Review

In vivo CYP phenotyping and predictive precision medicine

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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.

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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.[1,2] 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.[3] 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.[4]

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.[3] Hepatic CYP (hCYP) is particularly important as, in their lifetimes, humans are exposed to 1–3 million foreign substances, including drugs.[5] It can biotransform most of these substances, including approximately ¾ of all drugs in clinical use.[6] 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.[7,8] 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.[3,9–11] 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.[12] Poor activity of CYP for its substrate/drug slows down the biotransformation, causing an accumulation of the drug and drug toxicity. Rapid biotransformation,

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

CYP isozymes, in addition to their role in Phase 1 oxidation, catalyse 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 A2, and the degradation of vitamin D.[3,15–18] 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.[3,19–22] A CYP isoform can metabolize only one or a very limited number of substrates (e.g., CYP19), or may act on multiple substrates.[23] 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.[24] Families like CYP51 may not be present in some species.[11] The Human Genome Project has listed 57 CYP-encoding genes divided among 18 families and 43 subfamilies.[25,26] 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.[27] 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.[27] The University of Tennessee Health Science Centre CYP Homepage is a comprehensive resource on CYP nomenclature and sequence information.[26] 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.[3]

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.[28] 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%).[28] In humans, an estimated 90% of drugs involve 6 CYPs (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A4, and CYP3A5).[29] CYP3A4/5, CYP2C9, CYP2D6, and CYP2C19 account for about 79% of all drug oxidation reactions in humans.[28] CYP3A4 and CYP2D6 are the most significant.[30] Another CYP, CYP2B6, is important for its role in the metabolism of anticancer drugs cyclophosphamide and ifosphamide. 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.[31] CYP2B6 is induced by phenobarbital and cyclophosphamide, and inhibited by secobarbital.[32,33]

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.[18,34] 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.[35,36] 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).[37,38] 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.[39] 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.[40] 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.[6]

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.[41,42] 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.[43] 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).[44,45] An estimated 5–10% of Caucasians have a genetically determined decreased capacity for CYP2D6.[46] 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.[30,47,48] 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.[49] In a small study in a local cohort in India, 20% of the population was PM type for the drugs metabolized by CYP2B6.[31] 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.[50,51] Imatinib (used for the treatment of leukaemia) is both a substrate and an inhibitor of CYP3A4.[52] Co-administration of imatinib with another CYP3A inhibitor, simvastatin, can markedly increase the plasma concentrations of CYP3A4 substrates.[53] 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[62]	Carbamazepine, phenytoin, phenobarbital[63,64]	Inducers
	Simvastatin[65]	Erythromycin, clarithromycin, telithromycin[66]	Inhibitors
	Prednisone[67]	Diltiazem,[68,69] verapamil[70,71]	Inhibitors
	Buspirone, tandospirone[72,73]	Ketoconazole,[73] Grape fruit juice[74]	Inhibitors
CYP2D6	Risperidone[75]	Fluoxetine[76]	Inhibitor
	Tramadol[77,78]	Paroxetine[79]	Inhibitor
CYP2C9	Warfarin[80,81]	Amiodarone, desethylamiodarone[82]	Inhibitors*
		Metronidazole[83]	Inhibitor

*: Also inhibit CYP3A4

CYP3A4) increases supratherapeutic toxicity risk.[18] 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.[54,55]

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).[56,57] The antifungal drug terbinafine is metabolized by one isoform but inhibits another.[58] 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.[30] 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.[51,59–61]

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.[84] 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.[85] 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.[86] 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.[87] 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).[88] 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.[89]

CYP in Liver Cirrhosis and Other Chronic Conditions

Chronic liver diseases have been reported to impair CYP function.[90,91] 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.[91–93] In another study, a 20–40% reduction in CYP/mg liver tissue has been reported in liver cirrhosis.[94] CYP1A2, CYP2C19, and CYP3A4 in particular are sensitive to liver diseases.[90] Chronic hepatitis, liver cirrhosis, and HCC cause a significant change in CYP2C19, affecting the biotransformation of drugs metabolized by CYP2C19.[84,95] Investigations on hCYP are important as liver disease patients, especially cirrhosis and cancer patients, receive multiple medications for treatment and associated comorbidities.[96] 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.[97,98]

Immune-Mediated Hepatotoxicity

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

P2D6 is a major autoantigen in type 2 autoimmune hepatitis.[101] In INH-induced hepatotoxicity, anti-INH antibodies can be detected in patients' sera along with anti-CYP2E1, anti-CYP3A4, and anti-CYP2C9.[99] 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.[102] Several studies in the literature indicate this relationship between gut microbiota and host drug metabolism.[103–105] 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).[106–111] Drugs can be directly metabolized by the gut microbiota.[112] Antibiotics can also lower hCYP3A and 2B.[103,113] CYP3A4 (CYP3A11 in mice) has been implicated in the first-pass metabolism of many antibiotics and chemotherapeutics (irinotecan).[111,114–116] 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.[107] Studies on germ-free mice show a significant alteration in CYP, such as CYP3A11.[117,118] 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.[119] In Drosophila melanogaster, resistance against α-amanitin is attributed to CYP.[120–122] Whole-genome microarray of α-amanitin-resistant fly stock showed a constitutive upregulation of CYP.[122] 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.[123] 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.[124] Population-based studies in phenoconverted individuals with a genotype-phenotype mismatch have been reported in literature, such as CYP2B6 and CYP2D6.[31,125] 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 pharmacodynamics, particularly in the elderly population (slow metabolism), neonates (where several enzyme systems including the CYP system are not fully developed), and diseased individuals.[16,126–131] 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.[132] 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.[133] 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.[134,135] Intriguingly, women often experience increased drug exposure, a likely contributor to more adverse reactions in women than in men.[136–138] 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.[133] 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.[128,133,139] Susceptibility to drug-induced liver injury (DILI) also increases in old age.[140] 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-1β, TNF-α) downregulate many drug-metabolizing enzymes.[141] 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.[142] 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.[30,47,48] 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.[47]

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.[47] 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.[31] 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.[143] 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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References

1. Koo SH, Lo YL, Yee JY, Lee EJ. Genetic and/or non-genetic causes for inter-individual and inter-cellular variability in transporter protein expression: Implications for understanding drug efficacy and toxicity. Expert Opin Drug Metab Toxicol 2015;11(12):1821-1837.
2. Dang NL, Matlock MK, Hughes TB, Swamidass SJ. The metabolic rainbow: Deep learning phase I metabolism in five colors. J Chem Inf Model 2020;60(3):1146-1164.
3. Zhao M, Ma J, Li M, Zhang Y, Jiang B, Zhao X, et al. Cytochrome P450 enzymes and drug metabolism in humans. Int J Mol Sci 2021;22(23):12808.
4. Ogu CC, Maxa JL. Drug interactions due to cytochrome P450. Proc (Bayl Univ Med Cent) 2000;13(4):421-423.
5. Idle JR, Gonzalez FJ. Metabolomics. Cell Metab 2007;6(5):348-351.
6. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: Regulation of gene expression, enzyme activities, and impact of genetic variation. Pharmacol Ther 2013;138(1):103-141.
7. Persky L, Krohmer JS, Storaaslı JP. Clinical studies of excretion and localization of diethyl stilbestrol diphosphate labelled with radioactive phosphorus (P32). Surg Forum 1957;8:610-613.
8. Arnold H, Bourseaux F, Brock N. Chemotherapeutic action of a cyclic nitrogen mustard phosphamide ester (B 518-ASTA) in experimental tumours of the rat. Nature 1958;181(4613):931.
9. Kelly SL, Kelly DE. Microbial cytochromes P450: Biodiversity and biotechnology. Where do cytochromes P450 come from, what do they do and what can they do for us? Philos Trans R Soc Lond B Biol Sci 2013;368(1612):20120476.
10. Pandian BA, Sathishraj R, Djanaguiraman M, Prasad PVV, Jugulam M. Role of cytochrome P450 enzymes in plant stress response. Antioxidants (Basel) 2020;9(5):454.
11. Finnigan JD, Young C, Cook DJ, Charnock SJ, Black GW. Cytochromes P450 (P450s): A review of the class system with a focus on prokaryotic P450s. Adv Protein Chem Struct Biol 2020;122:289-320.
12. Rendic S, Guengerich FP. Survey of human oxidoreductases and cytochrome P450 enzymes involved in the metabolism of xenobiotic and natural chemicals. Chem Res Toxicol 2015;28(1):38-42.
13. Tao G, Huang J, Moorthy B, Wang C, Hu M, Gao S, et al. Potential role of drug metabolizing enzymes in chemotherapy-induced gastrointestinal toxicity and hepatotoxicity. Expert Opin Drug Metab Toxicol 2020;16(11):1109-1124.
14. Ortiz de Montellano PR. Cytochrome P450-activated prodrugs. Future Med Chem 2013;5(2):213-228.
15. Isin EM, Guengerich FP. Complex reactions catalyzed by cytochrome P450 enzymes. Biochim Biophys Acta 2007;1770(3):314-329.
16. Nebert DW, Wikvall K, Miller WL. Human cytochromes P450 in health and disease. Philos Trans R Soc Lond B Biol Sci 2013;368(1612):20120431.
17. Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. J Lipid Res 2014;55(1):13-31.
18. McDonnell AM, Dang CH. Basic review of the cytochrome p450 system. J Adv Pract Oncol 2013;4(4):263-268.
19. Kim SD, Antenos M, Squires EJ, Kirby GM. Cytochrome P450 2A5 and bilirubin: Mechanisms of gene regulation and cytoprotection. Toxicol Appl Pharmacol 2013;270(2):129-138.
20. Gazzin S, Zelenka J, Zdrahalova L, Konickova R, Zabetta CC, Giraudi PJ, et al. Bilirubin accumulation and Cyp mRNA expression in selected brain regions of jaundiced Gunn rat pups. Pediatr Res 2012;71(6):653-660.
21. Bansal S, Leu AN, Gonzalez FJ, Guengerich FP, Chowdhury AR, Anandatheerthavarada HK, et al. Mitochondrial targeting of cytochrome P450 (CYP) 1B1 and its role in polycyclic aromatic hydrocarbon-induced mitochondrial dysfunction. J Biol Chem 2014;289(14):9936-9951.
22. Khayeka-Wandabwa C, Ma X, Cao X, Nunna V, Pathak JL, Bernhardt R, et al. Plasma membrane localization of CYP4Z1 and CYP19A1 and the detection of anti-CYP19A1 autoantibodies in humans. Int Immunopharmacol 2019;73:64-71.

23. Paragas EM, Wang Z, Korzekwa K, Nagar S. Complex cytochrome P450 kinetics due to multisubstrate binding and sequential metabolism. Part 2. Modeling of experimental data. Drug Metab Dispos 2021;49(12):1100-1108.
24. Ingelman-Sundberg M. Human drug metabolising cytochrome P450 enzymes: Properties and polymorphisms. Naunyn Schmiedebergs Arch Pharmacol 2004;369(1):89-104.
25. Ingelman-Sundberg M. The human genome project and novel aspects of cytochrome P450 research. Toxicol Appl Pharmacol 2005;207(Suppl 2):52-56.
26. Nelson DR. The cytochrome p450 homepage. Hum Genomics 2009;4(1):59-65.
27. Nelson DR. Cytochrome P450 diversity in the tree of life. Biochim Biophys Acta Proteins Proteom 2018;1866(1):141-154.
28. Zanger UM, Turpeinen M, Klein K, Schwab M. Functional pharmacogenetics/genomics of human cytochromes P450 involved in drug biotransformation. Anal Bioanal Chem 2008;392(6):1093-1108.
29. Wilkinson GR. Drug metabolism and variability among patients in drug response. N Engl J Med 2005;352(21):2211-2221.
30. Lynch T, Price A. The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. Am Fam Physician 2007;76(3):391-396.
31. Varshney E, Saha N, Tandon M, Shrivastava V, Ali S. Prevalence of poor and rapid metabolizers of drugs metabolized by CYP2B6 in North Indian population residing in Indian national capital territory. Springerplus 2012;1:34.
32. Martin H, Sarsat JP, de Waziers I, Housset C, Balladur P, Beaune P, et al. Induction of cytochrome P450 2B6 and 3A4 expression by phenobarbital and cyclophosphamide in cultured human liver slices. Pharm Res 2003;20(4):557-568.
33. Hedrich WD, Hassan HE, Wang H. Insights into CYP2B6-mediated drug-drug interactions. Acta Pharm Sin B 2016;6(5):413-425.
34. Tang X, Chen S. Epigenetic regulation of cytochrome P450 enzymes and clinical implication. Curr Drug Metab 2015;16(2):86-96.
35. Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome P450 polymorphisms on drug therapies: Pharmacogenetic, pharmacoepigenetic and clinical aspects. Pharmacol Ther 2007;116(3):496-526.
36. van Schaik RH. CYP450 pharmacogenetics for personalizing cancer therapy. Drug Resist Updat 2008;11(3):77-98.
37. Phillips KA, Veenstra DL, Oren E, Lee JK, Sadee W. Potential role of pharmacogenomics in reducing adverse drug reactions: A systematic review. JAMA 2001;286(18):2270-2279.
38. Johansson I, Ingelman-Sundberg M. Genetic polymorphism and toxicology--with emphasis on cytochrome p450. Toxicol Sci 2011;120(1):1-13.
39. Riaz S, Muhammad Din S, Usman Tareen M, Tariq F, Latif Y, Siddiqi S, et al. Genetic polymorphism of CYP2C19 in Pakistani population. Iran J Pharm Res 2019;18(2):1097-1102.
40. Foster A, Mobley E, Wang Z. Complicated pain management in a CYP450 2D6 poor metabolizer. Pain Pract 2007;7(4):352-356.
41. Kirchheiner J, Schmidt H, Tzvetkov M, Keulen JT, Lötsch J, Roots I, et al. Pharmacokinetics of codeine and its metabolite morphine in ultra-rapid metabolizers due to CYP2D6 duplication. Pharmacogenomics J 2007;7(4):257-265.
42. Madadi P, Koren G, Cairns J, Chitayat D, Gaedigk A, Leeder JS, et al. Safety of codeine during breastfeeding: Fatal morphine poisoning in the breastfed neonate of a mother prescribed codeine. Can Fam Physician 2007;53(1):33-35.
43. Desta Z, Ward BA, Soukhova NV, Flockhart DA. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: Prominent roles for CYP3A and CYP2D6. J Pharmacol Exp Ther 2004;310(3):1062-1075.
44. Bunten H, Liang WJ, Pounder DJ, Seneviratne C, Osselton D. OPRM1 and CYP2B6 gene variants as risk factors in methadone-related deaths. Clin Pharmacol Ther 2010;88(3):383-389.
45. Sallee FR, DeVane CL, Ferrell RE. Fluoxetine-related death in a child with cytochrome P-450 2D6 genetic deficiency. J Child Adolesc Psychopharmacol 2000;10(1):27-34.
46. LLerena A, Dorado P, Berecz R, González AP, Peñas-LLedó EM. Effect of CYP2D6 and CYP2C9 genotypes on fluoxetine and norfluoxetine plasma concentrations during steady-state conditions. Eur J Clin Pharmacol 2004;59(12):869-873.
47. Varshney E, Tandon M, Saha N, Ali S. In vivo phenotyping of cytochrome 450 isoforms involved in the metabolism of anti-HIV and anti-tubercular drugs in human using cocktail approach: An LC-MS/MS analysis. J Pharm Biomed Anal 2019;164:698-705.
48. Gloor Y, Lloret-Linares C, Bosilkovska M, Perroud N, Richard-Lepouriel H, Aubry JM, et al. Drug metabolic enzyme genotype-phenotype discrepancy: High phenoconversion rate in patients treated with antidepressants. Biomed Pharmacother 2022;152:113202.
49. Fekete F, Mangó K, Déri M, Incze E, Minus A, Monostory K. Impact of genetic and non-genetic factors on hepatic CYP2C9 expression and activity in Hungarian subjects. Sci Rep 2021;11(1):17081.
50. Hakkola J, Hukkanen J, Turpeinen M, Pelkonen O. Inhibition and induction of CYP enzymes in humans: An update. Arch Toxicol 2020;94(11):3671-3722.
51. Zhou SF. Drugs behave as substrates, inhibitors and inducers of human cytochrome P450 3A4. Curr Drug Metab 2008;9(4):310-322.
52. Filppula AM, Neuvonen M, Laitila J, Neuvonen PJ, Backman JT. Autoinhibition of CYP3A4 leads to important role of CYP2C8 in imatinib metabolism: Variability in CYP2C8 activity may alter plasma concentrations and response. Drug Metab Dispos 2013;41(1):50-59.
53. Filppula AM, Laitila J, Neuvonen PJ, Backman JT. Potent mechanism-based inhibition of CYP3A4 by imatinib explains its liability to interact with CYP3A4 substrates. Br J Pharmacol 2012;165(8):2787-2798.
54. Poulsen L, Arendt-Nielsen L, Brøsen K, Sindrup SH. The hypoalgesic effect of tramadol in relation to CYP2D6. Clin Pharmacol Ther 1996;60(6):636-644.
55. Yasar U, Forslund-Bergengren C, Tybring G, Dorado P, Llerena A, Sjöqvist F, et al. Pharmacokinetics of losartan and its metabolite E-3174 in relation to the CYP2C9 genotype. Clin Pharmacol Ther 2002;71(1):89-98.
56. Daly AK, King BP. Pharmacogenetics of oral anticoagulants. Pharmacogenetics 2003;13(5):247-252.
57. Dean L. Metoprolol therapy and CYP2D6 genotype. 2017 Apr 4. In: Pratt VM, Scott SA, Pirmohamed M, Esquivel B, Kattman BL, Malheiro AJ, editors. Medical Genetics Summaries. Bethesda (MD): National Center for Biotechnology Information (US); 2012.
58. Ray WA, Murray KT, Meredith S, Narasimhulu SS, Hall K, Stein CM. Oral erythromycin and the risk of sudden death from cardiac causes. N Engl J Med 2004;351(11):1089-1096.
59. Nebert DW, Russell DW. Clinical importance of the cytochromes P450. Lancet 2002;360(9340):1155-1162.
60. Cascorbi I. Drug interactions - Principles, examples and clinical consequences. Dtsch Arztebl Int 2012;109(33-34):546-556.
61. Lee J, Beers JL, Geffert RM, Jackson KD. A review of cyp-mediated drug interactions: Mechanisms and in vitro drug-drug interaction assessment. Biomolecules 2024;14:99.
62. Palovaara S, Kivistö KT, Tapanainen P, Manninen P, Neuvonen PJ, Laine K. Effect of an oral contraceptive preparation containing ethinylestradiol and gestodene on CYP3A4 activity as measured by midazolam 1'-hydroxylation. Br J Clin Pharmacol 2000;50(4):333-337.
63. Doose DR, Wang SS, Padmanabhan M, Schwabe S, Jacobs D, Bialer M. Effect of topiramate or carbamazepine on the pharmacokinetics of an oral contraceptive containing norethindrone and ethinyl estradiol in healthy obese and nonobese female subjects. Epilepsia 2003;44(4):540-549.

64. Glue P, Banfield CR, Perhach JL, Mather GG, Racha JK, Levy RH. Pharmacokinetic interactions with felbamate. In vitro-in vivo correlation. Clin Pharmacokinet 1997;33(3):214-224.
65. Gruer PJ, Vega JM, Mercuri MF, Dobrinska MR, Tobert JA. Concomitant use of cytochrome P450 3A4 inhibitors and simvastatin. Am J Cardiol 1999;84(7):811-815.
66. Akiyoshi T, Ito M, Murase S, Miyazaki M, Guengerich FP, Nakamura K, et al. Mechanism-based inhibition profiles of erythromycin and clarithromycin with cytochrome P450 3A4 genetic variants. Drug Metab Pharmacokinet 2013;28(5):411-415.
67. Skauby RH, Bergan S, Andersen AM, Vethe NT, Christensen H. In vitro assessments predict that CYP3A4 contributes to a greater extent than CYP3A5 to prednisolone clearance. Basic Clin Pharmacol Toxicol 2021;129(6):427-436.
68. Sutton D, Butler AM, Nadin L, Murray M. Role of CYP3A4 in human hepatic diltiazem N-demethylation: Inhibition of CYP3A4 activity by oxidized diltiazem metabolites. J Pharmacol Exp Ther 1997;282(1):294-300.
69. Imani S, Jusko WJ, Steiner R. Diltiazem retards the metabolism of oral prednisone with effects on T-cell markers. Pediatr Transplant 1999;3(2):126-130.
70. Wang YH, Jones DR, Hall SD. Differential mechanism-based inhibition of CYP3A4 and CYP3A5 by verapamil. Drug Metab Dispos 2005;33(5):664-671.
71. Wang YH, Jones DR, Hall SD. Prediction of cytochrome P450 3A inhibition by verapamil enantiomers and their metabolites. Drug Metab Dispos 2004;32:259-266.
72. Zhu M, Zhao W, Jimenez H, Zhang D, Yeola S, Dai R, et al. Cytochrome P450 3A-mediated metabolism of buspirone in human liver microsomes. Drug Metab Dispos 2005;33(4):500-507.
73. Nishikawa H, Inoue T, Masui T, Izumi T, Koyama T. Effects of cytochrome P450 (CYP) 3A4 inhibitors on the anxiolytic action of tandospirone in rat contextual conditioned fear. Prog Neuropsychopharmacol Biol Psychiatry 2007;31(4):926-931.
74. Lilja JJ, Kivistö KT, Backman JT, Lamberg TS, Neuvonen PJ. Grapefruit juice substantially increases plasma concentrations of buspirone. Clin Pharmacol Ther 1998;64(6):655-660.
75. Zhang L, Brown SJ, Shan Y, Lee AM, Allen JD, Eum S, et al. CYP2D6 genetic polymorphisms and risperidone pharmacokinetics: A systematic review and meta-analysis. Pharmacotherapy 2020;40(7):632-647.
76. Spina E, Avenoso A, Scordo MG, Ancione M, Madia A, Gatti G, et al. Inhibition of risperidone metabolism by fluoxetine in patients with schizophrenia: A clinically relevant pharmacokinetic drug interaction. J Clin Psychopharmacol 2002;22(4):419-423.
77. Dean L, Kane M. Tramadol therapy and CYP2D6 genotype. In: Pratt VM, Scott SA, Pirmohamed M, Esquivel B, Kattman BL, Malheiro AJ, editors. Medical Genetics Summaries. Bethesda (MD): National Center for Biotechnology Information (US); 2012.
78. Gan SH, Ismail R, Wan Adnan WA, Zulmi W. Impact of CYP2D6 genetic polymorphism on tramadol pharmacokinetics and pharmacodynamics. Mol Diagn Ther 2007;11(3):171-181.
79. Laugesen S, Enggaard TP, Pedersen RS, Sindrup SH, Brøsen K. Paroxetine, a cytochrome P450 2D6 inhibitor, diminishes the stereoselective O-demethylation and reduces the hypoalgesic effect of tramadol. Clin Pharmacol Ther 2005;77(4):312–323.
80. Heimark LD, Wienkers L, Kunze K, Gibaldi M, Eddy AC, Trager WF, et al. The mechanism of the interaction between amiodarone and warfarin in humans. Clin Pharmacol Ther 1992;51(4):398-407.
81. Flora DR, Rettie AE, Brundage RC, Tracy TS. CYP2C9 genotype-dependent warfarin pharmacokinetics: Impact of CYP2C9 genotype on R- and S-warfarin and their oxidative metabolites. J Clin Pharmacol 2017;57(3):382-393.
82. Naganuma M, Shiga T, Nishikata K, Tsuchiya T, Kasanuki H, Fujii E. Role of desethylamiodarone in the anticoagulant effect of concurrent amiodarone and warfarin therapy. J Cardiovasc Pharmacol Ther 2001;6(4):363-367.
83. Kudo T, Endo Y, Taguchi R, Yatsu M, Ito K. Metronidazole reduces the expression of cytochrome P450 enzymes in HepaRG cells and cryopreserved human hepatocytes. Xenobiotica 2015;45(5):413-419.
84. Frye RF, Zgheib NK, Matzke GR, Chaves-Gnecco D, Rabinovitz M, Shaikh OS, et al. Liver disease selectively modulates cytochrome P450 - Mediated metabolism. Clin Pharmacol Ther 2006;80(3):235-245.
85. Diaz GJ, Murcia HW, Cepeda SM, Boermans HJ. The role of selected cytochrome P450 enzymes on the bioactivation of aflatoxin B1 by duck liver microsomes. Avian Pathol 2010;39(4):279-285.
86. Chandrashekar DV, DuBois BN, Rashid M, Mehvar R. Effects of chronic cirrhosis induced by intraperitoneal thioacetamide injection on the protein content and Michaelis-Menten kinetics of cytochrome P450 enzymes in the rat liver microsomes. Basic Clin Pharmacol Toxicol 2023;132(2):197-210.
87. Harjumäki R, Pridgeon CS, Ingelman-Sundberg M. CYP2E1 in alcoholic and non-alcoholic liver injury. Roles of ROS, reactive ıntermediates and lipid overload. Int J Mol Sci 2021;22(15):8221.
88. Linhart K, Bartsch H, Seitz HK. The role of reactive oxygen species (ROS) and cytochrome P-450 2E1 in the generation of carcinogenic etheno-DNA adducts. Redox Biol 2014;3:56–62.
89. Zhu L, Yang X, Feng J, Mao J, Zhang Q, He M, et al. CYP2E1 plays a suppressive role in hepatocellular carcinoma by regulating Wnt/Dvl2/β-catenin signaling. J Transl Med 2022;20(1):194.
90. Villeneuve JP, Pichette V. Cytochrome P450 and liver diseases. Curr Drug Metab 2004;5(3):273-282.
91. George J, Liddle C, Murray M, Byth K, Farrell GC. Pre-translational regulation of cytochrome P450 genes is responsible for disease-specific changes of individual P450 enzymes among patients with cirrhosis. Biochem Pharmacol 1995;49(7):873-881.
92. Fisher CD, Lickteig AJ, Augustine LM, Ranger-Moore J, Jackson JP, Ferguson SS, et al. Hepatic cytochrome P450 enzyme alterations in humans with progressive stages of nonalcoholic fatty liver disease. Drug Metab Dispos 2009;37(10):2087-2094.
93. Lelouët H, Bechtel YC, Paintaud G, Brientini MP, Miguet JP, Bechtel PR. Caffeine metabolism in a group of 67 patients with primary biliary cirrhosis. Int J Clin Pharmacol Ther 2001;39(1):25-32.
94. El-Khateeb E, Achour B, Scotcher D, Al-Majdoub ZM, Athwal V, Barber J, et al. Scaling factors for clearance in adult liver cirrhosis. Drug Metab Dispos 2020;48(12):1271-1282.
95. Ohnishi A, Murakami S, Akizuki S, Mochizuki J, Echizen H, Takagi I. In vivo metabolic activity of CYP2C19 and CYP3A in relation to CYP2C19 genetic polymorphism in chronic liver disease. J Clin Pharmacol 2005;45(11):1221-1229.
96. Lucena MI, Andrade RJ, Tognoni G, Hidalgo R, Sanchez de la Cuesta F; Spanish Collaborative Study Group on Therapeutic Management of Liver Diseases. Drug use for non-hepatic associated conditions in patients with liver cirrhosis. Eur J Clin Pharmacol 2003;59(1):71-76.
97. Duthaler U, Bachmann F, Suenderhauf C, Grandinetti T, Pfefferkorn F, Haschke M, et al. Liver cirrhosis affects the pharmacokinetics of the six substrates of the basel phenotyping cocktail differently. Clin Pharmacokinet 2022;61(7):1039-1055.
98. Albarmawi A, Czock D, Gauss A, Ehehalt R, Lorenzo Bermejo J, Burhenne J, et al. CYP3A activity in severe liver cirrhosis correlates with Child-Pugh and model for end-stage liver disease (MELD) scores. Br J Clin Pharmacol 2014;77(1):160-169.
99. Metushi IG, Sanders C; Acute Liver Study Group; Lee WM, Uetrecht J. Detection of anti-isoniazid and anti-cytochrome P450 antibodies in patients with isoniazid-induced liver failure. Hepatology 2014;59(3):1084-1093.

100. Manier JW, Chang WW, Kirchner JP, Beltaos E. Hepatotoxicity associated with ticrynafen - A uricosuric diuretic. Am J Gastroenterol 1982;77(6):401-404.
101. Christen U, Holdener M, Hintermann E. Cytochrome P450 2D6 as a model antigen. Dig Dis 2010;28(1):80-85.
102. Dempsey JL, Cui JY. Microbiome is a functional modifier of P450 drug metabolism. Curr Pharmacol Rep 2019;5(6):481-490.
103. Kuno T, Hirayama-Kurogi M, Ito S, Ohtsuki S. Effect of intestinal flora on protein expression of drug-metabolizing enzymes and transporters in the liver and kidney of germ-free and antibiotics-treated mice. Mol Pharm 2016;13(8):2691-2701.
104. Togao M, Kurakawa T, Tajima S, Wagai G, Ohta-Takada Y, Otsuka J, et al. Human gut microbiota influences drug-metabolizing enzyme hepatic Cyp3a: A human flora-associated mice study. J Toxicol Sci 2023;48(6):333-343.
105. Dikeocha IJ, Al-Kabsi AM, Miftahussurur M, Alshawsh MA. Pharmacomicrobiomics: Influence of gut microbiota on drug and xenobiotic metabolism. FASEB J 2022;36(6):e22350.
106. Jourová L, Vavreckova M, Zemanova N, Anzenbacher P, Langova K, Hermanova P, et al. Gut microbiome alters the activity of liver cytochromes P450 in mice with sex-dependent differences. Front Pharmacol 2020;11:01303.
107. Ishii M, Toda T, Ikarashi N, Kusunoki Y, Kon R, Ochiai W, et al. Gastrectomy increases the expression of hepatic cytochrome P450 3A by increasing lithocholic acid-producing enteric bacteria in mice. Biol Pharm Bull 2014;37(2):298-305.
108. Wilson ID, Nicholson JK. Gut microbiome interactions with drug metabolism, efficacy, and toxicity. Transl Res 2017;179:204-222.
109. Koppel N, Maini Rekdal V, Balskus EP. Chemical transformation of xenobiotics by the human gut microbiota. Science 2017;356(6344):eaag2770.
110. Togao M, Kawakami K, Otsuka J, Wagai G, Ohta-Takada Y, Kado S. Effects of gut microbiota on in vivo metabolism and tissue accumulation of cytochrome P450 3A metabolized drug: Midazolam. Biopharm Drug Dispos 2020;41(7):275-282.
111. Collins SL, Patterson AD. The gut microbiome: An orchestrator of xenobiotic metabolism. Acta Pharm Sin B 2020;10(1):19-32.
112. Zimmermann M, Zimmermann-Kogadeeva M, Wegmann R, Goodman AL. Mapping human microbiome drug metabolism by gut bacteria and their genes. Nature 2019;570(7762):462-467.
113. Kuno T, Hirayama-Kurogi M, Ito S, Ohtsuki S. Reduction in hepatic secondary bile acids caused by short-term antibiotic-induced dysbiosis decreases mouse serum glucose and triglyceride levels. Sci Rep 2018;8(1):1253.
114. Guengerich FP, Johnson WW, Ueng YF, Yamazaki H, Shimada T. Involvement of cytochrome P450, glutathione S-transferase, and epoxide hydrolase in the metabolism of aflatoxin B1 and relevance to risk of human liver cancer. Environ Health Perspect 1996;104(Suppl 3):557-562.
115. Smith NF, Figg WD, Sparreboom A. Pharmacogenetics of irinotecan metabolism and transport: An update. Toxicol In Vitro 2006;20(2):163-175.
116. Cheng J, Ma X, Krausz KW, Idle JR, Gonzalez FJ. Rifampicin-activated human pregnane X receptor and CYP3A4 induction enhance acetaminophen-induced toxicity. Drug Metab Dispos 2009;37(8):1611-1621.
117. Selwyn FP, Cheng SL, Klaassen CD, Cui JY. Regulation of hepatic drug-metabolizing enzymes in germ-free mice by conventionalization and probiotics. Drug Metab Dispos 2016;44(2):262-274.
118. González-Sarrías A, Azorín-Ortuño M, Yáñez-Gascón MJ, Tomás-Barberán FA, García-Conesa MT, Espín JC. Dissimilar in vitro and in vivo effects of ellagic acid and its microbiota-derived metabolites, urolithins, on the cytochrome P450 1A1. J Agric Food Chem 2009;57(12):5623-5632.
119. Bailey DG, Dresser GK. Interactions between grapefruit juice and cardiovascular drugs. Am J Cardiovasc Drugs 2004;4(5):281-297.
120. Erden A, Esmeray K, Karagöz H, Karahan S, Gümüşçü HH, Başak M, et al. Acute liver failure caused by mushroom poisoning: A case report and review of the literature. Int Med Case Rep J 2013;6:85-90.
121. Bonacini M, Shetler K, Yu I, Osorio RC, Osorio RW. Features of patients with severe hepatitis due to mushroom poisoning and factors associated with outcome. Clin Gastroenterol Hepatol 2017;15(5):776-779.
122. Mitchell CL, Saul MC, Lei L, Wei H, Werner T. The mechanisms underlying α-amanitin resistance in Drosophila melanogaster: A microarray analysis. PLoS One 2014;9(4):e93489.
123. Shah RR, Smith RL. Addressing phenoconversion: The Achilles' heel of personalized medicine. Br J Clin Pharmacol 2015;79(2):222-240.
124. Elemento O. The future of precision medicine: Towards a more predictive personalized medicine. Emerg Top Life Sci 2020;4(2):175-177.
125. Hongkaew Y, Gaedigk A, Wilffert B, Ngamsamut N, Kittitharaphan W, Limsila P, et al. Relationship between CYP2D6 genotype, activity score and phenotype in a pediatric Thai population treated with risperidone. Sci Rep 2021;11(1):4158.
126. Wynne H. Drug metabolism and ageing. J Br Menopause Soc 2005;11(2):51-56.
127. Stevens JC, Marsh SA, Zaya MJ, Regina KJ, Divakaran K, Le M, et al. Developmental changes in human liver CYP2D6 expression. Drug Metab Dispos 2008;36(8):1587-1593.
128. Nassan M, Nicholson WT, Elliott MA, Rohrer Vitek CR, Black JL, Frye MA. Pharmacokinetic pharmacogenetic prescribing guidelines for antidepressants: A template for psychiatric precision medicine. Mayo Clin Proc 2016;91(7):897-907.
129. Yang L, Li Y, Hong H, Chang CW, Guo LW, Lyn-Cook B, et al. Sex differences in the expression of drug-metabolizing and transporter genes in human liver. J Drug Metab Toxicol 2012;3(3):1000119.
130. Pikuleva IA, Waterman MR. Cytochromes p450: Roles in diseases. J Biol Chem 2013;288(24):17091-17098.
131. Kinirons MT, O'Mahony MS. Drug metabolism and ageing. Br J Clin Pharmacol 2004;57(5):540–544.
132. Cornelis MC, El-Sohemy A, Campos H. Genetic polymorphism of CYP1A2 increases the risk of myocardial infarction. J Med Genet 2004;41(10):758-762.
133. Trenaman SC, Bowles SK, Andrew MK, Goralski K. The role of sex, age and genetic polymorphisms of CYP enzymes on the pharmacokinetics of anticholinergic drugs. Pharmacol Res Perspect 2021;9(3):e00775.
134. Ismail Z, Black SE, Camicioli R, Chertkow H, Herrmann N, Laforce R Jr, et al; CCCDTD5 participants. Recommendations of the 5th Canadian Consensus Conference on the diagnosis and treatment of dementia. Alzheimers Dement 2020;16(8):1182-1195.
135. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet 2020;396(10248):413-446.
136. Degen LP, Phillips SF. Variability of gastrointestinal transit in healthy women and men. Gut 1996;39(2):299-305.
137. Vicente J, Simlund J, Johannesen L, Sundh F, Florian J, Ugander M, et al. Investigation of potential mechanisms of sex differences in quinidine-induced torsade de pointes risk. J Electrocardiol 2015;48(4):533-538.
138. Watson S, Caster O, Rochon PA, den Ruijter H. Reported adverse drug reactions in women and men: Aggregated evidence from globally collected individual case reports during half a century. EClinicalMedicine 2019;17:100188.
139. Spina E, de Leon J. Clinical applications of CYP genotyping in psychiatry. J Neural Transm (Vienna) 2015;122(1):-28.
140. Mitchell SJ, Hilmer SN. Drug-induced liver injury in older adults. Ther Adv Drug Saf 2010;1(2):65-77.
141. Aitken AE, Richardson TA, Morgan ET. Regulation of drug-metabolizing enzymes and transporters in inflammation. Annu Rev Pharmacol Toxicol 2006;46:123–149.
142. Rodieux F, Daali Y, Rollason V, Samer CF, Ing Lorenzini K. Practice of CYP450 genotyping and phenotyping in children in a real-life setting. Front Pharmacol 2023;14:1130100.
143. Lu Y, Cederbaum AI. Cytochrome P450s and alcoholic liver disease. Curr Pharm Des 2018;24(14):1502–1517.