Pharmacometabolomics in Drug Disposition, Toxicity and Precision Medicine

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Abstract

The precision medicine initiative has driven a substantial change in the way scientists and health care practitioners think about diagnosing and treating disease. While it has long been recognized that drug response is determined by the intersection of genetic, environmental and disease factors, improvements in technology have afforded precision medicine guided dosing of drugs to improve efficacy and reduce toxicity. Pharmacometabolomics aims to evaluate small molecule metabolites in plasma and/or urine to help evaluate mechanisms that predict and/or reflect drug efficacy and toxicity. In this mini review, we provide an overview of pharmacometabolomic approaches and methodologies. Relevant examples where metabolomic techniques have been used to better understand drug efficacy and toxicity in major depressive disorder and cancer chemotherapy are discussed. In addition, the utility of metabolomics in drug development and understanding drug metabolism, transport and pharmacokinetics is reviewed. Pharmacometabolomic approaches can help understand factors mediating drug disposition, efficacy and toxicity. While important advancements in this area have been made, there remain several challenges that must be overcome before this approach can be fully implemented into clinical drug therapy.

Significance Statement

Pharmacometabolomics has emerged as an approach to identify metabolites that allow for implementation of precision medicine approaches to pharmacotherapy. This review article provides an overview pharmacometabolomics including highlights of important examples.
Introduction

Precision medicine is an emerging field that involves treatment of disease tailored to specific patient and disease phenotypes. With respect to drug therapy, traditional medicine treats a condition using a uniform treatment that is thought will benefit most patients. In contrast, precision medicine considers patient factors underlying disease and tailors drug therapy to the specific patient. In effect, precision medicine moves away from the traditional top-down approach and instead attempts to understand the mechanisms behind drug response, nonresponse, and toxicity and design therapeutic regimens accordingly (Figure 1). Beyond clinical presentation of disease, patient genome, transcriptome, proteome, and metabolome can be used to optimize drug therapy. Emerging evidence has been elucidating these patient factors to improve drug efficacy and reduce toxicity. Pharmacometabolomics is an emerging technology where the patient’s metabolic profile is assessed and used to guide drug dosing and pharmacotherapy. The objective of this mini review is to discuss the historical context of precision medicine and highlight the emerging role of pharmacometabolomics in improving drug efficacy and reducing drug toxicity.

Brief Historical Perspective

The era of precision medicine was greatly accelerated following completion of the human genome project which enhanced our ability to identify genetic polymorphisms associated with disease and drug response(Carrasco-Ramiro et al., 2017). As of 2023, PharmGKB lists 26,311 variant annotations(PharmGKB.org) and there are 541 FDA drug labels describing drug-gene interactions(FDA, 2023). While pharmacogenomic guided drug dosing has had profound impact on precision medicine, drug therapy is influenced by epigenetics, diet, drug-drug interactions(Orr et al., 2012), environmental factors, and the microbiome(Sharma et al., 2019) in
addition to genotype. Accordingly, identification of metabolite signatures that reflect these factors can improve precision medicine guided drug dosing.

**Pharmacogenomics in Precision Medicine**

The premise for pharmacometabolomics stemmed from the success of pharmacogenomic approaches to assess drug efficacy and toxicity. Precision medicine and pharmacogenomics have seen significant use in cancer treatment (Friedman et al., 2015). Precision cancer medicine was kickstarted by the discovery of imatinib’s high efficacy in patients with chronic-phase BCR–ABL-mutated chronic myeloid leukemia. Chronic phase myeloid leukemia is a result of the expression of the BCR-ABL tyrosine kinase oncogene. This expression is typically the result of a reciprocal translocation between chromosomes 9 and 22, resulting in a shortened chromosome 22 known as the Philadelphia chromosome. Through this translocation, the ABL and BCR sequences are combined, resulting in the expression of a BCR-ABL protein-tyrosine kinase. The expression of this gene leads to chronic phase chronic myeloid leukemia (Talpaz et al., 2002). Imatinib was designed as an inhibitor of BCR-ABL protein-tyrosine kinase, which prevents cell proliferation. Imatinib’s target development was a milestone in cancer precision medicine and suggested that precision medicine may become a vital aspect of modern cancer treatment (Gambacorti-Passerini, 2008).

Precision medicine and pharmacogenomics have also seen use outside of cancer treatment. Patient genotype can have a significant impact on a drug’s pharmacokinetics and metabolism. For example, the opioid analgesic codeine has low affinity for the mu opioid receptor; however, affinity is greatly increased following metabolism by CYP2D6 to morphine. Polymorphisms in the CYP2D6 gene can greatly impact codeine’s metabolism into morphine (Ingelman-Sundberg et al., 2007). Normal metabolizers (i.e., those with the diplotypes *1/*10 and *1/*41), can follow the regular dosing regimen for codeine. Poor metabolizers (i.e., diplotypes *3/*4 and *4/*4), metabolize little to no codeine into morphine, and therefore gain little therapeutic benefit from
codeine. Ultrarapid metabolizers (i.e., those with diplotypes *1/*1xNc) and *1/*2xN) metabolize codeine into morphine at a much greater rate than normal metabolizers and may experience symptoms of opioid overdose.

For some drugs, pharmacogenomic evidence for genetically guided dosing recommendations is strong enough to be included in the product monograph. For example, 6-mercaptopurine is metabolized by thiopurine methyltransferase (TPMT) to inactive and non-toxic methylated metabolites. Patients with two inactive TPMT alleles experience severe myelosuppression as a result of decreased methylation and a subsequent increase in the accumulation of the active (yet toxic) thioguanine nucleotide metabolites. Following guidelines from the Clinical Pharmacogenetics Implementation Consortium (CPIC), patients homozygous for TPMT deficiency are recommended to start mercaptopurine therapy at 10% or less of the standard dose (Relling et al., 2013).

Pharmacometabolomics in Precision Medicine

Despite several successes using pharmacogenomic guided precision medicine, it’s widely recognized that drug response is mediated by several factors in addition to the patient's genotype. Some of these factors include diet, disease, age, co-prescribed medications and the gut microbiota. In 2006, Clayton et al. defined the term pharmacometabolomics as “the prediction of the outcome (for example, efficacy or toxicity) of a drug or xenobiotic intervention in an individual based on a mathematical model of pre-intervention metabolic signatures.” To illustrate this concept, they collected pre-post dose urine samples from rats treated with a single toxic dose of acetaminophen (paracetamol) and subjected the urine to 1H NMR spectroscopy (Clayton et al., 2006). They were able to determine that higher pre-dose urinary taurine was associated with no or minimal liver injury whereas higher pre-dose trimethylamine N-oxide (TMAO) and betaine were associated with more severe liver injury. Pharmacometabolomics was greatly accelerated following President Obama's precision
medicine initiative (Collins and Varmus, 2015) which facilitated the establishment of the Pharmacometabolomics Research Network. Pharmacometabolomics is now recognized as a field that complements and, in some cases, informs pharmacogenomics in the pursuit of understanding and optimizing drug therapy. An overview of pharmacometabolomic approaches to precision medicine is shown in Figure 1.

Overview of Metabolomics Methods

Metabolomics analysis is primarily done using either nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS) (Gonzalez-Covarrubias et al., 2022). MS methods are more commonly used, with MS typically being paired with liquid chromatography (LC-MS) or gas chromatography (GC-MS). GC-MS requires sample vaporization, achieved with the application of heat; thus, GC-MS is best suited for volatile compounds (Fiehn, 2016). As chemical compounds must be volatile for vaporization, not all chemical classes are amenable to GC-MS (Fiehn, 2016). In GC-MS metabolomics studies, chemical derivatization is necessary to increase compound volatility or thermal stability, complicating and lengthening the sample preparation process (Fiehn, 2016). Despite these limitations, GC-MS offers high resolution with a robust and reproducible fragmentation pattern induced by electron impact ionization; combined with comprehensive and standardized commercially available spectral libraries, these characteristic fragmentation patterns facilitate fast and reliable identification of unknown compounds (Emwas, 2015; Fiehn, 2016). On the other hand, LC-MS offers broad coverage of the metabolome by taking advantage of different column chemistries, without the need for derivatization nor the need for compounds to be volatile (Emwas, 2015). As derivatization is not necessary, sample preparation is generally simpler and quicker with LC-MS based metabolomics. LC-MS instrumentation tends to be more susceptible to matrix effects, as LC-MS machines often use electrospray ionization, a technique prone to ion suppression in the presence of co-eluting compounds (Gowda and Djukovic, 2014). Furthermore, spectral libraries
for LC-MS are also less developed and less standardized compared to GC-MS, making unknown identification more challenging (Fiehn, 2016). There are advantages to both NMR based and MS based metabolomics approaches. MS has a much higher sensitivity and accordingly can detect compounds at much lower concentrations than NMR (Dettmer et al., 2007). This increases the breadth of metabolites able to be analyzed. As mentioned previously, compounds must be ionized for detection by MS; compounds which ionize poorly will not be detected during MS analysis. While chemical derivatization is necessary for GC-MS, it can optionally be employed for LC-MS analysis, introducing an easily ionizable moiety onto some compounds that may otherwise ionize poorly (Zhao and Li, 2020). The need for derivatization for GC-MS or supplemental derivatization in LC-MS increases the complexity of sample preparation for MS based metabolomics. Conversely, NMR metabolomics is highly reproducible, nondestructive, and does not require ionization to analyze compounds. NMR has much less sophisticated sample preparation and does not require a molecule to ionize for detection (Emwas et al., 2019). NMR is inherently quantitative and structurally qualitative, unlike MS which can only quantify compounds through use of a standard curve, and only the retention time and mass-charge ratio (m/z) of compounds. Structural identification of compounds using MS requires the comparison of a compound’s m/z, fragmentation data, and retention time with analytical reference standards. Unlike NMR, MS analysis (especially LC-MS) is impacted by matrix effects such as ion suppression. NMR and MS based approaches each have their advantages and limitations (see Table 1) and the most comprehensive evaluations of the metabolome employ both technologies (Gathungu et al., 2020).

Metabolomics analysis can be split into two approaches: targeted metabolomics and untargeted metabolomics. Untargeted metabolomics is hypothesis generating and aims to identify novel small molecule biomarkers. All identifiable small molecules in a sample are analyzed, including novel, uncharacterized compounds. Multivariate analysis is performed on the collected data to
identify compounds that differ significantly between groups (e.g., patients without drug toxicity vs. patients with drug toxicity). The primary goal of untargeted metabolomics is to identify novel perturbations in biological pathways that may be clinically or mechanistically relevant to a drug’s action or a disease’s pathology.

In contrast, targeted metabolomics is hypothesis-driven and sometimes builds upon previously conducted untargeted metabolomics studies. Targeted metabolomics quantifies a specific set of previously identified, structurally and biochemically characterized metabolites. Targeted metabolomics studies observe metabolites associated with specific biochemical pathways and their relevance to disease and pharmacotherapy. Sample preparation can be optimized by using techniques such as derivatization and solid-phase extraction to maximize efficacy in extracting and analyzing a particular metabolite. Targeted metabolomics aims to build a metabolic fingerprint that can help predict or characterize drug response.

**Key Recent Advances**

**Drug Efficacy**

Pharmacometabolomics has been used to predict and monitor the efficacy of a pharmacological treatment. The ability to predict the efficacy of a drug by analyzing a patient’s metabolome could lead to patients receiving treatments that are more likely to be effective. In many diseases, such as cancers, rheumatoid arthritis (Nell et al., 2004) and neurodegenerative diseases (Mey et al., 2023), effective treatment provided early in the course of the disease is important to prevent further progression of disease. Being able to predict response/nonresponse to a drug may prevent polypharmacy approaches until the appropriate treatment regimen is found.

Major depressive disorder (MDD) is a common cause of disability and has a significant societal and economic burden (Otte et al., 2016). Selective serotonin reuptake inhibitors (SSRIs) are the primary treatment for MDD; however, a significant portion of MDD patients do not respond to
SSRI treatment, and a trial-and-error approach is often necessary, with patients having to try multiple SSRIs before achieving the desired therapeutic outcome (Alexander and Young, 2022). Furthermore, SSRIs can take 6 weeks to fully take effect, prolonging the time it takes to find a suitable treatment and achieve resolution of symptoms (Taylor et al., 2006). The significant heterogeneity among MDD’s symptomology, outcomes, and drug response make it a prime target for precision medicine.

The CAN-BIND is a program which studies biomarkers of depression. It consists of a group of Canadian researchers who take a multifaceted approach to the identification of markers of depression. CAN-BIND has investigated Interindividual differences in neurophysiology (Suh et al., 2021), symptomology (Khoo et al., 2022), EEG (Zhdanov et al., 2020) and metabolome (Caspani et al., 2021) as potential diagnostic measures as well as predictors of antidepressant treatment response. The pharmacometabolomics of treatment response to both escitalopram alone and escitalopram in combination with aripiprazole was investigated (Caspani et al., 2021). Escitalopram is an SSRI used in the treatment of MDD (Kennedy et al., 2009) and generalized anxiety disorder (GAD) (Dhillon et al., 2006). Aripiprazole is an atypical antipsychotic medication used as an augmentation to the treatment of MDD with an SSRI (Worthington et al., 2005). Metabolomic analysis of plasma and urine using H-NMR revealed metabolomic differences between patients exhibiting response/nonresponse to treatment (Caspani et al., 2021). Interestingly, significant sex differences were found. For escitalopram monotherapy, various pretreatment plasma metabolite levels were altered in males who responded to treatment. Altered metabolites were lipoproteins and apolipoproteins, and included apolipoprotein A1, HDL$_2$-apolipoprotein A2 and HDL$_3$-free cholesterol. No statistically significant predictive markers were found in female subjects. Conversely, post-treatment response to escitalopram with aripiprazole augmentation was able to be predicted in females but not males, with alterations in various baseline metabolite levels found to be indicative of treatment
response. Altered metabolites again included lipoproteins and apolipoproteins, such as VLDL$_4$-free cholesterol, VLDL$_2$-triglycerides, apolipoprotein A2, and HDL$_3$-cholesterol. Other studies have taken a combined pharmacometabolomic/pharmacogenomic approach to better understand escitalopram response (Ji et al., 2011). A pharmacometabolomic study revealed that glycine was negatively associated with escitalopram treatment response. This was followed up by genotyping single nucleotide variants associated with glycine synthesis and degradation and discovery of a variant (rs10975641) in the gene encoding glycine dehydrogenase that was associated with treatment outcome. Collectively, these data illustrate the interplay between pharmacometabolomics and pharmacogenomics and could be used to help optimize pharmacological treatment of MDD with escitalopram.

Triple negative breast cancer (TNBC) is characterized by its lack of expression of the progesterone, estrogen and HER2 receptors. It accounts for approximately 17% of breast cancer diagnoses (Lin et al., 2012). The lack of receptor targets, along with the considerable heterogeneity of TNBC has resulted in a lack of targeted therapies and poorer prognosis than other forms of breast cancer. Due to the lack of targeted therapies for TNBC, the chemotherapeutic agent doxorubicin is the primary treatment. A major challenge in treating TNBC is that some tumors are resistant to doxorubicin mediated chemotherapy. Untargeted metabolomics were used to identify biomarkers of doxorubicin efficacy in patient-derived xenograft mouse models of TNBC that were either resistant or sensitive to doxorubicin (Velenosi et al., 2022). Patient derived xenograft models of TNBC revealed elevated urinary levels of diacetyl spermine in TNBC tumors that were sensitive to doxorubicin. The increase in urinary diacetyl spermine was mediated by doxorubicin mediated upregulation of SAT1, the gene which codes for spermidine/spermine $N^1$-acetyltransferase 1, an enzyme responsible for the acetylation of polyamines in catabolic polyamine metabolism (Ou et al., 2016). Metabolomics also revealed that in some cancer cell lines, doxorubicin exposure induces SAT1 expression.
and therefore urinary diacetylspermine levels; however, in others, high baseline levels of SAT1 expression mask the induction of SAT1 by doxorubicin. Following the initial increase in diacetylspermine production, urinary diacetylspermine levels are correlated with tumor mass, with reductions in tumor mass resulting in reduction in urinary diacetylspermine levels. Urinary diacetylspermine levels could potentially be used as a biomarker of tumor mass in TNBC patients being treated with chemotherapeutic agents that do not induce SAT1 expression, in which a decrease in urine diacetylspermine concentration could be indicative of a reduction in tumor mass. Urinary diacetylspermine levels, therefore, have potential to serve as a minimally invasive way to evaluate the efficacy and progression of TNBC treatment using doxorubicin and other chemotherapeutic agents.

**Drug Toxicity**

Drug toxicity is a major limiting factor in the treatment of disease, especially during chemotherapy. Pharmacometabolomics can be used to predict or monitor drug toxicity and has the potential to reduce adverse outcomes. The identification of metabotypes associated with susceptibility to or occurrence of drug toxicity can be used to influence treatment plans, reduce adverse effects and maximize therapeutic effectiveness. In drugs where toxicity is dose-limiting, the pretreatment identification of risk for drug toxicity can be used to inform the dosage, and those with less risk of toxicity can potentially receive higher and more efficacious doses.

Cisplatin is a widely used platinum based chemotherapeutic drug which is used in the treatment of a variety of malignancies, including lung, breast, head, and neck cancers (Gold and Raja, 2023). Cisplatin is eliminated by and accumulates in the kidneys, where it exhibits significant dose limiting nephrotoxicity (Ozkok and Edelstein, 2014). Approximately one third of patients undergoing cisplatin therapy will develop acute kidney injury (AKI) (Miller et al., 2010). AKI is characterized by a significant, rapid decrease in kidney function, evident by a rise from baseline serum creatinine levels (Kellum et al., 2021). Novel biomarkers are needed to identify the initial
stages of cisplatin-induced AKI, as serum creatinine fails to identify kidney toxicity until damage has progressed enough for kidney function to be impaired. In mice administered cisplatin, untargeted metabolomics has been used to identify biomarkers that can identify AKI earlier in treatment than the conventional biomarker serum creatinine. 26 metabolites were identified that could be used to identify kidney damage prior to alterations in serum creatinine levels (Lim et al., 2023a). Cisplatin treatment caused changes in several metabolites associated with mitochondrial function, which is consistent with the known mechanisms of cisplatin's nephrotoxicity (Miller et al., 2010). For example, L-carnitine concentration was found to be increased in both urine and plasma following cisplatin administration. These changes occurred prior to the rise in serum creatinine. L-carnitine plays a significant role in fatty acid oxidation within the mitochondria (McCann et al., 2021). Other rodent studies have also found metabolites associated with mitochondrial dysfunction. An increase in metabolites associated with the TCA (tricarboxylic acid cycle) such as cis-aconitic acid and isocitric acid is found in rats following cisplatin administration, which may lead to increased ROS production and therefore oxidative stress to the kidneys (Qu et al., 2020). 3-methylcrotonylglycine, a metabolite implicated in fatty acid oxidation is also found to be raised following cisplatin administration (Wilmes et al., 2015).

The ACCENT study is a prospective observational study looking at metabolomic changes during cisplatin chemotherapy in pediatric and adult cancer patients (Jain et al., 2021). In a discovery phase of the ACCENT study, plasma and urine samples from adult head and neck cancer patients treated with cisplatin were subjected to untargeted metabolomics (Lim et al., 2023b). Early, late, and predictive biomarkers were identified which could act as prognostication of AKI following cisplatin administration. The altered biomarkers followed a similar trend to earlier mouse studies suggesting the importance of mitochondrial dysfunction. Glycine, 3-hydroxydecanedioic acid, hippuric acid sulfate and suberate were found to be significantly different in patients that went on to develop AKI in samples collected before cisplatin was infused. This suggests silent metabolic features that put some patients at higher risk of cisplatin
induced AKI. In samples collected 7 days following cisplatin administration, serum acylcarnitine levels differed between the non-AKI and AKI patient groups. Elevated serum acylcarnitine levels are implicated in abnormal fatty acid β-oxidation. These findings suggest that susceptibility to cisplatin induced AKI may be linked to decreased mitochondrial fatty acid oxidation.

Aside from direct investigations into drug efficacy and toxicity, pharmacometabolomics also has implications to support drug development (Mastrangelo et al., 2014). Metabolome informed in-vitro drug toxicity assays can be used to screen novel drugs for potentially toxic effects. Efficacious, high throughput toxicity assays could streamline the drug-development process and provide a method for early identification of harmful off-target effects. Metabolomics may prove useful in this area due to its ability to characterize a wide range of metabolites which provide an overall snapshot into cellular processes including exposome interactions. This approach has potential to reduce efforts into a drug candidate that later is found to have toxic effects that would prevent it from seeing clinical implementation. For example, a drug-induced cardiotoxicity pharmacometabolomic assay has been developed (Palmer et al., 2020). Initially, untargeted metabolomics experiments were performed on cardiomyocyte cell cultures exposed to drugs previously known to induce cardiotoxicity through a variety of mechanisms, as well as drugs not known to produce cardiotoxic effects. Subsequent data analysis elucidated metabolites which were altered following exposure to cardiotoxic drugs. These prospective biomarkers were then used to develop a targeted metabolomics platform which could robustly predict a drug’s in-vivo cardiotoxicity. Following optimization, the final model used only four metabolites: arachidonic acid, 2′-deoxycytidine, lactic acid, and thymidine. The final model was able to robustly discriminate between cardiotoxic drugs such as amsacrine, nilotinib, paclitaxel, vandetanib and non-cardiotoxic drugs such as acyclovir, biotin, erlotinib. This assay serves as another tool for investigating drug safety and efficacy.

**Drug Transporters**
Drug transporters play a significant role in the pharmacokinetics of drugs and drug elimination. Variation in drug transporter activity can account for significant interindividual differences in drug disposition (Tirona, 2011). Furthermore, certain drugs can inhibit or induce expression of various transporters, leading to drug-drug interactions which can significantly alter the safety and efficacy of a drug when co-administered with a substrate of the transporter (Konig et al., 2013). Metabolomics can be used to identify and quantify endogenous substrates of drug transporters. These compounds can serve as biomarkers of variation in transporter activity that could impact drug efficacy or lead to adverse drug reactions.

The organic anion transporting polypeptides (OATPs) are a family of solute carrier proteins expressed in various tissues and organs. OATPs mediate the transmembrane movement of drugs and endogenous compounds. Variability in OATP expression and activity has been implicated in clinically relevant variation in the pharmacokinetics of drugs. OATP1B1 is expressed in the liver and mediates the influx of drugs into hepatocytes from blood, allowing for metabolism by liver enzymes (Konig et al., 2013). Polymorphisms in the OATP1B1 gene are known to impact the pharmacokinetic profiles of drugs (Mori et al., 2019). Co-administered drugs can also profoundly impact OATP1B1 transport activity. For example, the tyrosine-kinase inhibitors axitinib, pazopanib, nilotinib have a significant inhibitory effect on OATP1B1 (Hu et al., 2014). Untargeted metabolomics has been used to identify chenodeoxycholic acid 24-acyl-β-D-glucuronide (CDCA-24G) as a biomarker of OATP1B1 function (Li et al., 2022). This study initially used untargeted metabolomics to compare the metabolic profiles of wild-type and Oatp1b2 (the murine orthologue of both OATP1B1 and OATP1B3 in humans) knockout mice, which initially identified CDCA-24G as a potential biomarker of OATP1B inhibition. To further investigate CDCA-24G as a biomarker, wild-type mice were administered known OATP1B inhibitors, where it was found that mice administered OATP1B inhibitors had higher plasma concentrations of CDCA-24G than those that were not. An in vitro uptake experiment confirmed
that CDCA-24G is a substrate of human OATP1B transporters and that administration of known OATP1B inhibitors reduced its uptake into cells. Finally, a clinical study observed changes in plasma concentrations of CDCA-24G following paclitaxel administration in cancer patients. It was found that the administration of paclitaxel, a known OATP1B inhibitor, resulted in increased plasma CDCA-24G levels in a dose dependent manner. Measuring CDCA-24G levels in patients could potentially reduce the risk of OAT1B1 mediated DDIs during polypharmacy and would be helpful in elucidating potential DDIs during drug development.

In addition to CDCA-24G, other metabolomic markers of OATP1B1 function were identified by leveraging GWAS studies. For example, the common OATP1B1-Val174Ala variant (rs4149056) (Tirona et al., 2001) was associated with 20 endogenous metabolites(Yee et al., 2016). This variant reflects decreased OATP1B1 mediated hepatic uptake, so metabolites identified in this study are likely substrates for OATP1B1 transport. Metabolites identified included bile acids and steroids but novel OATP1B1 markers were also identified including fatty acid dicarboxylates (tetradecanedioic acid, hexadecanedioic acid, octadecanedioic acid) and lysolipids (1-arachidonoylglycerophosphoethanolamine and 1-arachidonoylglycerophosphoinositol). These metabolites may be useful for determining if an OATP1B1 mediated drug-drug interaction is occurring during polypharmacy.

Biomarkers of renal transporter activity have also been an area of active investigation. N₁-methylnicotinamide (NMN) is a product of tryptophan metabolism and has been evaluated as a biomarker of the multidrug and toxin extrusion protein (MATE) transporters that facilitate drug excretion from proximal tubule cells to the tubular lumen(Ito et al., 2012a). A clinical study evaluated the effect of rifampin (OATP inhibitor), verapamil (P-gp inhibitor), probenecid (OAT inhibitor) and cimetidine (OCT2, MATE1, MATE2-K inhibitor). They found cimetidine mediated a reproducible decrease in NMN renal clearance(Muller et al., 2023). While cimetidine has been shown to inhibit OCT2 and MATE1/MATE2-K mediated transport, studies have shown it has a
greater effect on MATE transport activity than OCT2 transport suggesting NMN is a biomarker of MATE transporter activity (Ito et al., 2012b; Muller et al., 2018). Collectively, these studies highlight that endogenous metabolites reflect hepatic and kidney mediated transporter activity and may be useful in evaluating DDIs and minimizing variation in drug disposition.

**Cytochrome P450 Mediated Metabolism**

Cytochrome P450 (CYP) mediated drug-drug interactions are a significant source of concern in polypharmacy. CYP enzymes are responsible for the metabolism of approximately 70-80% of marketed drugs (Zanger and Schwab, 2013; Peng et al., 2021). Many xenobiotics induce or inhibit CYP enzymes, which can alter the pharmacokinetics of CYP substrates. Polymorphisms in CYP genes can also impact the activity of the enzyme in a clinically significant manner (Ingelman-Sundberg et al., 2007). CYP2D6 is considered one of the most clinically relevant CYP isoforms, responsible for the metabolism of approximately 20% of clinically used drugs (Zanger and Schwab, 2013). Accordingly, CYP2D6 is the source of many drug-drug and drug-gene interactions (Nofziger et al., 2020). Due to the widely variable activity of CYP2D6 the ability to easily monitor its activity could prove useful in precision medicine, especially in polypharmacy. The use of genetic testing to identify CYP2D6 diplotype has been used in the context of codeine therapy (Madadi et al., 2013). While testing for CYP2D6 diplotype is useful, it does not consider variations in activity brought on by exogenous inhibitors and inducers. An untargeted metabolomics approach has been used to identify endogenous substrates of CYP2D6 that can act as biomarkers of CYP2D6 activity. Urine and plasma from patients of various CYP2D6 diplotypes was collected prior to CYP2D6 phenotyping using dextromethorphan as a probe substrate. Subjects were then administered 20mg of paroxetine daily for 7 days. Paroxetine is a potent CYP2D6 inhibitor (Magliocco et al., 2021). Urine and plasma samples were collected after 7 days of paroxetine administration to phenotype CYP2D6. Two unknown endogenous metabolites were identified as potential biomarkers that could be
correlated with CYP2D6 activity. The identities of these two metabolites were not identified beyond their m/z. This is a limitation of LC-MS based metabolomics approaches and highlights the difficulty in identifying the structure of compounds using mass spectrometry. Interestingly, a biomarker of dietary origin, solanidine, was also identified. Solanidine is a steroidal alkaloid found in potatoes and is a known substrate of CYP2D6. Subjects with reduced CYP2D6 activity due to genetics or paroxetine administration had higher levels of solanidine and lower levels of its metabolites. Solanidine’s metabolites, once normalized to solanidine levels to account for dietary variation, could predict CYP2D6 activity. The use of solanidine and its metabolites as biomarkers of CYP2D6 has been further investigated (Wollmann et al., 2023a; Wollmann et al., 2023b).

CYP3A4 metabolizes approximately 30% of marketed small molecule drugs (Zanger and Schwab, 2013). Midazolam hydroxylation is the typical in vivo probe of CYP3A4 activity and has been used to probe CYP3A4 in several clinical studies (Thummel et al., 1996). Efforts have been made to correlate endogenous biomarkers to midazolam clearance as surrogate markers of CYP3A4 activity. The urinary ratio of 6β-hydroxycortisol/cortisol has been used to detect CYP3A4 induction or inhibition but has been deemed to not be sufficiently reliable to reflect CYP3A4 activity (Galteau and Shamsa, 2003). Plasma concentration of 4β-hydroxycholesterol has also been shown to be reflective of CYP3A activity including evaluation of CYP3A5 genotype and following induction with rifampin. One limitation of using 4β-hydroxycholesterol is its long half-life (~17 days) (Diczfalusy et al., 2009; Diczfalusy et al., 2011). Shin et al. used multiple endogenous metabolites and CYP3A5 genotype to predict midazolam clearance. They found using 7β-hydroxy-DHEA/DHEA, 6β-hydroxycortisone/cortisone and CYP3A5 genotype was able to predict midazolam clearance (Shin et al., 2013). More recently, an untargeted metabolomics analysis identified urinary ω- (or ω-1)-hydroxylated medium chain acylcarnitines as novel CYP3A4 biomarkers (Kim et al., 2018).
Current Challenges and Knowledge Gaps

Despite the progress made in the area of pharmacometabolomics over the last decade, there are still significant challenges. A notable strength of pharmacometabolomic approaches is that metabolomics reflects factors such as diet, disease, and environmental exposure. While this is noted as a strength it is also a challenge with respect to timing of sample collection. For example, an untargeted metabolomics analysis revealed that 15% of identified metabolites in plasma and saliva showed circadian variability and this was independent of sleep or food intake (Dallmann et al., 2012). Food intake can also have a prominent impact on the metabolome. For example, fasting results in lower insulin and glucose levels with a corresponding increase in lactate production. In addition, fasting causes an increase in plasma non-esterified fatty acids and branched chain fatty acids with an increase in urinary levels of β-aminoisobutyrate. Free carnitine and acetylcarnitine show opposite effects induced by fasting with plasma free carnitine concentrations decreasing during fasting with a corresponding increase of acylcarnitines such as acetylcarnitine (Krug et al., 2012). The effect of sex on the metabolome has also been investigated and some notable differences in metabolite profile between female and male subjects described. Males have been shown to have higher plasma levels of phenylalanine, glutamine, glutamate, kynurenine, tyrosine, methionine and proline than females (Lawton et al., 2008; Jove et al., 2016; Vignoli et al., 2018; Andraos et al., 2021; Costanzo et al., 2022). Similarly, small chain acylcarnitines such as free carnitine, propionylcarnitine and isovalerylcarnitine are higher in males than females (Trabado et al., 2017). Collectively, these studies highlight the need for careful study design and interpretation when relating metabolomic changes to drug response and/or toxicity.

Another challenge in LC-MS based pharmacometabolomics approaches is identification of metabolites. Pharmacogenomics has been successful owing largely to the sequence of the human genome being complete and openly accessible. Investigators performing LC-MS based
metabolomics tend to use different analytical conditions (e.g., LC column, mobile phase gradient) making standardization of metabolite identification by retention time impossible. The other challenge is identifying metabolites that have not yet been characterized. While the sequence of the human genome is known, there are some metabolites that have only recently been characterized (Zhang et al., 2017; Velenosi et al., 2019; Sidor et al., 2023). While there are likely many metabolites that remain uncharacterized, fortunately the tools available to researchers to help identify metabolites from LC-MS derived data are constantly improving. Spectral metabolite libraries with low energy and high energy mass spectra are available through the human metabolome database (HMDB, https://hmdb.ca), MassBank (https://massbank.eu/MassBank/) and metlin (https://metlin.scripps.edu).

**Perspective on Future Directions**

The metabolome sits downstream of other -omic technologies including genomics, transcriptomics, and proteomics. Accordingly, changes in the metabolome can reflect changes upstream and may be very useful in identifying metabolic changes that reflect drug disposition, efficacy, or toxicity; some examples highlighting the utility of pharmacometabolomics are summarized in Table 2. Pharmacometabolomic studies have shed light on mechanisms that impact variability in drug response; however, metabolomic approaches are seldomly used clinically. It’s likely that metabolomic approaches could be useful in improving drug therapy but have to be targeted to conditions where the impact of additional tests will outweigh the risk for severe adverse events. In addition to further research and validation of findings, the economics of these approaches must be evaluated, and validated assays designed to support clinical implementation of pharmacometabolomics. In order to advance this field, future studies need to demonstrate the impact of pharmacometabolomic approaches at improving the outcome (e.g., efficacy and/or toxicity) of drug therapy. Similar challenges were faced at the early stages of pharmacogenomic testing with several drugs (e.g., warfarin, mercaptopurine) now having high
level evidence supporting the positive impact of genetic testing on patient outcomes. Assessment of metabolite profiles have been successful in identifying inborn errors of metabolism and future collaboration between clinical chemists, researchers and health care practitioners will be necessary to advance the study of pharmacometabolomics into the clinic.
Data Availability Statement
The authors declare that all the data supporting the manuscript are contained within the paper.

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Authorship Contributions
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References

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PharmGKB.org.


Legends for Figures

Figure 1. Comparison of standard medicine and precision medicine approaches to drug therapy. Created with BioRender.com.
Table 1. Comparison of NMR and LC-MS based metabolomic approaches.

<table>
<thead>
<tr>
<th></th>
<th>NMR</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Non-destructive</td>
<td>Destructive (requires ionization)</td>
</tr>
<tr>
<td>Derivatization</td>
<td>Not required</td>
<td>GC-MS - Requires derivatization</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LC-MS - optional</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>Less sensitive</td>
<td>More sensitive</td>
</tr>
<tr>
<td>Qualitative</td>
<td>Structural identification</td>
<td>m/z identification</td>
</tr>
<tr>
<td>Sample Preparation</td>
<td>Simple sample preparation</td>
<td>Complex sample preparation (esp. for GC-MS)</td>
</tr>
<tr>
<td>Quantitative</td>
<td>Inherently Quantitative</td>
<td>Requires standard curve</td>
</tr>
<tr>
<td>Reproducibility</td>
<td>Higher</td>
<td>Lower</td>
</tr>
</tbody>
</table>
Table 2. Summary of biomarkers associated with pharmaceutical compounds and their clinical significance.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Clinical Setting</th>
<th>Study Subjects</th>
<th>Biomarker</th>
<th>Utility</th>
<th>Clinical Significance</th>
</tr>
</thead>
</table>
| Citalopram/Escalotram | Major depressive disorder                  | Clinical cohort (Caspani et al., 2021)      | Plasma lipoproteins, apolipoproteins | Prediction of drug efficacy | • Lower baseline plasma lipoprotein and apolipoprotein levels were predictive of response to escitalopram in male patients  
• Higher baseline plasma lipoprotein and apolipoprotein levels were predictive of response to escitalopram + aripiprazole in female patients |
|                   |                                           | Clinical cohort (Ji et al., 2011)           | Plasma glycine                     | Prediction of drug efficacy | • Baseline plasma glycine levels were negatively associated with response to citalopram/escitalopram  
• Subsequent genotyping revealed a SNP in the gene encoding glycine dehydrogenase (rs10975641) to be associated with response to citalopram/escitalopram |
| Doxorubicin       | Triple negative breast cancer (TNBC)       | Patient-derived xenograft mouse model (Velenosi et al., 2022) | Urine diacetylspermine             | Prediction of drug efficacy | • Elevated urine levels of diacetylsperrmine found in mice bearing doxorubicin-sensitive TNBC tumors  
• Elevation of diacetylsperrmine was mediated by doxorubicin-induced SAT1 expression  
• Urine diacetylsperrmine may be a potential biomarker of doxorubicin effectiveness in TNBC |
| Cisplatin         | Acute kidney injury (AKI)                  | Mouse model (Lim et al., 2023a)             | Metabolites associated with mitochondrial bioenergetics | Monitoring of drug toxicity | • Serum creatinine is a poor marker of AKI due to late presentation following kidney injury  
• 26 metabolites were found to be altered prior to elevations in creatinine levels – potential early markers of cisplatin-induced AKI  
• Metabolites altered by cisplatin were associated with fatty acid β-oxidation, TCA cycle, and overall mitochondrial health |
|                   |                                           | Clinical cohort (Lim et al., 2023b)         | Urine glycine, 3-hydroxydecanedioic acid, hippuric acid sulfate, suberate | Prediction of drug toxicity | • Baseline urine levels of glycine, 3-hydroxydecanedioic acid, hippuric acid sulfate, and suberate were significantly different between adult head and neck cancer patients who developed AKI following cisplatin therapy vs. patients who did not develop AKI  
• Four potential urine markers to predict patient risk for cisplatin-induced AKI prior to cisplatin |
<table>
<thead>
<tr>
<th>Drug development</th>
<th>Cardiotoxic drugs</th>
<th>In vitro drug toxicity assay (Palmer et al., 2020)</th>
<th>Arachidonic acid, 2'-deoxy-cytidine, lactic acid, thymidine</th>
<th>Prediction of drug toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>OATP1B inhibitors</td>
<td>Drug development</td>
<td>Oatp1b2 knockout mice (Li et al., 2022)</td>
<td>Plasma CDCA-24G</td>
<td>Prediction of transporter-mediated drug-drug interactions (DDIs)</td>
</tr>
<tr>
<td>Drug development</td>
<td>Drug development</td>
<td>GWAS study + clinical cohort (Yee et al., 2016)</td>
<td>Plasma bile acids, steroids, fatty acid dicarboxylates, lysolipids</td>
<td>Prediction of transporter-mediated DDIs</td>
</tr>
<tr>
<td>Paroxetine</td>
<td>Personalized medicine</td>
<td>Clinical cohort (Magliocco et al., 2021)</td>
<td>Plasma and urine solanidine</td>
<td>Monitoring of CYP activity</td>
</tr>
</tbody>
</table>

- Susceptibility to cisplatin-induced AKI may be associated with pre-existing baseline differences in mitochondrial fatty acid β-oxidation
- Screening of novel drugs for cardiotoxicity using pharmacometabolomics
- Metabolomics used to identify metabolites altered by cardiotoxic drugs in cardiomyocytes
- Arachidonic acid, 2'-deoxy-cytidine, lactic acid, and thymidine were selected to generate a model for the prediction of cardiotoxicity for novel drugs
- Metabolomic comparison of Oatp1b2 knockout mice and wildtype mice identified CDCA-24G as a marker of OATP1B function
- CDCA-24G was confirmed as a substrate of OATP1B transporters using in vitro and in vivo studies
- Changes in plasma concentrations of CDCA-24G following paclitaxel administration (known OATP1B inhibitor) were observed clinically in cancer patients
- GWAS studies of the human metabolome revealed OATP1B1 variant (rs4149056) was associated with higher plasma levels of 20 metabolites; of these, 12 metabolites were significantly higher in healthy subjects receiving OATP1B1 inhibitor cyclosporine A
- Bile acids, steroids, fatty acid dicarboxylates, and lysolipids represent potential in vivo markers of transporter-mediated DDIs
- Metabolomics using healthy volunteers genotyped for CYP2D6 to identify potential biomarkers for CYP2D6 activity
- Subjects receiving paroxetine (CYP2D6 inhibitor) exhibited higher levels of solanidine and lower levels of solanidine metabolites
- Subjects with the poor metabolizer CYP2D6 phenotype exhibited higher levels of solanidine and lower levels of solanidine
<table>
<thead>
<tr>
<th>Personalized medicine</th>
<th>Clinical cohort (Kim et al., 2017)</th>
<th>Urine ω- or (ω-1)-hydroxylated medium chain acylcarnitines</th>
<th>Monitoring of CYP activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoconazole + rifampicin</td>
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</tr>
</tbody>
</table>

- Metabolomics analysis of healthy subjects receiving ketoconazole (CYP3A inhibitor) and rifampicin (CYP3A inducer) along with midazolam (CYP3A probe substrate)
- ω- or (ω-1)-hydroxylated medium chain acylcarnitines were identified as endogenous metabolites associated with CYP3A inhibition and induction
- A predictive model was generated using ω- or (ω-1)-hydroxylated medium chain acylcarnitines to predict midazolam clearance with high correlation ($r^2 = 0.894$)
**Figure 1**

Standard Medicine

- Clinical Presentation
- Treatment
- Varied Treatment Response
  - Adverse Effects
  - Nonresponse
  - Desired Response

Precision Medicine

- Metabolome
- Proteome
- Transcriptome
- Genome
- Clinical Presentation

- Altered Metabolite Levels
- Altered Protein Expression/Dynamics
- Altered Transcription
- Genetic Variation

- Data Analysis
- Informed Treatment
- Desired Response