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Individualized Pharmacotherapy Utilizing Genetic Biomarkers and Novel In Vitro Systems As Predictive Tools for Optimal Drug Development and Treatment

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ABSTRACT

In the area of drug development and clinical pharmacotherapy, a profound understanding of the pharmacokinetics and potential adverse reactions associated with the drug under investigation is paramount. Essential to this endeavor is a comprehensive understanding about interindividual variations in absorption, distribution, metabolism, and excretion (ADME) genetics and the predictive capabilities of in vitro systems, shedding light on metabolite formation and the risk of adverse drug reactions (ADRs). Both the domains of pharmacogenomics and the advancement of in vitro systems are experiencing rapid expansion. Here we present an update on these burgeoning fields, providing an overview of their current status and illuminating potential future directions.

SIGNIFICANCE STATEMENT

There is very rapid development in the area of pharmacogenomics and in vitro systems for predicting drug pharmacokinetics and risk for adverse drug reactions. We provide an update of the current status of pharmacogenomics and developed in vitro systems on these aspects aimed to achieve a better personalized pharmacotherapy.

Introduction

Precision medicine is a cutting-edge approach to healthcare that tailors medical treatment and interventions to the unique characteristics of each individual. It marks a departure from traditional prescribing practices by taking into account genetic, environmental, pathologic, and comedication aspects to customize healthcare strategies. This field relies heavily on advances in genomics, molecular biology, and data analytics to provide a more nuanced understanding of disease and treatment response. Regarding the genetic aspect, it is evident that variations within genes encoding drug transporters and drug-metabolizing enzymes play a paramount role, frequently resulting in substantial differences in the pharmacokinetics of drugs. Conversely, in terms of quantity, pharmacogenomic aspects rooted in pharmacodynamic variation are comparatively less frequent.

The utilization of genetic variation for drug therapy holds distinct significance across various therapeutic domains. Notably, oncology (Chan et al., 2019; Dávila-Fajardo et al., 2019), psychiatry (Jukic et al., 2022), and cardiovascular diseases (Ross et al., 2023) emerge as primary clinical areas where the integration of pharmacogenomic information yields substantial benefits. A crucial tool facilitating the integration of pharmacogenomics into clinical practice is the use of pharmacogenomic labels. These labels highlight genetic variants that are pertinent to the prescription of various drugs, offering information categorized by pharmacogenetic associations. The inclusion of data supporting recommendations for therapeutic management, with the potential to impact safety or efficacy, is a key feature (https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations#section1). Additionally, these labels may emphasize a potential influence on pharmacokinetic properties. The selection of these genetic variants is grounded in clinical studies conducted across different sites, with a careful consideration of factors such as validity, quality, and reproducibility. Notably, when assessing the polymorphic influence on pharmacokinetics, particular attention is given to the significance of CYP2C19 and CYP2D6, as these genes play a pivotal role in this context (https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations#section3).

Thus, the pharmacokinetic aspect holds significant importance in the field, serving as the historical starting point for pharmacogenomics. The roots of the polymorphic pharmacokinetics can be traced back to the early identification of polymorphisms in *N*-acetyltransferase and cytochromes P450 (P450s) during the period from the 1950s

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ABBREVIATIONS: ADME, absorption, distribution, metabolism, and excretion; CNS, central nervous system; DILI, drug-induced liver injury; MPCC, micropatterned coculture; MPS, microphysiological system; P450, cytochrome P450; TEER, transepithelial electrical resistance; UM, ultrarapid metabolizer.

to the 1970s (cf. Müller and Rizhanovsky, 2020). A pivotal moment in this trajectory occurred in 1988 with the cloning of *CYP2D6* and the identification of its major defective allele (Gonzalez et al., 1988). However, the overall development of the field proceeded at a relatively slow pace until the landmark identification of the draft human genome sequence in 2003. This description catalyzed accelerated growth, as evidenced by the noteworthy increase in publications on pharmacogenomics in PubMed, surging from 230 in 1999 to 2,300 in 2022.

Polymorphisms in genes governing drug-metabolizing enzymes and drug transporters are pervasive (Osanlou et al., 2018; Lauschke et al., 2024). Preemptive genotyping of variants that impact the susceptibility to adverse drug reactions is of potential importance; however, the current evidence for cost-benefit is country specific and remains limited to a few specific gene-drug associations, as highlighted by Turongkaravee et al. (2021). Importantly, pharmacogenomic studies greatly benefit from experimental approaches that can functionally test and thereby provide a mechanistic fundament for identified associations. Over the last years, the in vitro tool kit has expanded drastically. In addition to recombinant expression systems that can evaluate the effects of candidate genes and variants on drug metabolism and cytotoxicity, emerging organotypic and microphysiological 3D models provide novel opportunities to unravel the intricate interplay between endogenous genetic variation and adverse drug reactions. By leveraging increasingly large biobanks and repositories of patient-derived material, these models allow for faithfully replicating the impact of individual gene variants and even polygenic risk scores and elucidating the toxicogenomic mechanisms behind adverse drug reactions. This overview aims to present the current and prospective systems adept at addressing these complex tasks.

The Roots of Pharmacogenomics

Due to the drug-metabolizing genes' capacity to process toxic substances such as plant alkaloids, it is hypothesized that the polymorphic nature of these genes conferred evolutionary benefits (Ingelman-Sundberg, 2005; Pandian et al., 2020). This polymorphism appears to facilitate adaptation to new environments, with indications that dietary-based selection of animals occurred around 400 million years ago, when animals transitioned to terrestrial habitats (Alt et al., 2022). Substantiating this theory is evidence of dietary-based genetic selection in North Africa approximately 5,000 to 10,000 years ago, leading to the proliferation of alleles with duplicated and multiduplicated *CYP2D6* genes. This adaptation provided a heightened potential for detoxification of plant toxins, owing to the detoxifying capacity of the CYP2D6 enzyme (Ingelman-Sundberg, 2005).

Moreover, tracing the evolutionary timeline reveals a shared *CYP2C* haplotype encompassing the *CYP2C9*3* and *CYP2C9*2* alleles back to the Neanderthals (Haeggström et al., 2022). This historical perspective underscores the integral role of drug-metabolizing genes in the adaptive evolution of species, particularly in response to dietary and environmental challenges. Nevertheless, a high portion of polymorphic absorption, distribution, metabolism, and excretion (ADME) gene variants has arisen through genetic drift, followed by their uneven distribution among diverse ethnic populations. This phenomenon has been widespread, primarily facilitated by the lack of crucial physiologic roles for many ADME genes. This absence of functional constraints has led to the retention of numerous variants, even those with potentially detrimental effects.

Biologic Effects of Polymorphic P450 Gene Variants

The polymorphic nature of P450 not only impacts drug metabolism but also plays a crucial role in tissue ontogeny. Specifically, P450 genes expressed during fetal development can influence the metabolism of steroids, thereby affecting the development of important organs such as the brain (Adhya et al., 2018). For instance, the conversion of androgens to estrogen by CYP19A1 (aromatase) is pivotal in shaping the sexual differentiation of the male brain and behavior (Hutchison et al., 1997).

In the context of drug metabolism, a compelling example is provided by CYP2C19, which metabolizes different steroids (Niwa et al., 2021). This gene is expressed in the brain during fetal stages but not in adult life. Overexpression of CYP2C19 in the fetal brain has been linked to smaller hippocampus and cerebellar size, which correlates with an increased susceptibility to depression and anxiety in adulthood. This highlights the intricate connection between P450 function, drug metabolism, and neurologic outcomes, emphasizing the importance of understanding these relationships for both medical and developmental considerations (Jukić et al., 2017; Milosavljević et al., 2023). Although these effects were observed in mice possessing multiple copies of CYP2C19, analogous distinctions are evident in humans, particularly between those lacking CYP2C19 and those expressing high levels in the liver (Persson et al., 2014; Jukić et al., 2017; Stingl et al., 2021). Notably, individuals classified as normal or ultrarapid metabolizers (UMs) of CYP2C19 exhibit a diminished hippocampal volume, consequently presenting a higher risk for depression, anxiety, and suicide (Sim et al., 2010; Persson et al., 2014; Jukić et al., 2017). The mechanism governing these phenomena remains elusive but appears to be preprogrammed during fetal development, coinciding with the expression of the *CYP2C19* gene in the brain. As mentioned, steroids, recognized substrates for P450s, play a pivotal role in brain development (Ferguson and Tyndale, 2011; Adhya et al., 2018). Consequently, it remains to be elucidated whether polymorphisms in other P450 genes encoding steroid metabolism are associated with variations in brain development. In adulthood, the exclusive expression of the *CYP2C19* gene in the liver contributes significantly to clinically relevant differences in the metabolism of various drugs, including antidepressants and other central nervous system (CNS)-active drugs (Fig. 1).

Pharmacogenomics in Exemplary Therapeutic Areas

Pharmacogenomics has gained significant attention in recent years, with a growing focus on different specific therapeutic areas. Although its initial application was broad, the field now emphasizes specific gene-drug pairs where genetic variations have been established that play crucial roles in shaping clinical outcomes. Notably, organizations like the Clinical Pharmacogenetics Implementation Consortium (CPIC) and the Dutch Pharmacogenetics Working Group (DPWG) continually update recommendations for the clinical use of pharmacogenomics. Of paramount importance is the US Food and Drug Administration (FDA), which regularly releases updated lists of pharmacogenomic labels within different drug product descriptions. Currently, approximately 140 drugs carry these labels, providing essential information on pharmacogenomic associations. The most clinically important drug-gene pairs in pharmacogenomics are visualized in Fig. 2. As seen, genes encoding drug-metabolizing enzymes and transporters dominate, whereas the pharmacodynamic aspect is of less clinical importance due to the relatively rare occurrence of such mutations (Zhou et al., 2021).

Pharmacogenomics in Oncology. In the area of oncology, the primary focus lies in leveraging somatic genome variations to tailor individualized pharmacotherapy. Somatic mutations that result in altered expression or activity of growth factor receptors, along with their associated kinases and phosphatases, hold considerable significance for guiding targeted cancer therapy (Waarts et al., 2022). Furthermore, the diversity in the pharmacokinetics of anticancer drugs, influenced by

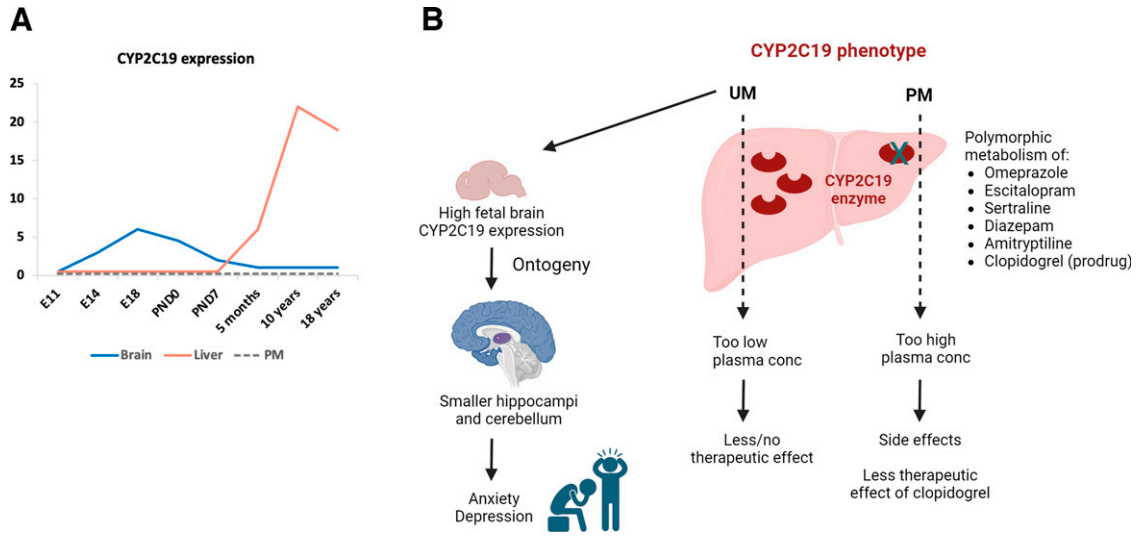

Fig. 1. Pivotal role of CYP2C19 in both fetal brain development and in adult human liver metabolism of clinically significant drugs influenced by *CYP2C19* gene polymorphism. (A) CYP2C19 expression in brain (mice) and livers (human). CYP2C19 is expressed during fetal brain development but becomes silenced after birth. By contrast, the hepatic expression starts after birth and is maximal at 10 years of age. Data from Koukouritaki et al. (2004) and Persson et al. (2014). (B) CYP2C19 expressed in liver is responsible for the metabolism of several clinically important drugs. Lack of the enzyme (PM phenotype) causes higher plasma concentrations of the drugs metabolized by CYP2C19 with exception for clopidogrel, which is a prodrug. Opposite effects are seen in subjects carrying the UM phenotype. Mice with overexpressed CYP2C19 during fetal life exhibit in adult life shrinkage of the cerebellum and hippocampus, making them more susceptible to depression and anxiety. Similar phenotypes are observed in humans who are rapid metabolizers (RMs) for CYP2C19, respectively. (Persson et al., 2014; Jukić et al., 2017; Stingl et al., 2021; Milosavljević et al., 2023) (figure made by Biorender.com).

germline polymorphisms in genes such as *DPYD*, *NUDT15*, *TPMT*, and *TYMS* plays a crucial role for optimizing drug exposures. Despite being less common, these polymorphisms often exert a significant impact on the overall success of drug therapy (Miteva-Marcheva et al., 2020). Somatic mutations, integral to therapeutic strategies, are typically incorporated based on pathologic examinations of tumors. In contrast, the kinetic aspects crucial for enhancing drug therapy efficiency are predominantly addressed by pharmacists.

Within the pharmacokinetic domain, certain gene-drug pairs take precedence, such as 5-fluorouracil and *DPYD*, mercaptopurine, azathioprine and thioguanine versus *TPMT* and *NUDT15*, and to some extent, irinotecan and *UGT1A1* (https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations). The significance of *CYP2D6* polymorphisms in the activation of tamoxifen to endoxifen remains a topic of controversy, especially concerning long-term effects (Mulder et al., 2021). The importance of whole exome sequencing, particularly for *DPYD* variants, cannot be overstated in relation to the necessity of considering numerous rare genetic variants that contribute to the formation of the active DPYD enzyme (De Mattia et al., 2022).

Pharmacogenomics in Psychiatry and Neurology. As previously mentioned, the polymorphism of *CYP2C19* and *CYP2D6* plays a crucial role in determining the pharmacokinetics of a wide range of drugs, with particular significance in the field of psychiatry. A meta-analysis has highlighted the clinically relevant impact of *CYP2C19* polymorphism on the metabolism and effects of antidepressants, specifically escitalopram and sertraline, as well as the

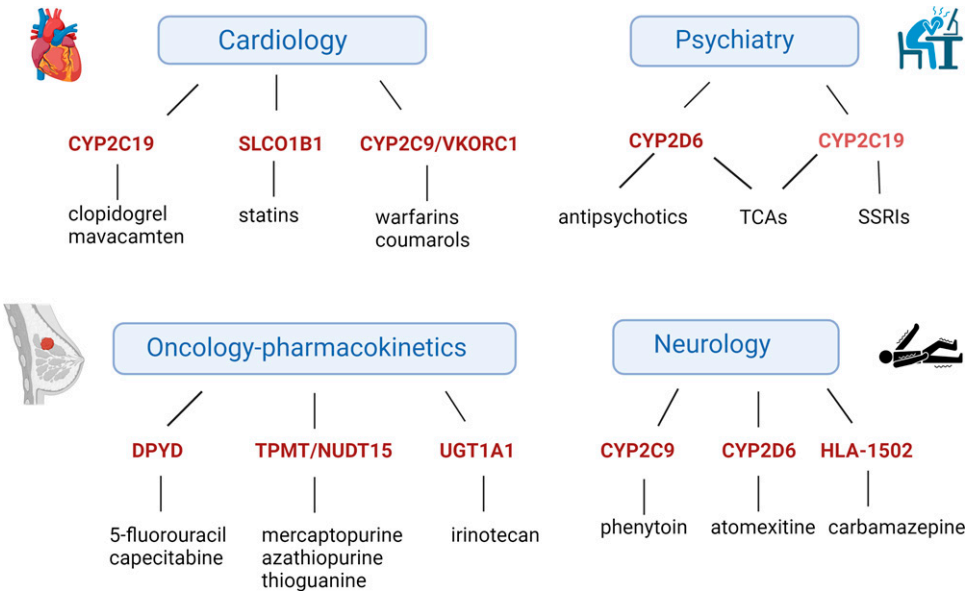

Fig. 2. Overview of clinically important drug-gene pairs in pharmacogenomics (figure made by Biorender.com).

role of CYP2D6 in the treatment response to antipsychotic drugs such as haloperidol, risperidone, and aripiprazole (Milosavljevic et al., 2021).

In the context of depression research, a comprehensive meta-analysis conducted by Brown et al. (2022) revealed a substantial effect size of 1.3, indicating significant improvement through patient genotyping during the administration of selective serotonin reuptake inhibitors (SSRIs). This finding was further corroborated by Jukic et al.'s (2022) analyses. However, a more recent meta-analysis challenges this perspective, suggesting a diminished overall effect of preemptive genotyping and highlighting the importance of considering diverse study designs among various randomized controlled trials (RCTs) published (Milosavljević et al., 2024).

It becomes apparent that the variability in conditions across different studies poses a significant challenge for arriving at definitive conclusions. In contrast, an intriguing study conducted in British Columbia stands out, where preemptive genotyping in psychiatry has been extensively used for two decades. In this work, Ghanbarian et al. (2023) conducted a comprehensive analysis of a cohort comprising 194,149 adults with major depression and eligible for pharmacological treatment. They found that pharmacogenomics-guided treatment led to a remarkable 37% reduction in treatment-resistant cases. Additionally, there were 1869 fewer deaths and 21,346 fewer hospital admissions over the 20-year period, resulting in a substantial cost reduction of $4926 per patient. Although the exact contribution of placebo effects versus genuine genetic prediction remains unclear, the data strongly indicate an opportunity to achieve significant benefits for both individual patients and society at large through preventive genotyping in the treatment of major depressive disorder (MDD).

Indeed, a substantial proportion of pharmacogenomic drug labels, as designated by the FDA, is associated with the treatment of central nervous system (CNS) disorders (https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations). This category encompasses not only antidepressants and antipsychotics but also includes various antiepileptic drugs that serve as substrates for polymorphic P450s, such as *CYP2C9* and *CYP2C19*. Furthermore, *HLA-1502*–mediated adverse events are seen after treatment with carbamazepine (Chen et al., 2011). We anticipate a significant expansion in the application of pharmacogenomics-based drug treatments in this domain in the forthcoming years. Navigating this terrain necessitates prospective clinical trials, yet these endeavors are fraught with challenges. Several pitfalls complicate the landscape of clinical trials in this therapeutic area, including the influence of comedications, the pervasive impact of extensive placebo effects, and the intricacies of distinguishing drug-induced side effects from the symptoms of the underlying disease. Addressing these complexities is imperative in order to advance our understanding and application of pharmacogenomic approaches in CNS disorder treatment.

Pharmacogenomics in Cardiovascular Disease. Over the past decade, substantial strides have been made in pharmacogenomic research within the cardiovascular disease domain, marked by a plethora of randomized trials (Duarte and Cavallari, 2021; McDonough, 2021; Nogueiras-Álvarez, 2023; cf. Ingelman-Sundberg and Pirmohamed, 2024). A significant emphasis has been placed on statins, particularly in elucidating the risk of myopathy development due to restricted transport into the liver in individuals harboring the *SLCO1B1*5* variant. This allele poses a heightened susceptibility to statin-induced myopathy, with the greatest risk observed in cases of high-dose simvastatin administration. Furthermore, numerous trials have explored the interplay between warfarin response and polymorphisms in *CYP2C9* and *VKORC1*, specifically examining their impact on bleeding incidence (Nogueiras-Álvarez, 2023). Although warfarin is currently being replaced by protein inhibitors [direct oral anticoagulants (DOACs)], treatment with warfarin is still important for kids and in patients where DOACs are contraindicated such as those with mechanical heart valves. The recent development of mavacamten, aimed at reducing hypertrophic cardiomyopathy, introduces a new dimension, as its metabolism is influenced by the polymorphic CYP2C19 enzyme. Patients classified as poor metabolizers (PMs) may experience elevated systemic concentrations, potentially elevating the risk of heart failure (Raymond et al., 2021). The activation of antiplatelet agents, notably clopidogrel, by the CYP2C19 enzyme has also been a focal point. The efficacy of clopidogrel is diminished in individuals lacking this enzyme, shedding light on the importance of CYP2C19 status in tailoring antiplatelet therapy. Additionally, considerable attention has been directed toward understanding the impact of *CYP2D6* polymorphism on the effectiveness of β-blockers metabolized by this enzyme. However, current consensus suggests that preemptive *CYP2D6* genotyping is not associated with clinically significant benefits (Thomas and Johnson, 2020).

Future Use of Pharmacogenomics

Undoubtedly, the field of pharmacogenomics stands to benefit significantly from future extensive randomized prospective studies employing closed labeling. Genetic variations contributing to interindividual differences in drug response are highly gene and substrate specific, necessitating a focus on a select number of drugs in such studies. The execution of large-scale clinical studies encounters substantial challenges due to confounding factors. A pivotal issue involves the imperative for closed-label studies, where blinding is constrained to limited patient information and blinded data analyses. This is essential to provide prescribing physicians with comprehensive information while mitigating biases. Notably, the impact of placebo effects in randomized controlled trials (RCTs) is exemplified by SSRIs, where placebos alone can contribute up to 70% to the observed effects (Khan and Brown, 2015).

Confounding variables, particularly issues like polypharmacy and potential drug-drug interactions, present significant challenges, especially in the case of elderly patients. Furthermore, studies relying on low-frequency genotype and/or phenotype occurrences are prevalent, contributing to the emergence of ambiguous or erroneous conclusions of different studies. The pivotal role of liver and kidney function, along with the selection of individuals exhibiting a relevant phenotype for the specific study, cannot be overstated. For meaningful comparisons, it is thus critical that studies are meticulously designed to encompass a sufficient number of patients within the therapeutic intervention context of interest that harbor actionable genetic variations pertinent to each drug.

The challenge of unraveling the molecular basis of heritability persists, with up to 50% of heritable variations in drug pharmacokinetics having unknown genetic bases. The consideration of fixed endpoints, such as a thorough analysis of differences in drug concentrations during pharmacokinetic assessments, is paramount for effective research design. In recent years, the development of advanced human in vitro models has provided new avenues for the mechanistic evaluation of pharmacogenetic effects.

Overview of Human In Vitro Model Diversity

In vitro models have been a long-standing research tool for the evaluation of pharmacogenomic associations as well as for drug testing. In vitro evaluation of the effects of pharmacogenetic candidate variants using recombinant expression systems has been highly successful in confirming the functional effect of variants in drug metabolizing enzymes (Muroi et al., 2014; Shrestha et al., 2018; Siamoglou et al., 2022) and drug transporters (Seitz et al., 2015; Russell et al., 2020) as well as for determining the molecular and structural basis of drug resistance (Zhou et al., 2021; Gao et al., 2023). However, these models require overexpression of candidate variants or the knockout/knockdown of gene

products of interest. Thus, they are not useful to investigate indirect variant consequences (e.g., via altered gene regulation) or to test drug effects on complex cellular phenotypes. For these purposes, in vitro models have historically employed either animal or human cell lines that were derived from tumors or transformed so that the cells could be expanded for a virtually unlimited number of passages. The use of such immortalized cell lines is cost effective and the cells are typically easy to culture, allow for replicate experiments between laboratories and across time using ostensibly identical material, and bypass most ethical concerns. However, transformation and long-term culture under in vitro conditions result in the accumulation of genetic and functional aberrations and such cell lines therefore reflect the molecular phenotypes of their tissue of origin only poorly. As a consequence, results obtained in cell lines often do not hold in the physiologic tissue, contributing to what is often referred to as the "translational gap."

The use of primary fully differentiated human cells promises to narrow this gap, as cellular phenotypes are, at least at the start of culture, highly physiologic. However, when cultured in traditional monolayer systems, primary cells experience rapid alterations in their transcriptome and proteome and lose their tissue-specific function in a process called dedifferentiation. Consequently, the use of primary cells requires the use of conducive culture methods that prevent or at least delay dedifferentiation in order to take full advantage of their benefits.

Over the past decades, a multitude of diverse methods have been developed to support the maintenance of cellular phenotypes and functions (Youhanna et al., 2022). The methods of choice differ between tissues and applications (Table 1). Spheroids describe the 3D culture of mature fully differentiated cells, typically isolated from tissue resections, biopsies, or donated organs. In this culture method, a defined number of cells are seeded in such a way that they cannot attach to a culture substrate and thus attach to each other, forming a spheroidal aggregate. In contrast to spheroids, which are assembled from mature cells, organoids are 3D structures that are established from either pluripotent or tissue-resident stem cells or progenitor cells (Zhao et al., 2022). Organoid culture is initiated from one or few undifferentiated cells and, upon treatment with an often-complex regimen of medium additives, these cells proliferate and eventually differentiate into one or more cell types that make up the tissue of interest.

Besides a classification of methods based on differences in microtissue assembly and composition, methods can also be distinguished into static and perfused systems. The latter is often referred to as "organ-on-a-chip" or microphysiological systems (MPSs). A multitude of different MPSs have been presented that can be broadly distinguished into systems in which medium is recirculated versus chips with single-pass perfusion. One common single-pass model involves the culture of cells in a quasi-2D configuration on one or both sides of a membrane, which separates two flow paths (Kim and Ingber, 2013). However, MPSs can also support perfusion culture of 3D tissue models, thereby combining organ-on-a-chip technology with spheroid (Zandi Shafagh et al., 2022) or organoid culture. In the following, we will discuss recent advances in the use of 3D human tissue models of liver and intestine for the assessment of human drug absorption, distribution, metabolism, and excretion (ADME). For a more comprehensive overview of available tissue models and their advantages and limitations with regards to toxicity, metabolism, and absorption studies, we refer the interested reader to recent reviews (Lauschke et al., 2019; Zhou et al., 2019; Shen et al., 2020; Brooks et al., 2021; Franco et al., 2021; Yadav et al., 2021; Youhanna and Lauschke, 2021; Bouwmeester et al., 2023).

Hepatotoxicity Studies. Hepatic in vitro models are mainstay tools for the investigation of drug metabolism and toxicity. Human liver spheroids consisting of primary human hepatocytes (PHHs) have been shown to overall retain the molecular phenotypes of their in situ counterparts for multiple weeks at the transcriptomic, proteomic, and metabolomic level (Bell et al., 2016, 2017; Vorrink et al., 2017; Messner et al., 2018). Furthermore, they maintain key metabolic functions such as albumin secretion, urea cycle, and xenobiotic metabolism, rendering them useful tools for hepatotoxicity studies. Specifically, in separate screens, each comprising >100 compounds implicated in drug-induced liver injury (DILI) and nontoxic controls, liver spheroids correctly identified 60%–70% of hepatotoxic compounds with very low numbers of false positive (92%–100% specificity) (Proctor et al., 2017; Vorrink et al., 2018). Differentiated human liver cells can also be cultured as micropatterned cocultures (MPCCs) in which liver cells are seeded on islands of extracellular matrix surrounded by murine stromal fibroblasts (Khetani and Bhatia, 2008). Although test panels were overall smaller (45 compounds), MPCCs yielded similar overall predictive accuracies, with sensitivity and specificity of 66% and 90%, respectively (Khetani et al., 2013). Furthermore, both liver spheroids and MPCCs have been successfully used for the delineation of diverse genotoxic, mitochondrial, and cholestatic toxicity mechanisms using toxicogenomic approaches (Hendriks et al., 2016, 2019; Bell et al., 2017; Ware et al., 2017). Both methods allow the culture of fully differentiated cells that are, if at all, only slowly dividing, mimicking their long lifespan in vivo (around 1–3 years for human hepatocytes) (Heinke et al., 2022), thereby

TABLE 1

Main differences between spheroid and organoid culture methods for ADME relevant tissues

	Organoids	Spheroids
Starting material	Stem cells	Fully differentiated cells
Liver	+	+++
Intestine	+++	—
Kidney	++	+
Scaffold	Mostly yes	Mostly no
Media	Complex, often including serum, growth factors, and small molecules	Often basal and chemically defined
Time required for generation of cultures	Multiple weeks	3–7 days
Stability in cultures	Days to few weeks	Weeks to months
Main advantages	Useful to reconstruct complex architecture. Possible to generate isogenic organoids for different tissues. Possible to generate stem cell lines from patients with rare genotypes of interest.	Use of fully differentiated mature cells with high phenotypic relevance. Spheroids are highly homogeneous, facilitating HTS applications.
Main disadvantages	High heterogeneity between organoids. Protocols require complex differentiation regimens and culture medium compositions.	Primary mature cells are difficult to obtain. Material is finite and cannot be expanded.

—, data not available; HTS, high-throughput screening.

enabling chronic repeated-dose exposure studies. Besides PHHs, both models are also compatible with the coculture with nonparenchymal liver cells (NPCs), including Kupffer cells, stellate cells, and liver sinusoidal endothelial cells. Notably, the presence of NPCs can modulate toxicity responses as shown for acetaminophen (Bell et al., 2020), demonstrating that cellular complexity can have important roles even for the toxicity of compounds with hepatocellular mechanisms.

Besides primary human cell–based models, hepatic organoids have also developed into platforms that can faithfully detect DILI risk. Multiplexed evaluation of viability and cholestatic and mitochondrial toxicity of 238 drugs in stem cell–derived liver organoids revealed 89% sensitivity and specificity for DILI prediction, which was overall similar compared with the use of primary cell models (Shinozawa et al., 2021). Furthermore, by using single-cell sequencing, liver organoids have been useful for the identification of toxicity mechanisms, including for bosentan and its associated genetic risk factors and tenofovir-inarigivir (Shinozawa et al., 2021; Zhang et al., 2023). A unique opportunity of organoids is the development of polygenic risk scores for DILI susceptibility based on genome-wide association studies. By assessing organoids from multiple donors treated with different drugs, this approach allows for identifying at-risk individuals and recapitulating complex genetic predispositions in vitro (Koido et al., 2020).

In Vitro Studies of Hepatic Drug Metabolism and Pharmacokinetics. Liver microsomes and hepatocyte suspension cultures have been the main experimental models for metabolite identification and clearance prediction. Besides toxicity investigations, 3D liver models constitute emerging tools for the determination of hepatic metabolism and clearance predictions. Metabolic profiles of spheroids recapitulated the major primary and secondary metabolites observed in vivo for a range of chemically diverse compounds (Kanebratt et al., 2021; Novak et al., 2023). Moreover, spheroids established from donors with different genetic *CYP2D6* polymorphisms recapitulated the differing metabolic fluxes of the CYP2D6 substrate dextromethorphan, providing proof of principle that genetically encoded interindividual differences in drug metabolism can be mimicked in vitro (Vorrink et al., 2017). Spheroids can also identify P450 induction with increased accuracy compared with isogenic 2D cultures and have been useful in identifying novel noncanonical induction pathways (Hendriks et al., 2020; Oliva-Vilarnau et al., 2023). Furthermore, when using regression-based correction, spheroids accurately predicted the in vivo intrinsic clearance of >90% of tested compounds within 3-fold, which importantly includes slowly metabolized compounds whose clearance is challenging to measure using conventional microsomes or suspension cultures due to their rapid functional deterioration that limits assay time to <2–4 hours (Kanebratt et al., 2021; Riede et al., 2021; Preiss et al., 2022). Reliable clearance prediction is also possible in MPCCs (Chan et al., 2013), demonstrating that maintenance of hepatic functionality rather than culture method constitutes the main criterion that determines model suitability for long-term studies. These results suggest that advanced models of primary human liver cells show promise in drug discovery to study both short- and long-term metabolism, including for unknown and complex mechanisms.

Modeling of Intestinal Absorption. Determination of intestinal absorption constitutes an important step in the development of compounds with an intended oral administration. Enterocytes are the main absorptive cell type of the intestinal epithelium. The most widely used models for intestinal absorption were developed in the late 1980s and early 1990s and are comprised of a cell line–based epithelium, most commonly based on Caco-2 cells cultured on a transwell membrane that separates two fluid compartments (Hidalgo et al., 1989; Artursson, 1990). Over the years, detailed protocols have been developed that standardize measurements and allow for reliable quantifications of permeability coefficients (Hubatsch et al., 2007). Furthermore, the system is extensively benchmarked with regard to permeability using large sets of training compounds (Cheng et al., 2008; Turco et al., 2011). The model has been gradually expanded to include HT29 cells, which can be differentiated into mucus-producing goblet-like cells. Although Caco-2 monolayers show transepithelial electrical resistance (TEER), which is substantially higher than in vivo, HT-29 cocultures exhibited more physiologically relevant values (500–1000 Ω*cm^2 for Caco-2 monolayers; 200 Ω*cm^2 for Caco-2–HT-29 cocultures compared with 50–100 Ω*cm^2 for intestine in vivo) (Hilgendorf et al., 2000; Lopez-Escalera and Wellejus, 2022). Triculture with intestinal M cells (Raji B cells) further reduced TEER and increased paracellular absorption (Araújo and Sarmento, 2013). Overall, Caco-2 transwell cultures based on human colorectal adenocarcinoma cell lines provide a well established system that allows for accurate identification of the extent of in vivo absorption for compounds that permeate the intestinal epithelium via transcellular mechanisms (typically hydrophobic and lowly ionized compounds). Coculture with HT-29 and Raji B further increases and extends the utility of the model to predict paracellular permeability (mostly hydrophilic molecules) and transcytosis (macromolecules).

Intestinal organoids can be differentiated from individual stem cells and form hollow structures that self-organize into villi and cryptlike domains containing the entire cellular complexity of the intestinal epithelium, including enterocytes, Goblet cells, Paneth cells, enteroendocrine cells, and a self-renewing stem cell compartment (Almeqdadi et al., 2019). They have been used with great success for intestinal development and modeling of gastrointestinal disease; however, their utilization for absorption studies has been limited, particularly because the organoid lumen is not directly accessible, complicating TEER measurements and quantifications of epithelial transport. To combine the cellular complexity of organoids with the accessibility of transwell cultures, organoids can be dissociated and used as the cellular substrate for monolayer cultures (Wang et al., 2017; Workman et al., 2017; Kasendra et al., 2018). These cultures retain their cellular composition and constitute a physiologically relevant model for intestinal studies. However, whether they are more accurate in the prediction of intestinal absorption than the simpler cell line–derived transwell models remains to be determined.

Perfused and Integrated Tissue Models. Over the past years, there have been major developments to emulate more and more complex phenotypes and functions using in vitro systems. One of the areas with the most active development is microfluidics in which 2D or 3D cell culture models are subject to controlled perfusion. This approach can further increase physiologic relevance by adding relevant shear forces, which are particularly important for epithelial and endothelial cells, and by facilitating tight control of the supply and replenishment of fresh nutrients and media components. For the liver, a multitude of diverse setups with a wide range of complexities have been presented, which are excellently reviewed elsewhere (Dalsbecker et al., 2022). Liver cells in these models retain physiologic phenotypes and functions to a comparable extent as in 3D systems; however, despite some promising results, the added value of the drastic increase in complexity remains controversial (Rubiano et al., 2021; Ewart et al., 2022). For intestinal models, which constitute "natural" epithelia, perfusion provides polarized flow, which, when combined with cyclic stretching of the membrane on which cells are cultured, can result in villi formation, drastically improved phenotypes, and physiologically relevant TEER (Kim et al., 2012; Schweinlin et al., 2016; Nikolaev et al., 2020).

Integration of different tissue models constitutes an important frontier of contemporary tissue engineering. Specifically, current trends go toward the development of individual tissue modules that comprise the cellular complexity of the respective organ, which can then be combined in a plug-and-play–like fashion. A multitude of such constellations have

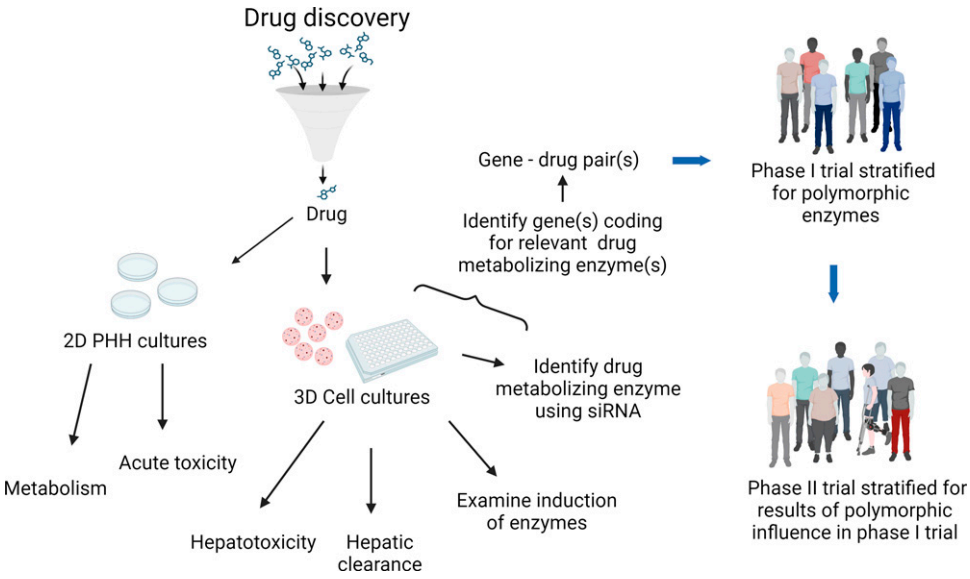

Fig. 3. In drug development, the integration of in vitro cell systems and pharmacogenomics has become instrumental. Candidate drugs can in in vitro systems undergo thorough screening processes to assess factors such as toxicity, clearance, and drug-drug interactions, including potential enzyme induction and identification of enzymes responsible for metabolism. This can effectively be done by small interfering RNA (siRNA) silencing of different enzymes in the 3D system used. Building upon these results, a compound is carefully chosen for clinical trials. If this compound is recognized as a substrate for polymorphic enzyme(s), the clinical trial design may incorporate stratification based on the genetics of the trial participant (figure made by Biorender.com).

already been presented, including liver-intestine (Tsamandouras et al., 2017), liver-pancreas (Bauer et al., 2017; Zandi Shafagh et al., 2022), liver-heart (Zhang et al., 2017; Oleaga et al., 2018; Yin et al., 2021), liver-kidney (Li et al., 2018), and liver-lung (Bovard et al., 2018; Schimek et al., 2020). Important consideration for integration of such modules is that the individual tissue models are compatible with a single medium composition. This is particularly complicated for the integration of organoid cultures since these often rely on complex and highly tissue-specific media formulations. In contrast, primary cells and cell lines can typically be cultured in basal media without complex additives.

Bioengineering of tissues with importance for drug metabolism, disposition, and toxicity continues to receive considerable interest. This has resulted in tremendous developments that have given rise to a highly heterogeneous landscape of cell models and culture systems. We argue that it is time to consolidate these activities and emphasize the importance of careful multicenter benchmarking of available systems to develop standardized setups and approaches that provide robust, reproducible, and reliable results along time and between study sites. Only then will it be possible to fully leverage the power of these models and provide added value for pharmacological and toxicological applications in drug discovery and development.

Conclusions

The fields of pharmacogenomics and in vitro systems for prediction of drug metabolism and toxicity are experiencing rapid development, and their utilization in drug development is summarized in Fig. 3. However, several complicating factors need consideration. In pharmacogenomics, challenges arise from incorporating patient populations without disease or subtype stratification, overlooking environmental and pathophysiological factors (such as liver and kidney pathology), neglecting drug-drug interactions, and insufficiently accounting for placebo effects in clinical trials. Additionally, there is a significant challenge of missing heritability, with up to 50% of the heritable variation in drug pharmacokinetics having an unknown genetic basis. For future progress, it is advisable to concentrate clinical investigations on well defined large cohorts and to focus on specific drug-gene pairs. Chiseling away part of the missing heritability can be achieved through the novel ultrarapid methods for genome sequencing combined with improved AI-based algorithms for interpretation of variations in open reading frames and in regulatory regions of gene importance for alterations in drug metabolism and toxicity.

Concerning in vitro systems, many efforts are still directed at models lacking relevant phenotypes, leading to ambiguous data that is challenging to extrapolate into the in vivo situation. Efforts to improve the fidelity of in vitro models are crucial for advancing the ability to predict drug action and toxicity. Emphasis should be placed on further developing new human 3D, high-throughput screening (HTS)-compatible models that reliably mimic in vivo properties of different tissues both individually and in combination.

Data Availability

This review article contains no datasets generated or analyzed during the current study.

Authorship Contributions

Wrote or contributed to the writing of the manuscript: Ingelman-Sundberg, Lauschke.

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