper vigilance and postmarketing surveillance in this population.

Concomitant intake of potentially interacting substances/drugs, an existing chronic disease, or an environmental cue could be among common non-genetic modifiers of hCYP activity. Diseases generally have a negative effect on drug metabolism. Advanced-stage liver disease patients are particularly vulnerable to the consequences of impaired drug metabolism due to CYP, especially CYP3A, CYP2C19, and CYP1A, while CYP2C9, CYP2D6, and CYP2E1 are less affected. Pro-inflammatory cytokines (IL-6, IL-1 $\beta$ , TNF- $\alpha$ ) downregulate many drug-metabolizing enzymes.<sup>[141]</sup> In addition, pharmacokinetics vary widely in children (due to ontogeny), as in old age individuals and individuals suffering from chronic liver disease. Studies on CYP phenotyping in children to assess the correlation between CYP genotype and phenotype in real-life settings have shown significant differences in actual and genotype-based predicted values, depending on the assessed CYP.<sup>[142]</sup> In several cases in this study, the phenotype did not correspond to the genotype. It is, therefore, important to determine the actual activity of a CYP in physiological conditions and under the influence of a combination of extrinsic factors, including co-administration of drugs, diet, smoking, alcohol consumption, etc. In literature, significant discrepancies have been reported between genetically-predicted and actual activity levels of hCYP1A2, hCYP2B6, hCYP2C9, hCYP2C19, hCYP2D6, and hCYP3A4.<sup>[30,47,48]</sup> The actual activity level determination or *in vivo* phenotyping of CYP isoforms can be done by measuring the plasma concentration of the drug (metabolized by that CYP) and its metabolite. The metabolite/drug ratio in plasma is then used to calculate the actual enzyme activity *in vivo*, independent of the genotype.<sup>[47]</sup>

In literature, the *in vivo* phenotyping of CYP isoforms involved in the metabolism of anti-HIV and antitubercular drugs has been reported and suggested to help determine therapeutic dose levels in HIV/AIDS-

TB coinfections.<sup>[47]</sup> In this study, the plasma metabolite/drug ratio of three drugs, bupropion, losartan, and dapsone, metabolized by CYP2B6, CYP2C9, and NAT2, respectively, was determined in human volunteers by administering a cocktail of these drugs. CYP2B6 is the main catalyst of the anti-HIV drug efavirenz, while CYP2C9 has a significant association with antitubercular drug-induced reactions. NAT2 is involved in the metabolism of the antitubercular drug isoniazid. This small study in a local cohort reported a significant number (20.56%) of PM phenotype for the drugs metabolized by CYP2B6, highlighting the clinical relevance of *in vivo* phenotyping and therapeutic dose optimization.<sup>[31]</sup> Other examples where *in vivo* phenotyping can be used in therapeutic dose decisions may include CYP2A6, a CYP isoform which is induced by alcohol in a CYP2E1-dependent Nrf2-regulated process. Intriguingly, alcohol also induces CYP2A5, a mouse analogue, but, unlike CYP2E1, CYP2A5 protects against alcohol toxicity.<sup>[143]</sup> In literature, most protocols focus on 8 CYP isoforms, namely CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5 for genetic analysis, but *in vivo* phenotyping data for these enzymes are scanty and need to be looked upon for effective personalized treatment in vulnerable populations or those suffering from liver disease affecting CYP activity.

## Conclusions

Cytochrome P450/CYP proteins metabolize a number of drugs and substances. Phenoconversion of a genotypically extensive metabolizer to a poor metabolizer phenotype, as a result of a combination of non-genetic reasons, may significantly alter the genotype-based clinical presumption, compromise drug efficacy, and affect any potential for advancing the prospects of precision or personalized therapy and predictive precision medicine. Population-based studies in phenoconverted individuals with a genotype-phenotype mismatch have been reported for CYPs like CYP2B6 and CYP2D6. Determining the actual activity level of a CYP by determining the plasma metabolite/drug ratio in an individual in physiological conditions (*in vivo* phenotyping) can help advanced physicians to take appropriate measures in drug dose optimization and disease management in cases of treatment failure and drug dose toxicity.

## Recommendations

Phenoconversion due to non-genetic reasons and *in vivo* phenotyping of hepatic CYP by calculating the plasma (drug) metabolite/drug ratio in vulnerable individuals, such as those suffering from a chronic liver ailment or taking a combination therapy, can be beneficial in making drug dose decisions in cases of treatment failure or drug toxicity at a normal dose level. *In vivo* CYP phenotyping can be a useful tool in the hands of advanced physicians in optimizing drug dose in personalized therapy.

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