Multi-factorial pharmacokinetic interactions: unraveling complexities in precision drug therapy

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Abstract

Introduction: Precision drug therapy requires accounting for pertinent factors in pharmacokinetic (PK) inter-individual variability (*i.e.,* pharmacogenetics, diseases, polypharmacy, and natural product use) that can cause sub-therapeutic or adverse effects. Although each of these individual factors can alter victim drug PK, multi-factorial interactions can cause additive, synergistic, or opposing effects. Determining the magnitude and direction of these complex multi-factorial effects requires understanding the rate-limiting redundant and/or sequential PK processes for each drug.

Areas covered: Perturbations in drug metabolizing enzymes and/or transporters are integral to single- and multi-factorial PK interactions. Examples of single factor PK interactions presented include gene-drug (pharmacogenetic), disease-drug, drug-drug, and natural product-drug interactions. Examples of multi-factorial PK interactions presented include drug-gene-drug, natural product-gene-drug, gene-gene-drug, disease-natural product-drug, and disease-gene-drug interactions. Clear interpretation of multi-factorial interactions can be complicated by study design, complexity in victim drug PK, and incomplete mechanistic understanding of victim drug PK.

Expert opinion: Incorporation of complex multi-factorial PK interactions into precision drug therapy requires advances in clinical decision tools, intentional PK study designs, drug metabolizing enzyme and transporter fractional contribution determinations, systems and computational approaches (*e.g.,* physiologically-based pharmacokinetic modeling), and PK phenotyping of progressive diseases.

Keywords

complex interactions; disease-drug interaction; drug-drug interaction; multi-factorial; natural product-drug interaction; pharmacogenetics; pharmacokinetics; precision medicine; transporter-mediated drug interaction

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1.1 Introduction

Precision medicine is a rapidly expanding approach to healthcare that seeks to account for all pertinent factors affecting health, including inter-individual variability (*i.e.*, genes, environment, and lifestyle), to maximize patient benefit and minimize patient harm. Recent advances in -omics technologies and mechanistic understanding of pharmacokinetic (PK) and pharmacodynamic (PD) mediators have broadened the application of precision medicine. In 2015, the National Institutes of Health began the most expansive precision medicine effort, the *Precision Medicine Initiative,* with a goal of characterizing factors that affect health, including those that contribute to altered PK and PD, by recruiting at least one million people living in the United States into the *All of Us* Research Program [1].

The major inter-individual variability factors that can affect PK and PD include genetics (*i.e.,* pharmacogenetics), environment (*i.e.,* diseases and polypharmacy), and lifestyle (*i.e.,* diet and natural product use). Additionally, other factors such as age, sex, and other physiological differences are known to affect PK and PD, however, these factors are not the focus of this article and have been thoroughly reviewed elsewhere [2–5]. Extensive evidence has demonstrated that each factor alone can alter PK and PD (Figure 1A and sections 2.1–2.4), but individual factors have not been able to explain the entirety of inter-individual variability. As our understanding of individual factors grows, evidence is emerging of multi-factorial interactions involving the combination of two or more factors with, additive, synergistic, or opposing PK effects (Figure 1B) [6,7]. The magnitude and direction of the multi-factorial effects are context dependent based on the rate-limiting PK processes for each drug. These processes typically involve functional redundant and/or sequential pathways wherein more than one metabolic enzyme or transporter is capable of metabolizing or transporting the same drug (Figure 2). This review will focus on single- and multi-factorial PK interactions involving drug metabolizing enzymes and transporters integral to PK [8–10].

To clarify what is meant by "interactions" and how they are written in this review, the perpetrating factors are always listed at the beginning of the interaction and are separated by hyphens. The "drug" listed last in the interactions always represents the victim drug. Rather than suggesting that the perpetrating factors influence each other, this review is focused on how single or multiple perpetrators affect a victim drug.

1.2 Intricacies of multi-factorial interactions

To understand the intricacies of multi-factorial PK interactions, two areas of complexity require further discussion: redundant/sequential PK processes and complex inter-individual variability factors.

Functionally redundant and/or sequential PK processes are characterized by more than one enzyme or transporter (PK mediators) that determine drug absorption, distribution, metabolism, and excretion (ADME) (Figure 2). For these PK processes, a factor changing only a single PK mediator will be less likely to alter the victim drug PK. In contrast, more

complex effects that alter more than one PK mediator in a redundant or sequential process may have a greater impact on PK.

Complex inter-individual variability factors are characterized by a single factor that affects multiple PK mediators. For example, a genetic polymorphism typically only affects a single PK mediator, whereas disease states may decrease expression or function of multiple PK mediators. In addition, a drug-drug interaction (D-DI) precipitated by a single drug may inhibit a few PK mediators, whereas a natural product (NP) that is a complex mixture of compounds may inhibit the function of many PK mediators, although some single drugs can elicit complex interactions [11,12]. Therefore, diseases and natural products may have a greater impact on drug disposition due to their ability to affect multiple sites of drug ADME.

Understanding these complexities and appropriately accounting for each factor will maximize drug efficacy and minimize toxicity leading to improved overall patient outcomes. This article will review single- and multi-factorial PK interactions and provide an expert opinion on the future of precision drug therapy.

2   Single factor PK interactions

Table 1 summarizes examples of single factor PK interactions.

2.1   Drug-drug interactions

D-DIs are a major concern in patients experiencing polypharmacy, the concurrent use of multiple drugs, accounting for nearly 30% of all reported adverse drug reactions [13]. Between 2013 and 2016, over 51% of the 103 drugs approved by the U.S. Food and Drug Administration (FDA) were victims of at least one D-DI, 14 of which produced a   5-fold increase in area under the plasma concentration time curve (AUC) [14]. Anti-viral and chemotherapeutic drugs are the most common groups of drugs to fall victim to D-DIs, and these patient populations are particularly prone to polypharmacy [14]. As the number of approved and repurposed drugs continues to grow, the number of potential interactions involving drug metabolizing enzymes and transporters will also continue to grow.

Metabolizing enzyme-mediated D-DIs are extensively documented [15–17]. The cytochrome P450 (CYP) superfamily of metabolizing enzymes have broad substrate specificity and are majors targets for D-DIs [18]. For example, the CYP3A family, which is expressed in the liver and intestine, is involved in an approximately two-thirds of D-DIs among recently approved drugs [14]. Several anti-fungal azole drugs are metabolized by and inhibit CYP3A enzymes at plasma concentrations below 1 μM [19], and co-administration of the benzodiazepine midazolam with fluconazole increased midazolam plasma AUC 2- to 3-fold, significantly increasing PD effects as observed by the digit symbol substitution test, critical flicker fusion test, and subjective drowsiness [20]. UDP-glucuronosyltransferases (UGTs) are also involved in D-DIs, however, these interactions are less common due to the smaller number of selective inhibitors and inducers, lower inhibitor affinity, and isoform functional redundancy [10,21]. Thus, UGT-mediated D-DIs rarely produce an AUCi/AUC ratio greater than 2-fold [10].

Transporter-mediated D-DIs can affect drug distribution and excretion [21]. The FDA and the International Transporter Consortium (ITC) have highlighted multiple important transporters including organic anion transporting polypeptides (OATPs), organic anion transporters (OATs), organic cation transporters (OCTs), breast cancer resistance protein (BCRP), p-glycoprotein (P-gp), and multidrug and toxin extrusion proteins (MATEs) [21–23]. Rifampin is a canonical inhibitor of hepatic OATP1B1 and OATP1B3 uptake transporters. Intravenous infusion of 600 mg rifampin along with oral administration of 40 mg atorvastatin to healthy volunteers increased atorvastatin parent and metabolite $AUC_{0-\infty}$ $682 \pm 241\%$ and $167 \pm 73\%$, respectively [24]. While transporters have traditionally garnered less attention than metabolizing enzymes in the area of D-DIs, the efforts of the FDA and ITC as well as the increasing number of review articles focusing on transporter-mediated D-DIs illustrate an increased interest and relevance in the field [23,25–27].

The above D-DI examples describe interactions between a single perpetrating drug, a single victim drug, and specific enzymes or transporters, but the pathway through the body for many drugs is often more complex and requires careful consideration to understand the D-DI mechanism. As the drug enters the body and is distributed, it can be a substrate for multiple transporters before reaching its pharmacological site of action and/or site of metabolism. Likewise, the parent drug and its metabolites can be metabolized by multiple enzymes before undergoing efflux and excretion. In addition, D-DIs can be exploited to benefit the patient. An example of this complexity is illustrated by the D-DI between the anti-viral drugs paritaprevir and ritonavir. Ritonavir is an inhibitor of, while paritaprevir is a substrate for, multiple enzymes and transporters (*e.g.,* CYP3A, OATP1B1, OATP1B3, BCRP and P-gp). Co-administration of these two drugs increased paritaprevir AUC (47-fold), C_{max}, and trough concentrations, thereby improving treatment for chronic hepatitis C infection [14,28]. Another important consideration for D-DIs is the specific molecular mechanism, which can involve different types of inhibition (*e.g.,* competitive, uncompetitive, mechanism-based) and/or gene induction. Assiduous attention to detail is important in patients experiencing polypharmacy because the complexity of some D-DIs can create risks for adverse drug interactions and/or provide opportunities for improved pharmacotherapy.

2.2 Natural product-drug interactions

Natural product-drug interactions (NP-DIs) pose a complex challenge to the advancement of precision medicine. Natural product use continues to increase in the United States, as evident by natural product herbal supplement sales increasing 8.6% to an estimated $9.6 billion in 2019 [29]. NP use also contributes to adverse drug events with over 23,000 estimated annual emergency department visits between 2004 and 2013 reported to be associated with NP use [30]. Robust NP-DI research is complicated by inconsistent product compositions between different brands of the same NP and between different lots of the same brand because of variability in how products are sourced, processed, and formulated [31]. In contrast to D-DIs, which usually involve one specific perpetrating compound, NPs are often a complex mixture of compounds potentially capable of inhibiting multiple PK mediators. The complex nature of NP-DIs precludes a comprehensive review of this area of precision medicine, but a brief example to illustrate the challenges in this area is outlined here.

Grapefruit juice (GFJ) perpetrates a set of well-characterized NP-DIs that involve multiple perpetrating compounds, victim drugs, and PK mediators. The main perpetrating constituents in GFJ are flavonoids and furanocoumarins (FCs), which inhibit intestinal CYPs [32–34]. Thus, GFJ increased AUC of multiple statins (*e.g.*, simvastatin by ~1,610%, atorvastatin acid by ~250%, and atorvastatin lactone by ~330%) *via* CYP3A4 inhibition, and irreversibly inhibited intestinal CYP2B6 and CYP3A5 [6,34–37]. GFJ flavonoids and FCs also inhibit multiple transporters such as OATPs and P-gp [38]. However, unlike the irreversible inhibition seen with the aforementioned intestinal CYPs, transporter inhibition is reversible [38]. GFJ highlights the complexity of NP-DIs because each of the multiple constituents can affect drug disposition in different ways (*e.g.*, FCs inhibit CYP3A4 while the flavonoids inhibit intestinal OATP-mediated uptake). Several review articles provide in-depth analysis of the complex nature of GFJ-mediated NP-DIs [33,39,40].

The complex nature of NP-DIs has created often perplexing and contradictory results in the scientific literature. The Center of Excellence of Natural Product Drug Interaction Research (NaPDI Center) was formed to address these complexities and devise a series of recommended approaches for NP-DI research [41–43]. Adherence to these, or similar, recommended approaches will improve the quality of NP-DI research and facilitate incorporation of NP-DIs into multi-factorial interactions.

2.3 Pharmacogenetic-drug interactions

Pharmacogenetics, or gene-drug interactions (G-DIs), are well-documented to influence efficacious and safe pharmacotherapy, and genetic testing is a cornerstone to precision medicine. Genetic polymorphisms that alter the expression and/or function of PK mediators can involve a single nucleotide polymorphism (SNP) or multiple nucleotide polymorphisms that form a polymorphic allele. These polymorphisms can be classified into different functional phenotypes for metabolizing enzymes (*i.e.,* poor, intermediate, extensive, and ultrarapid metabolizers) and transporters (*i.e.,* low, intermediate, and normal function). Implementation of pharmacogenetics often involves dose adjustments or medication changes, and has been observed to reduce the number of re-hospitalizations by 53% and emergency room visits by 42%[44]. Open-source databases cataloging the characteristics of several of these pharmacogenomic interactions are established, such as Pharmacogenomic Knowledge Base (PharmGKB) and the Pharmacogene Variation (PharmVar) Consortium for example. Additionally, the Clinical Pharmacogenetics Implementation Consortium (CPIC) compiles information and data to support relevant and updated clinical decision-making guidelines to aid clinicians on known G-DIs.

CYPs are highly polymorphic (*e.g.,* CYP2D6 has over 100 variant alleles), and the number of clinically actionable CYP polymorphisms continues to grow [45]. CYP2D6, CYP2C9, and CYP2C19 represent the most common metabolizing enzymes with respect to polymorphic expression, thus are of greatest regulatory interest when assessing potential G-DIs [46]. For example, CYP2D6 polymorphisms are responsible for significant variations in exposure to the antipsychotic aripiprazole, where poor metabolizers saw a 50% increase in AUC compared to extensive metabolizers [47]. Such increases suggest a dose adjustment may be necessary for some populations, however the FDA does not currently mandate

CYPD2D6 genotyping [47]. CYP2C19 polymorphisms are also known to affect exposure and efficacy of the anti-fungal voriconazole. Voriconazole AUC was 48% and 85% lower in healthy male volunteers expressing an ultra-rapid metabolizer phenotype compared to the extensive metabolizer and poor metabolizers, respectively [48]. This is particularly notable because trough concentrations of voriconazole < 1 mg/L are associated with sub-therapeutic effects, and may warrant dose adjustments or medication changes [48,49]. Another established G-DI involves CYP2C9 and the anticoagulant warfarin. Warfarin is administered in a racemic mixture comprising both the S- and R-enantiomers, with the S-enantiomer producing a 3- to 5-fold higher anticoagulant effect [50]. Elimination of the S-enantiomer is mediated primarily by CYP2C9. Therefore, CYP2C9 poor metabolizers are prone to increased systemic exposure, and can experience uncontrolled internal bleeding [50,51]. Comprehensive CYP-based G-DI reviews are available in the literature [52–55].

Some metabolizing enzyme polymorphisms affect specific classes of drugs. For example, the dihydropyrimidine dehydrogenase (*DPYD*) gene, which encodes for dihydropyrimidine dehydrogenase (DPD) and is responsible for the catabolism of the anti-cancer agent 5-fluorouracil (5-FU), has multiple variant alleles that decrease DPD activity [56]. 5-FU has serious dose-dependent toxicities that occur due to decreased DPD activity and increased 5-FU plasma accumulation [57]. These variants have three times greater prevalence in African populations compared to Caucasian populations [56,58]. The CPIC has established guidelines for 5-FU dose adjustments in patients expressing *DPYD* variant alleles [57].

Polymorphisms in uptake and efflux transporters can directly affect the absorption, distribution, and excretion of a victim drug, and may indirectly affect the metabolism of that drug when transport is the rate-limiting step of its elimination [8]. For example, the c.421C>A nonsynonymous SNP in ATP-binding cassette G2 (*ABCG2*), the gene encoding BCRP, significantly increased the exposure of the investigational anti-cancer agent diflomotecan by 299% in patients heterozygous for the SNP [59]. The underlying mechanism for the c.421C>A SNP dysfunction is believed to be a result of decreased protein expression [60,61]. The clinical importance of transporter polymorphisms on PK is expected to grow as functional characterization of polymorphic transporters expands [62–65].

G-DIs are distinct from D-DIs and NP-DIs because polymorphisms generally affect a single metabolizing enzyme or transporter, whereas D-DIs and NP-DIs have a greater potential to affect multiple ADME processes. Thus, drug interactions are most impactful for drugs that are dependent on a single drug metabolizing enzyme or transporter in the ADME of the drug. In contrast, drug substrates with broader selectivity may be impacted in multi-factorial PK interactions when the redundant mechanisms that compensate for the dysfunctional polymorphic protein are also perturbed. The importance of complex inter-individual variability factors is discussed further in the multi-factorial sections (see Sections 3.1–3.4).

2.4 Disease-drug interactions

Diseases of the major ADME organs (*i.e.,* intestine, liver, and kidney) can alter PK through mechanisms involving organ structure and/or function. The liver will be the focus of this section to illustrate examples of disease-drug interactions (Dis-DIs). The FDA has issued a guidance document for assessing PK in patients with hepatic impairment and held a

workshop in October to discuss ways to improve these assessments [66]. Conditions that elicit an immune response can alter CYP expression and activity. For example, interferons decreased CYP expression [67], and interleukin-6 production in the days following surgery decreased CYP3A4 activity 20–60% [68]. Other biological insults that elicit similar immune responses, such as autoimmune diseases, acute infections, and severe trauma, also decreased hepatic CYP3A4 activity [69]. Likewise, hepatic CYP3A activity decreased in patients with advanced malignancies, which is noteworthy considering the number of chemotherapeutic agents metabolized by the CYP3A family and the often narrow therapeutic window for these drugs [70]. In fact, altered CYP3A activity is believed to account for some of the interindividual variability in chemotherapy efficacy [70]. Dysfunction of other CYP enzymes have also been reported in chronic liver diseases [71,72].

Transporters are also subject to liver disease-mediated changes in function. For example, NTCP and OATP1B3 protein expression decreased in livers from nonalcoholic steatohepatitis (NASH) patients. In contrast, MRP2 protein expression increased in NASH [11], although MRP2 was shown to undergo a mislocalization event in NASH, resulting in reduced MRP2 function [73]. The changes in OATP1B3 and MRP2 function decreased ^{99m}Tc-mebrofenin uptake clearance from the blood to liver and clearance from liver to bile [74]. Protein expression of other efflux transporters such as MRP1, MRP3, P-gp, and BCRP also increased in NASH [73]. Hepatitis C (HCV) is also reported to affect hepatic transporter expression. Uptake transporters OCT1, OATP1B1, and OATP1B3, and efflux transporters MATE1, MDR1, MRP1, MRP2, MRP4, and BCRP all had elevated mRNA levels in cirrhotic HCV livers compared to healthy livers [75]. The increase in transporter transcription could be associated with the increased transcription of tumor necrosis factor-alpha (TNF-α) or possibly nuclear factor erythroid 2-related factor (Nrf2), both of which have been shown to affect transcription of transporters [76,77]. Interestingly, NASH and HCV are also reported to alter NP PK. For example, silymarin flavonolignan AUC and C_{max} were increased in NASH and HCV patients [78,79]. A review of liver disease effects on hepatic transporter mRNA and protein expression can be found elsewhere [80].

Diseases of ADME organs frequently occur as co-morbidities and affect multiple steps in sequential and/or redundant PK processes. Although hepatic impairment was the focus of this section, impaired renal function can have similar effects on drug metabolism and transport and is the subject of a recent FDA guidance document [66]. As stated above, diseases often affect more than a single gene or protein. However, as noted by the ITC, broad specificity of potential victim drugs may mitigate the PK-mediated effects associated with diseases through redundant transport processes [81]. Thus, Dis-DIs may be most impactful in a multi-factorial PK interaction.

3   Multi-factorial PK interactions

Table 2 summarizes examples of multi-factorial PK interactions.

3.1   Drug-gene-drug interaction and natural product-gene-drug interaction

Multi-factorial interactions between exogenous compounds (*e.g.,* drugs or natural products) and genetic polymorphisms have several layers of complexity that determine clinical effects.

Central to this complexity is the sequential and/or redundant nature of the specific PK processes for the victim drug. Several examples of multi-factorial effects on sequential or redundant PK processes are reviewed in this section. In addition, the polymorphism effect on intrinsic clearance (*i.e.,* V_{max}/K_m) and/or inhibition kinetics (*i.e.,* IC_{50} or K_i) can influence the clinical multi-factorial effect. The details for each victim drug, polymorphism, and perpetrating compound(s) should be considered to determine the risk of these multi-factorial interactions.

Sequential PK processes involving the combination of transporter polymorphisms and D-DIs can alter victim drug plasma concentrations greater than each factor alone through a drug-gene-drug interaction (D-G-DI). For example, repaglinide is a substrate for OATP1B1 and CYP2C8. The *SLCO1B1* c.521T>C SNP encoding the *SLCO1B1*5* polymorphism (homozygous) increased repaglinide plasma AUC 1.8-fold, while inhibition of CYP2C8 *via* gemfibrozil increased repaglinide plasma AUC 8.2-fold [82]. The combination of *SLCO1B1*5* (homozygous) and gemfibrozil-mediated CYP2C8 inhibition increased repaglinide plasma AUC 11.1-fold [82]. These data suggest that patients with the *SLCO1B1*5* allele who experience CYP2C8 inhibition will have an elevated risk of increased exposure to repaglinide, potentially compromising glycemic control [82].

Another example of a sequential D-G-DI involves CYP3A4 inhibition and OATP1B1 and P-gp polymorphisms on the disposition of simvastatin. Simvastatin is metabolized into its active metabolite, simvastatin acid, by CYP3A4, which undergoes hepatic uptake *via* OATP1B1 [83,84]. Pre-treatment with amlodipine alone, a weak CYP3A4 inhibitor, increased simvastatin and simvastatin acid AUC 80% and 40%, respectively [85]. Subjects heterozygous for the *SLCO1B1*5* allele had a 40% increase in simvastatin acid AUC [85]. The combination of amlodipine and heterozygous *SLCO1B1*5* caused a 90% increase in simvastatin acid AUC relative to control groups homozygous for the reference *SLCO1B1* allele [85]. No combined effect was observed in the presence of the *MDR1* c.1236T>C, 2677G>T(A), or 3435C>T polymorphisms, suggesting P-gp is not as integral to simvastatin acid disposition, however conflicting data exist [85,86]. Perturbations in simvastatin PK may cause elevated levels of simvastatin acid leading to myopathy and potential termination of statin treatment, increasing the risk of cardiovascular disease [86–90].

Redundant PK processes involving the combination of transporter polymorphisms and D-DIs can have divergent effects based on substrate specificity. For example, hexadecanedioate (HDA), tetradecanedioate (TDA), coproporphyrin I (CP-I), and coproporphyrin III (CP-III) are considered endogenous biomarkers of hepatic OATP1B1/OATP1B3 function [91,92]. Polymorphic *SLCO1B1* increased plasma AUC of all four substrates, but compounded PK effects were only observed for CP-I and CP-III in homozygous *SLCO1B1*5* subjects [93]. The lack of combined effects of the polymorphism and the D-DI may be due to the fact that HDA and TDA are also substrates of OAT1 and OAT3, whereas CP-I and CP-III are primarily substrates of hepatic OATPs [94]. Another study co-administrated fevipiprant with either rosuvastatin or simvastatin in patients expressing the *SLCO1B1*5* allele, and reported an additive effect only for simvastatin acid C_{max} [95]. This may be explained by the fact that rosuvastatin is a substrate for multiple transporters, including NTCP, whereas simvastatin acid is more dependent on OATP1B1 for hepatic uptake [88]. Importantly, both studies

described here suffered from small sample sizes for the individuals carrying the polymorphism and require further investigation to confirm the results.

Physiologically based pharmacokinetic (PBPK) modeling has been used to evaluate multi-factorial PK interactions. For example, the modeling of pitavastatin and atorvastatin exposure in the context of *SCLO1B1*5* polymorphisms in combination with multiple known OATP inhibitors (itraconazole, erythromycin, and gemfibrozil) provided encouraging results [96]. However, the model continued to under-predict the statin exposure even after adjustments to scaling factors were applied [96]. It is believed that the under-predictions were due, in part, to inconsistencies in the inhibition kinetic data between *in vitro* and *in vivo* systems [96]. The authors noted similar under-predictions reported by other groups modeling similar systems [96–98]. The reduction in K_i values of the inhibitors corrected some of the prediction, but highlights the need for quality *in vivo* data to validate PBPK models [96].

Some polymorphisms reduce the effect of the perpetrating drug or NP on victim drug PK. An example of this is the interaction between baicalin and rosuvastatin. Hyperbilirubinemia occurs in individuals suffering from total or substantial loss of OATP1B1 and OATP1B3 function, a rare disease known as Rotor's Syndrome [99]. The NP baicalin can partially rescue *SLCO1B1* polymorphic transporter dysfunction by decreasing bilirubin levels, suggesting potential induction of OATPs by baicalin [100]. A PK NP-gene-drug interaction (NP-G-DI) study investigated the effect of baicalin on rosuvastatin disposition in subjects with *SLCO1B1* polymorphisms. *SLCO1B1*15/*15* subjects had a modest increase in rosuvastatin $AUC_{0-\infty}$ compared to *SLCO1B1*1b/*1b* subjects. Administration of 50 mg baicalin three times daily for 14 days reduced rosuvastatin $AUC_{0-\infty}$ in *SLCO1B1*1b/*1b* subjects by 41.9% and *SLCO1B1*1b/*15* subjects by 23.9%, whereas baicalin did not affect rosuvastatin $AUC_{0-\infty}$ in *SLCO1B1*15/*15* subjects [100]. The authors suggest hepatic OATP induction may explain reduced rosuvastatin AUC, although no data to support this claim were presented. Alternatively, baicalin inhibits OATP2B1, which could reduce rosuvastatin AUC after baicalin administration [101]. The mechanism for reduced baicalin effect in subjects carrying one or two copies of *SLCO1B1*15* is unclear and requires further investigation. A limitation to this study is the absence of reference allele participants (*SLCO1B1*1a/*1a*). This study demonstrates the importance of identifying the perpetrator target transporter(s), the major PK processes for the victim drug, and where the two intersect in order to clearly characterize the multi-factorial interaction.

As with hepatic PK mediators, intestinal metabolizing enzymes and transporters are also susceptible to multi-factorial NP-G-DIs involving fruit juices, such as grapefruit, orange, and apple [102]. For example, co-administration of apple juice with fexofenadine reduced fexofenadine AUC and increased $t_{1/2}$ in all *SLCO2B1* genotypes, although to a slightly lesser extent in individuals with the c.1457C>T allele, suggesting reduced inhibitory effect in the presence of the polymorphism and greater dependence on OATP2B1 for fexofenadine uptake [103]. In contrast, apple juice decreased atenolol AUC in a dose-dependent manner, but the *SLCO2B1* c.1457C>T polymorphism had no effect, suggesting that an OATP2B1 redundant transporter may be involved in atenolol uptake in the intestine [104]. Inconsistent

genotype-dependent effects emphasize the importance of substrate specificity and redundant processes with respect to these intestinal transporters.

An additional layer of complexity to potential multi-factorial interactions between exogenous compounds and polymorphisms is altered perpetrator inhibition kinetics for polymorphic alleles. Polymorphisms in *CYP2D6* and *CYP3A4* increased or decreased IC_{50} values for several inhibitors in a polymorphism specific manner. For example, *CYP2D6*2* decreased terbinafine IC_{50} for the substrate venlafaxine (607 nM to 93 nM) [105]. Decreased IC_{50} values will increase the risk of an NP-DI or D-DI, while increased IC_{50} values will decrease the risk of an NP-DI or D-DI. These changes in CYP IC_{50} values are relevant because a retrospective analysis of 1,143 individuals reported 217 individuals (19%) may experience a multi-factorial D-G-DI involving CYP2C9, CYP2C19, or CYP2D6 [106]. More research is needed to determine how these changes in inhibition kinetics effect multi-factorial PK interactions.

These data highlight the complexity of metabolizing enzyme and transporter specificities, and the relevance of redundant and/or sequential uptake, metabolism, and efflux processes. To account for NP use in precision drug therapy, clinicians first need to accurately capture the quantity and content of NP consumption, which requires full patient disclosure and accurate product characterization. Additionally, this requires an understanding of which NPs are potentially clinically relevant for the multitude of ADME processes of the drugs taken by the patient. Application of pharmacogenomic testing, especially in polypharmacy populations, as part of the clinical decision making process has been shown to decrease rehospitalization [107]. Therefore, by expanding pharmacogenetic testing and increasing our understanding of these discrete PK processes, better clinical decisions can be made to maximize precision drug therapy.

3.2   Gene-gene-drug interactions

Genetic polymorphisms in two or more drug metabolizing enzymes or transporters occur in a predictable manner based on allele copy number frequencies. These complex genetic interactions differ from other multi-factorial interactions because these will never involve a 'double hit' on a single rate-limiting PK mediator, whereas the combination of a natural product inhibitor and a polymorphism could impact the same metabolizing enzyme or transporter. Thus, gene-gene-drug interactions (G-G-DIs) will always involve sequential and/or redundant PK processes.

An example of such an interaction is SN-38, the active anti-cancer metabolite of the prodrug irinotecan, which is a sequential substrate of OATP1B1 and UGT1A1 (metabolism is an inactivation step) [108]. *SLCO1B1* and *UGT1A1* polymorphisms create an additive PK effect, increasing plasma SN-38 and causing severe toxicity [109–112]. Notably, individuals heterozygous for *SLCO1B1*5* and *UGT1A1*28* had toxicity risks similar to patients that are homozygous for either polymorphism, and patients with two or more *SLCO1B1*5* and *UGT1A1*28* polymorphisms had an odds ratio of 4.15 for grade 3/4 neutropenia compared to reference patients [109]. Another study found that these polymorphisms were associated with severe irinotecan toxicity, which necessitated termination of cancer treatment. These data demonstrate that additive PK effects can have a deleterious effect on patient outcomes

[110]. The parent drug, irinotecan, is hydrolyzed by carboxylesterases (primarily CES2) into its metabolite SN-38, which is 100–1000-fold more toxic than the parent drug [108]. While CES2 polymorphisms have been investigated, data suggests the polymorphic effect on irinotecan metabolism is not significant [113–115].

The anticonvulsant phenytoin exhibits a small therapeutic index with substantial inter-individual variability requiring dose monitoring to avoid serious toxicities. Phenytoin is primarily metabolized by CYP2C9 and CYP2C19 and is a substrate for P-gp, therefore involving both redundant and sequential PK processes. In one study *CYP2C9*3* and *MDR1* c.3435C>T polymorphisms were associated with altered phenytoin plasma concentrations, but *CYP2C19*2* had no effect [116]. *CYP2C19*3*, which increased phenytoin exposure in another study, was not tested because it was not present in the study participants [116]. Phenytoin plasma concentrations were associated with the number of polymorphic alleles (*i.e.,* homozygous reference < heterozygous < homozygous polymorphic). Likewise, phenytoin metabolite to parent ratio was associated with the number of polymorphic alleles (*i.e.,* homozygous reference: 1.83 ± 0.67, heterozygous: 1.34 ± 0.38, and homozygous: 0.76 ± 0.48 polymorphic) [116]. Inclusion of both *CYP2C9*3* and *MDR1* c.3435C>T alleles improved prediction of phenytoin plasma concentration variability from 9.2% to 15.4% [116].

One limitation of these studies is the inability to genotype and account for every metabolizing enzyme and transporter involved in the victim drug's disposition, thereby disregarding how the genotypes of redundant processes contributed to the observed changes in drug disposition. Genetic testing is already a mainstay in precision drug therapy, and will continue to play a major role as our knowledge of important polymorphisms grows and patient genotyping becomes more common. In addition, as genomic data continue to become more affordable and accessible, retrospective investigation of potential G-G-DIs will become more practical. Access to massive datasets, such as the *All of Us* research program, will facilitate this process and open research opportunities. In addition, continued refinement of mechanistic PK processes will allow for hypothesis testing for specific sequential and/or redundant PK processes.

3.3   Disease-natural product-drug interactions

NPs are marketed with structure-function claims that target specific disease populations, suggesting that patients with diseases may experience disease-NP-drug interactions (Dis-NP-DIs). Unfortunately, most NP-DI studies are completed in healthy volunteers because designing and executing NP-DI studies involving patients with a specific disease can be logistically and ethically challenging [117]. Thus, there are limited clinical data for these multi-factorial PK interactions. As an alternative to clinical studies, animal models can recapitulate certain diseases, however, not every disease condition is able to be replicated in animal models, and interspecies variability in transporters and metabolizing enzymes is a significant limitation when translating findings to clinical populations. Nonetheless, animal models can provide valuable PK interaction data if the preclinical models are selected judiciously and the data are interpreted and translated with caution. As covered in Sections 2.2 and 2.4, disease states can alter the structure and/or function of ADME organs, and NPs

are complex mixtures of potential NP-DI perpetrators. Thus, the combination of a disease with a NP can potentially have a multi-factorial PK effect.

Multi-factorial PK interactions can also have opposing effects, wherein the effect of each factor is negated when the factors are combined. An example of this is illustrated by the individual and combined effects of Nisha Amalaki (NA), which is a formulation of *Curcuma longa* and *Phyllanthus emblica,* and streptozotocin-induced diabetes on metformin PK in Wistar rats. The NP-DI between NA and metformin caused a significant increase in metformin C_{max} (70.0%), t_{max} (43.8%), and AUC (53.0%) in healthy animals. The authors attributed the increased metformin systemic exposure to inhibition of renal Oct2 [117]. Diabetes alone increased metformin C_{max} (164%), t_{max} (67.8%), and AUC (60.3%) compared to healthy rats [117]. The authors speculated that the diabetes-associated increase in metformin systemic exposure was due to altered hepatic CYP2C11 metabolism, however, metformin is minimally metabolized and this is unlikely to explain the increase in exposure. Rather, various streptozotocin doses and treatment times are reported to alter intestinal, hepatic, and renal transporter expression [118–120]. There are two probable transporter changes that could explain increased metformin exposure in the diabetic group; either decreased renal and/or hepatic uptake transporters, or increased intestinal uptake transporters. Interestingly, the combination of NA and diabetes decreased metformin C_{max} (62.1%) and AUC (42.7%) when compared to diabetic rats receiving only metformin, and were comparable to metformin C_{max} and AUC in healthy rats [117]. The first probable scenario for the effect of diabetes described above is not supported by the effect of NA on metformin in diabetes; rather, induction of an intestinal transporter that is susceptible to NA inhibition could explain the combined effect of NA and diabetes on metformin exposure. Unfortunately, these mechanisms were not explored, and more research is needed for this multi-factorial interaction in patient populations. This example illustrates the complexity and sometimes counteractive nature of multi-factorial PK interactions involving NPs.

The multi-factorial effect of silymarin and NASH on pitavastatin disposition illustrates a potential additive Dis-NP-DI. Pitavastatin plasma concentrations are heavily dependent on hepatic OATP1B1/OATP1B3 transporters. NASH is known to affect the expression and/or glycosylation of OATP1B1 and OATP1B3 in patients and Oatp1b2 in rats [11,12], and clinically relevant silymarin flavonolignan concentrations are known to inhibit OATP activity [121,122]. In this study, the combination of silymarin and methionine and choline deficient diet-induced NASH altered intravenous pitavastatin PK in rats [12]. According to a two-way ANOVA test, NASH and silymarin had significant effects on pitavastatin $AUC_{0-120min}$ without having a significant interaction effect. The latter effect suggests an additive interaction between NASH and silymarin [12]. Pitavastatin is a widely prescribed drug to reduce the risk of cardiovascular disease and it is used as a probe drug to represent a larger class of drugs dependent on hepatic OATP1B1/OATP1B3 uptake. Thus, these data demonstrate a potential risk of a hepatic OATP-mediated Dis-NP-DI, although more research is needed to demonstrate this interaction in patient populations.

Disease populations experience polypharmacy and are more likely to take one or more dietary supplement [123,124]. A proactive approach needs to be taken to identify potential at-risk populations to ensure proper monitoring of medications and NP use and avoid Dis-

NP-DIs. More research is needed to define how the multi-factorial mechanisms combine to elicit additive, synergistic, or opposing PK effects.

3.4 Disease-gene-drug interactions

Multi-factorial disease-gene-drug interactions (Dis-G-DIs) can complicate precision drug therapy. As previously discussed, a polymorphism in a single PK mediator can alter victim drug disposition, whereas a disease can impact multiple proteins involved in PK. In addition, diseases are typically progressive, and often have heterogeneity in PK mediator function across and within disease stages [125]. Thus, the combination of these two factors can have divergent effects depending on the different PK mediators involved in each step of victim drug PK.

Multiple polymorphic CYPs exhibited a genotype-driven modulation in *in vitro* substrate kinetics. A distinct genotype effect was observed to alter many, but not all, substrate kinetic parameters (K_m, V_{max}, and CL_{int}) in both healthy and hepatocellular carcinoma (HCC) human liver microsomes [126]. Although no statistical comparison was attempted between the healthy and HCC groups, examination of the data suggests increased *CYP2D6* reference allele V_{max} in the HCC samples compared to healthy samples of the same genotype. *CYP2D6*10* appeared to counteract the HCC-associated increase in V_{max} because the polymorphism decreased V_{max} only in the HCC samples. Further statistical tests are required to test this hypothesis. In addition, this study did not provide clinical data and noted inconsistencies with previously published data, suggesting a lack of uniformity in the selection criteria for the HCC patients [126].

Dis-D-DIs can involve transporters in redundant and/or sequential processes. For example, the combination of methionine and choline deficient diet-induced NASH and *Slco1b2* knockout in mice produced a synergistic increase in pravastatin plasma AUC, potentially due to changes in redundant transporters [127]. In this study, NASH alone did not alter pravastatin PK, while *Slco1b2* knockout had a modest effect. A potential mechanism for the synergistic interaction is the combined effects of genetic loss of a primary transporter along with downregulation of multiple redundant hepatic Oatp transporters. A similar study was performed in healthy *versus* NASH patients with or without the *SLCO1B1*15* polymorphism. NASH increased [99mTc] mebrofenin AUC in the blood and the liver potentially due to decreased expression or function of the sequential hepatic transporters OATP1B3 and MRP2 [74]. Hepatic uptake clearance was progressively lower from healthy subjects with normal OATP1B1 function to healthy subjects with intermediate/low OATP1B1 function to NASH subjects with normal OATP1B1 function. Unfortunately, this study was not able to confirm the synergistic effect observed in rodents due to insufficient NASH subjects with intermediate/low OATP1B1 function. Another example of redundant transporter processes in Dis-G-DIs involves the combination of methionine and choline deficient diet-induced NASH and *Bcrp* knockout on SN-38 PK. SN-38 is the active metabolite of the anti-cancer agent irinotecan, and is an established substrate of the hepatic efflux transporter BCRP [128]. However, SN-38 biliary AUC did not change in *Bcrp* knockout animals, suggesting a redundant canalicular efflux transporter contributes to SN-38 biliary elimination [129]. Although Bcrp expression increased in NASH, *Bcrp* wild-type

NASH rodents had no change in SN-38 biliary disposition [129]. Interestingly, although each factor in isolation did not change SN-38 disposition, the combination of the *Bcrp* knockout and NASH significantly decreased SN-38 biliary efflux by 68.1% [129]. These data illustrate the complex nature of Dis-G-DIs, and emphasize the importance of understanding all transporters involved in each step of a specific drug's disposition.

The complexity of Dis-G-DIs is highlighted by the multiple examples provided. As illustrated, the combination of two factors, that may or may not have clinical impact in isolation, can cause significant changes in victim drug PK. Another challenge for these complex interactions is accurately categorizing these multi-factorial patient populations. Identifying genetic polymorphisms is becoming more affordable and common, but disease diagnosis and staging can be imprecise, although diagnostic methods continue to improve. Consideration of liver or renal impairment has been included in precision drug therapy for many years and will continue to be integral when accounting for inter-individual variability in PK and improving patient outcomes. Finally, it is important to note that polymorphisms in metabolizing enzymes and transporters increase susceptibility or severity of liver disease, thus potentially placing these specific disease populations at a unique risk for exacerbated or idiosyncratic liver toxicity [130–132].

4 Expert opinion

Incorporation of multi-factorial PK interactions into precision drug therapy requires better mechanistic understanding of redundant and sequential PK processes, deconvolution of complex factors, characterization of combined effects, and advancing methodological and clinical approaches (Figure 3).

4.1 Mechanistic determination of redundant and/or sequential PK processes

Knowing the substrate specificity and fractional contribution of each metabolizing enzyme or transporter to rate-limiting PK processes is central to predicting complex PK interactions. Fraction metabolized (f_m) for drug metabolizing enzymes and fraction transported (f_t), and relative activity factors (RAF) or relative expression factors (REF) for transporters are important fractional contribution metrics. Critical areas for continued improvement to these metrics include expansion of specific inhibitors and substrates, advances in *in vitro* models (*e.g.,* sandwich cultured hepatocytes and microfluidics cultures), and advances in protein quantification methods [133–135]. A limitation to RAF calculations include not accounting for changes in protein expression, which can be influenced by polymorphisms and disease. In contrast, REF calculations do account for relative protein expression, but have a limitation of not distinguishing between functioning and non-functioning transporters due to technical limitations of protein quantification. Both approaches provide useful data in select contexts, but neither alone are universally accepted for determining relative contribution [135]. Therefore, prudence should be exercised when selecting the appropriate method.

Protein quantification for transporters is further complicated by functional regulation through post-translational modifications, plasma membrane localization, and oligomerization [136]. Transporter phosphorylation has also been suggested to alter localization and function of ABC transporters, however the direction and magnitude of the

effect is inconsistent making PK interaction predictions challenging [137]. Thus, further research is needed to determine the mechanism of phosphorylation-mediated transporter regulation, and whether these changes will impact clinical PK (*e.g.,* pharmacological phosphorylation modulators). Likewise, transporter glycosylation affects transporter localization and function, but unlike phosphorylation, impaired glycosylation consistently decreases transporter plasma membrane localization and function [138]. For example, transporter glycosylation decreased in liver tissue of NASH patients, potentially causing decreased uptake transporter function and contributing to decreased hepatic uptake clearance of ^{99m}Tc-mebrofenin as described in Section 2.4. Accounting for post-translational regulation of transporter function is challenging because current LC-MS/MS methods do not capture transporter phosphorylation or glycosylation without performing specific assays [138]. As research tools and our knowledge of these molecular processes continue to expand, better predictions can be made for single- and multi-factorial PK interactions to improve patient outcomes.

4.2   Deconvolution of complex factors

The major complex factors discussed in this review are NPs and diseases in ADME organs. These complex factors make identification of the perpetrating compound or perturbed physiological process more difficult because it may not be readily apparent which factor is primarily mediating the interaction. Each factor carries a unique set of challenges and opportunities that need to be addressed in unique and deliberate ways to appropriately incorporate them into precision drug therapy. For NPs, the important information includes a complete knowledge of all NP consumption for each patient, knowing the NP composition and dose, and following robust and reproducible experimental approaches. This is complicated by under-reporting of NP use and by variability in NP composition, formulations, and dosing. For diseases in ADME organs, complexities include mechanistic understanding of underlying disease states and accurately and consistently diagnosing the disease stage. As a disease progresses, changes in PK mediators may be heterogeneous within and across diagnostic categories. Additionally, subjectivity in diagnostic procedures further complicates the ability to accurately and consistently stage disease progression. For example, the gold standard for NAFLD diagnosis and staging utilizes pathologist scoring of liver biopsy for histological features such as steatosis, lobular inflammation, and ballooning [139]. Liver biopsy is limited by a small sample volume collected *via* an invasive procedure, and histological scoring carries an inherent level of variability [140]. Improved diagnosis and PK phenotyping of progressive diseases will enable clearer predictions and prescribing practices in these patients.

4.3   Characterizing the combined effects of multi-factorial interactions

Multi-factorial PK interactions cannot necessarily be predicted based on the effect of each factor alone. Examples presented in this review demonstrate that multi-factorial interactions can have combined (*i.e.,* additive or synergistic) or opposing effects. Additive and opposing effects are the most intuitive, but synergistic effects, where one or more factor individually has no significant effect but there is a greater combined effect, are more difficult to explain. Thus, it is critical that the potential combined effects are accounted for and utilized in prediction models and clinical trials.

4.4 Future directions

The future of precision drug therapy must go beyond single factor interaction studies, and take deliberate steps to incorporate pertinent multi-factorial interactions. This can be accomplished by accounting for the complexities discussed above, advancing quantitative systems pharmacology (QSP), and developing clinical decision support tools. QSP is an emerging field of systems biology incorporating exposure level parameters with PD target biology. PBPK modeling is an established and integral tool in drug development and can be used to predict drug interactions. Similar to preclinical and clinical studies, these models have traditionally focused on single factor interactions, but as the granularity and accuracy of PBPK input parameters improve, multi-factorial interaction models are becoming more common [141,142]. Therefore, continued advancements in understanding how each inter-individual variability factor, discrete pathway, and redundant/sequential process integrate to affect PK for individual patients are integral to the incorporation of PBPK and QSP and into precision drug therapy. Clinical decision support tools are designed to incorporate pertinent inter-individual variability factors discussed throughout this review into patient care. Important improvements to clinical decision support tools include expanded pharmacogenomic testing, improved disease staging, and accurate reporting of NP content and use. The immediate future of multi-factorial PK interaction research and its clinical implementation will hinge tightly on employment of the concepts discussed. Unraveling the complexities described here will inevitably lead to more questions but will also advance precision drug therapy.

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Articles of special interest have been highlighted as either of interest (*) or of considerable interest (**) to readers.

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Article highlights

- Genetics (*i.e.,* pharmacogenetics), environment (*i.e.,* diseases and polypharmacy), and lifestyle (*i.e.,* diet and natural product use) are factors that contribute to inter-individual variability in pharmacotherapy.

- Combinations of these factors cause additive, synergistic, or opposing effects on drug PK.

- Functionally redundant and/or sequential PK processes dictate the direction and magnitude of these effects on drug disposition.

- Advances in clinical decision tools, drug metabolizing enzyme and transporter fractional contribution determinations, PBPK modeling, and PK phenotyping of progressive diseases will facilitate precision drug therapy.
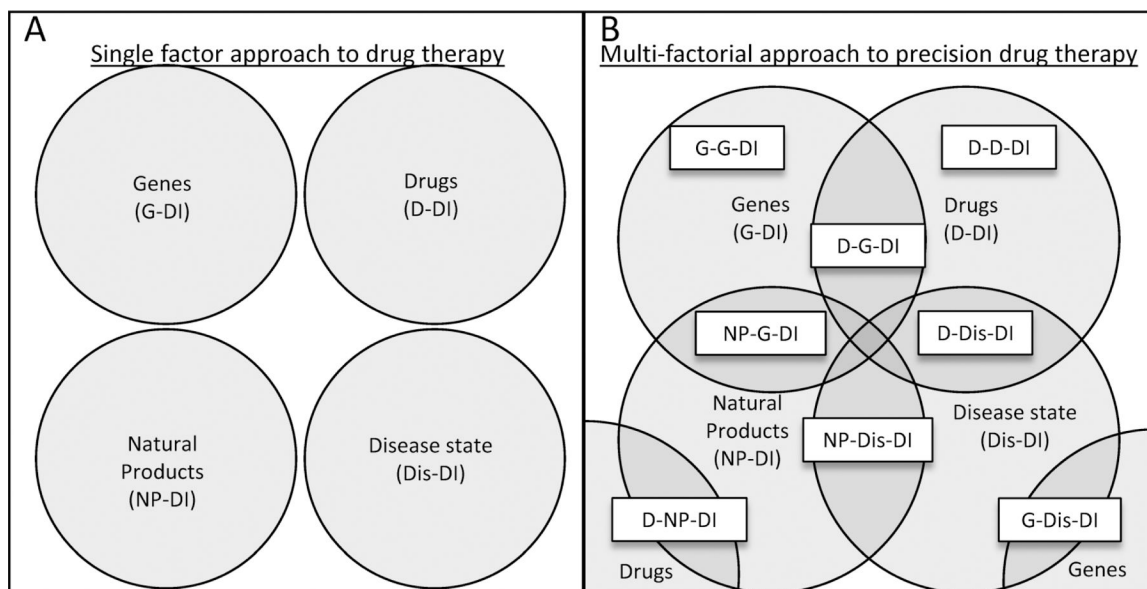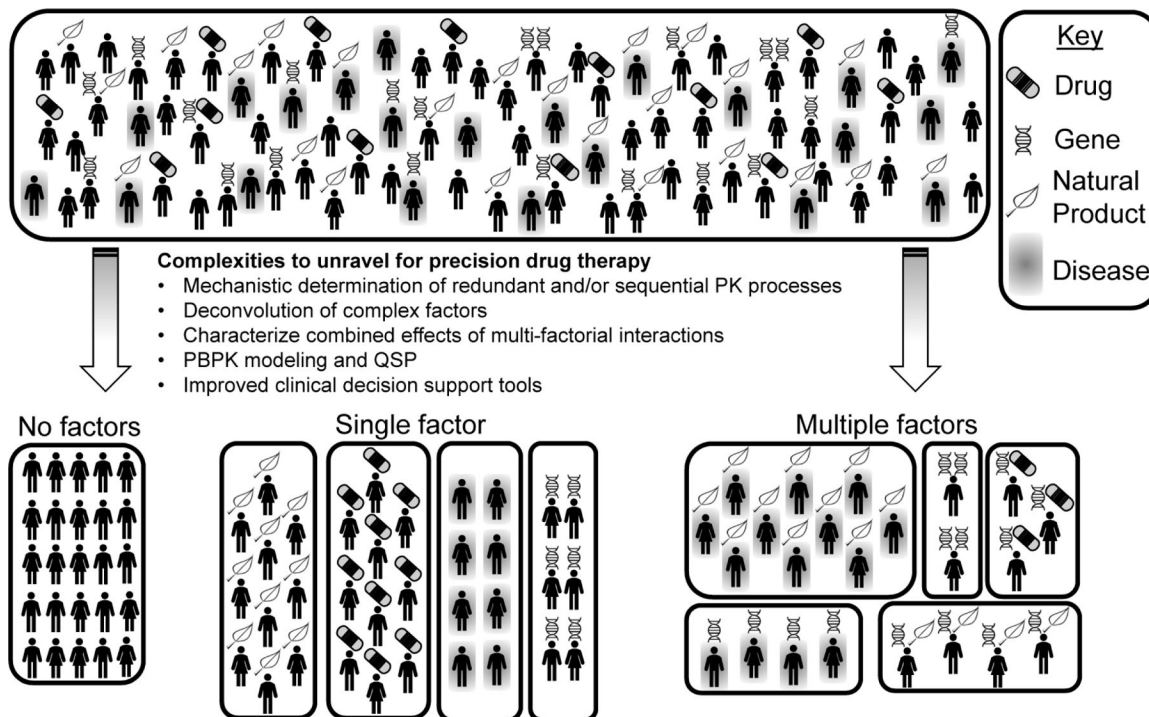
Figure 1:

(A) Identification and testing of factors in inter-individual PK variability often take a single factor approach (i.e., genes, drugs, natural productNPs, and disease states). (B) A multi-factorial approach to precision drug therapy attempts to account for the sub-populations of patients who experience more complex PK interactions. The overlapping portions of the circles indicate all possible 2-, 3-, and 4-way interactions between factors. The 2-way interactions are shown in white textboxes at the intersection of two circles (except for G-G-DI and D-D-DI, which represent interactions between two polymorphisms in different genes and complex drug interactions, respectively). The relevance of each interaction will depend on the specific metabolizing enzymes or transporters involved and will be determined on a case-by-case basis.
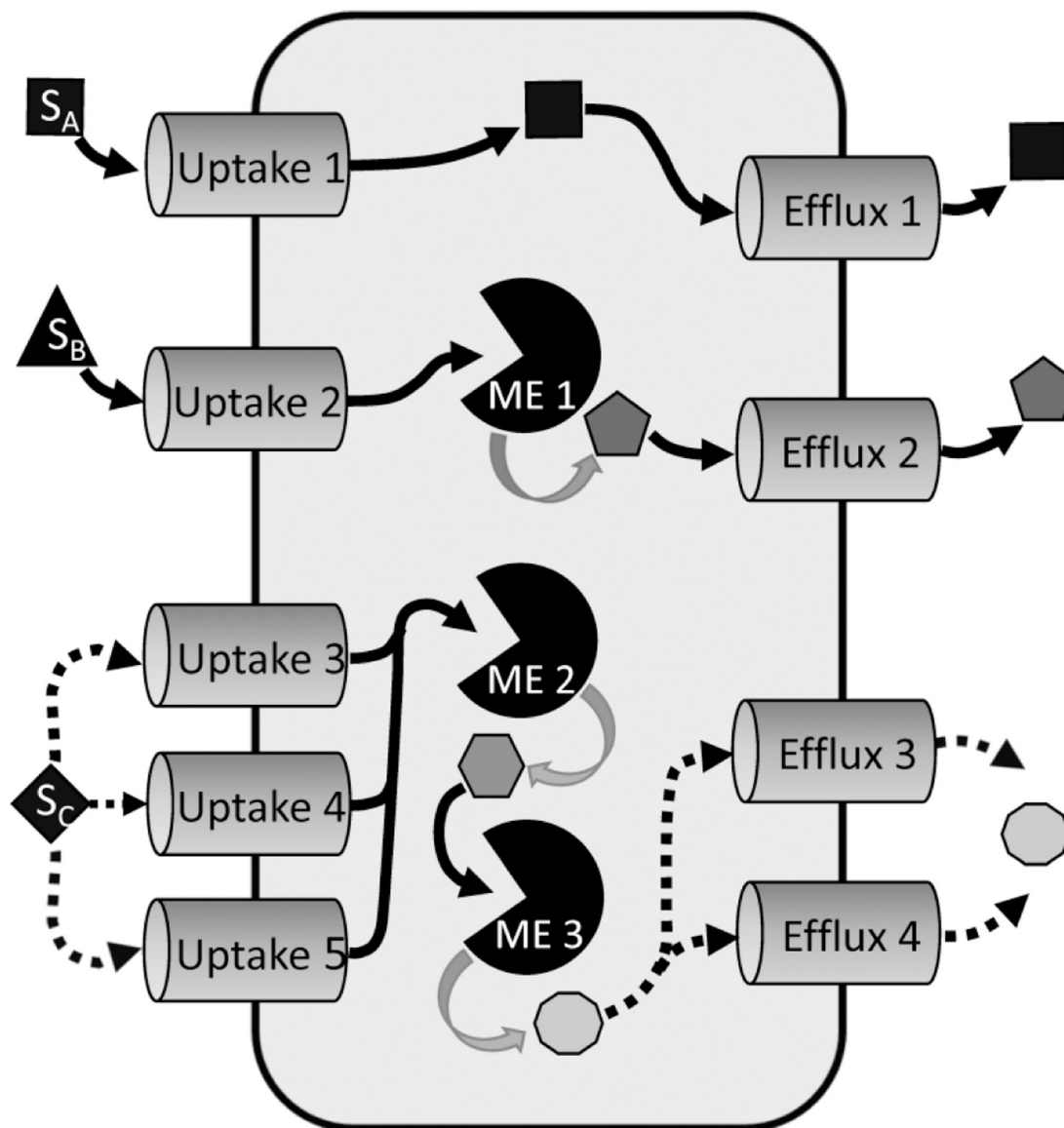
Figure 2.
Functionally redundant and sequential PK processes are characterized by more than one PK mediator determining drug absorption, distribution, metabolism, and excretion. A drug substrate (S_A) may enter a cell via a single uptake transporter (Uptake 1), then leave via a single efflux transporter (Efflux 1) without undergoing any metabolism. Another drug substrate (S_B) may enter the cell via a single uptake transporter (Uptake 2), then undergo metabolism by a single metabolizing enzyme (ME 1) before export via a single efflux transporter (Efflux 2). A third drug substrate (S_C) may enter the cell via functionally redundant uptake transporters (Uptake 3, Uptake 4, or Uptake 5), then undergo sequential metabolism (ME2 then ME3) before being exported from the cell via functionally redundant efflux transporters (Efflux 3 or Efflux 4). Only three scenarios are depicted, but any combination of redundant and/or sequential transport and/or metabolism is possible, and may include passive diffusion across the membrane.

Figure 3.

A hypothetical population of patients is depicted (top). Unraveling the complexities involved in pharmacotherapy such as accounting for redundant and sequential PK processes, deconvoluting complex factors, characterizing combined effects, improving physiologically-based pharmacokinetic (PBPK) modeling and quantitative systems pharmacology (QSP), determining the combined effects of multiple factor, and utilizing advanced clinical decision support tools will facilitate division of patients into unique sub-populations (bottom) and administration of precision drug therapy.

Table 1:

Single Factor PK Interactions

Victim Drug	Drug Class	PK-mediators	Perpetrator	Mechanism	PK effect	Ref.
Midazolam	Benzodiazepine	CYP3A	Fluconazole	CYP3A inhibitor	↑AUC 200–300%	[20]
Atorvastatin	Statin	CYP3A4 OATP1B	Rifampin	OATP1B inhibitor	↑AUC 682%	[24]
			GFJ	CYP3A inhibitor	↑AUC 250% (acid)	[143]
			GFJ	CYP3A inhibitor	↑AUC 330% (lactone)	[143]
			SLCO1B1 poly.	↓OATP1B1 function	↑AUC 52% / ↑AUC 144% (acid)	[144]
Paritaprevir	Anti-viral	CYP3A OATP1B BCRP P-gp	Ritonavir	CYP3A4 and P-gp inhibitor	↑AUC 4700%	[14]
			Cyclosporine	Potential CYP3A4 and OAT1B inhibitor	↑AUC 72%	[145]
			Tacrolimus	NA	↓AUC 43%	[145]
			Carbamazepine	CYP3A4 inducer	↓AUC 70%	[146]
Simvastatin	Statin	CYP3A4 OATP1B1	GFJ	CYP3A4 inhibitor	↑AUC 1600%	[36]
Aripiprazole	Antipsychotic	CYP2D6 CYP3A4	CYP2D6 poly.	↓CYP2D6 activity	↑AUC 50%	[44]
			Paroxetine	CYP2D6 inhibitor	↑AUC 140%	[147]
			Fluvoxamine	CYP3A4 inhibitor	↑AUC 60%	[147]
			Itraconazole	CYP3A4 inhibitor	↑AUC 48%	[148]
Voriconazole	Anti-fungal	CYP2C19 CYP2C9 CYP3A4	CYP2C19 poly.	↑CYP2C19 activity	↓AUC 48%	[45]
Warfarin	Anticoagulant	CYP2C9	CYP2C9 poly.	↓CYP2C9 activity	↓clearance 90%	[47]
5-fluorouracil	Anti-cancer	DPD OAT2	DPYD poly.	↓DPD activity	Accumulation	[55]
			Cimetidine	Unknown	↑AUC 72%	[149]
Diflomotecan	Anti-cancer	CYP3A BCRP	ABCG2 poly.	↓BCRP expression	↑AUC 299%	[56]
Repaglinide	Anti-diabetic	OATP1B1 CYP2C8	Rifampin	CYP3A4 inducer	↑AUC 57%	[80]

Abbreviations and symbols: AUC- area under the plasma concentration-time curve; PK- pharmacokinetics; Poly.- polymorphism; Ref.- reference(s);

↑ - increased;

$\downarrow$ - decreased.

Table 2:

Multi-factorial PK Interactions

Victim Drug	Drug Class	PK-mediators	Perpetrator	Mechanism	Single PK effect	Combined PK effect	Ref.
Repaglinide	Anti-diabetic	OATP1B1 CYP2C8	*SLCO1B1* poly.	↓OATP1B1 function	↑AUC 180%	↑AUC 1110%	80
			Gemfibrozil	CYP2C8 inhibitor	↑AUC 820%		
Simvastatin	Statin	CYP3A4 OATP1B1	Amlodipine	CYP3A4 inhibitor	↑AUC 80% ↑AUC 40% (acid)	↑AUC 90%	83
			SLCO1B1 poly. (hetero.)	↓OATP1B1 function	↑AUC 40% (acid)		
Pravastatin	Statin	OATP1B1	*SLCO1B1* poly.	↓OATP1B1 function	↑AUC 88%[1]	↑AUC 531%[1]	91
			Cyclosporine	OATP1B1 inhibitor	↑AUC 500%		
CP-I	Endogenous biomarker	OATP1B1	Cyclosporine[2]	OATP1B1 inhibitor	↑AUC 171%	↑AUC 400%[1]	91
			SLCO1B1 poly.[2]	↓OATP1B1 function	↑AUC 158%[1]		
Rosuvastatin	Statin	OATP1B1 CYP2C9	Baicalin	↑OATP1B1 function	↓AUC 42%	NS	98
			SLCO1B1 poly.[3]	↓OATP1B1 function	↑AUC 45%[1]		
Fexofendaine	Antihistamine	OATP2B1	Apple juice	OATP2B1 inhibitor	↓AUC 20%	↓AUC 83%	102
			SLCO2B1 poly.	↓OATP2B1 function	↓AUC 37%		

Abbreviations and symbols: AUC- area under the plasma concentration-time curve; CP-I- coproporphyrin-I; Hetero.- heterozygous; NS- not significant; PK- pharmacokinetics; Poly.- polymorphism; Ref.-reference(s);

↑ - increased;

↓ - decreased.

[1] Calculation based on published data without determination of statistical significance.

[2] Pravastatin was also administered to the subjects in this study.

[3] Comparison of homozygous *SLCO1B1*1b* and homozygous *SLCO1B1*15*.