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THEMED ISSUE REVIEW

Therapeutic drug monitoring, liquid biopsies or pharmacogenomics for prediction of human drug metabolism and response

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Pharmacokinetics plays a central role in understanding the significant interindividual differences that exist in drug metabolism and response. Effectively addressing these differences requires a multi-faceted approach that encompasses a variety of tools and methods. In this review, we examine three key strategies to achieve this goal, namely pharmacogenomics, therapeutic drug monitoring (TDM) and liquid biopsy-based monitoring of hepatic ADME gene expression and highlight their advantages and limitations. We note that larger cohort studies are needed to validate the utility of liquid biopsy-based assessment of hepatic ADME gene expression, which includes prediction of drug metabolism in the clinical setting. Modern mass spectrometers have improved traditional TDM methods, offering versatility and sensitivity. In addition, the identification of endogenous or dietary markers for CYP metabolic traits offers simpler and more cost-effective alternatives to determine the phenotype. We believe that future pharmacogenomic applications in clinical practice should prioritize the identification of missing heritable factors, using larger, well-characterized patient studies and controlling for confounding factors such as diet, concomitant medication and physical health. The intricate regulation of ADME gene expression implies that large-scale studies combining long-read next-generation sequencing (NGS) of complete genomes with phenotyping of patients taking different medications are essential to identify these missing heritabilities. The continuous integration of such data into AI-driven analytical systems could provide a comprehensive and useful framework. This could lead to the development of highly effective algorithms to improve genetics-based precision treatment by predicting drug metabolism and response, significantly improving clinical outcomes.

KEYWORDS
ADME, adverse drug reactions, exosomes, missing heritability, post-translational regulation, precision medicine

1 | INTRODUCTION

It has been shown that personalized drug therapy based on genetic variation of drug-metabolizing enzymes, drug transporters and drug targets can improve the efficacy of treatment with certain drugs and reduce adverse drug reactions.[1–4] Tools to achieve this goal include (i) predictive drug treatment based on genetic analysis (pharmacogenomics), (ii) therapeutic drug monitoring (TDM), where both genetic and non-genetic variability, e.g. individual physiology, pathophysiology and potentially interacting comedication(s) are taken into account, and (iii) more recently suggested, liquid biopsy-based monitoring of hepatic absorption, distribution, metabolism and excretion (ADME) gene expression and drug metabolism. Here we compare and discuss these methods as tools to optimize drug therapy in health care.

One of the challenges in drug therapy is thus the considerable variability in the way people metabolize and respond to drugs. Factors such as genetics, age, liver and kidney function, concomitant medications and lifestyle can influence a person's response to a drug. Therefore, TDM allows for personalized dose adjustment based on the specific drug concentrations in the blood of the patient. TDM is particularly valuable in the treatment of chronic diseases such as epilepsy,[5] rheumatoid arthritis,[6,7] transplantation[8] infection[9,10] and psychiatric disorders.[3,11] Here, pharmacokinetic variability is huge and both insufficient effects and side effects may be severe. In epilepsy, for example, maintaining a stable concentration of antiepileptic drugs within the therapeutic range may be important to prevent seizures while avoiding side effects.[5] TDM of antiepileptic drugs is therefore common, including **valproic acid** which is indicated for treatment of both epilepsy (target range 50–100 μg/mL) and bipolar disorder (target range 50–125 μg/mL) and where guidelines define the highest grade of evidence for TDM (Level 1), i.e. 'Strongly recommended'.[4] In addition, with psychiatric medications such as antidepressants and antipsychotics, there is often considerable interindividual variation in response to the medication, where individualization through TDM helps to adjust drug dosing.[3,6] Furthermore, short-term treatment with certain antibiotics may require TDM to ensure effective treatment while minimizing adverse effects.[9,10]

The introduction of pharmacogenomics as a tool for predicting personalized drug treatment highlights that TDM and pharmacogenomics can be integrated as complementary tools into routine clinical care, as shown in Figure 1. Here, pharmacogenomics can be helpful in prescribing by predicting initial dose requirements whereas TDM can subsequently be used for dose fine-tuning to achieve blood levels complying with the levels required for optimal treatment.

As pointed out by Jukic et al.,[3] the increasing application of high-resolution accurate mass spectrometry (HR-MS), exemplified by Orbitrap detectors, has notably enhanced the significance of TDM in precision medicine and metabolomics studies. This technological advancement, characterized by improved resolution and mass accuracy, enables the discrimination of analytes with equal molecular masses. As a result, HR-MS detectors enhance sensitivity and specificity, facilitating the acquisition and storage of comprehensive full-scan data. This capability allows for phenotyping at very low drug levels. TDM, with its advantages in optimizing treatment outcomes and safety, is not without drawbacks, including costs, invasiveness, the need for meticulous evaluation, and the absence of predictive dose estimations, as TDM occurs post-treatment initiation.

The merits and demerits of TDM encompass its ability to[1–6]:

- Enable personalized dosing by considering factors such as age, weight, diet, genetics and drug interactions;
- Ensure that concentrations are within ranges most likely providing beneficial effects without serious side effects;
- Identify patients not adhering to prescribed medication (nonadherence);
- Incur expenses due to the necessity for specialized equipment and trained staff;
- Often involve waiting for laboratory results, potentially delaying treatment decisions, which may not be suitable for acute situations;
- Primarily apply to drugs with a narrow therapeutic range or significant interindividual variability in response;
- Typically involve single-point concentration measurements, possibly not reflecting total drug exposure (AUCs) at prescribed dosages;
- Mandate strict conditions on sampling time to assess the expected clinical response in relation to target concentration ranges.

Regarding psychiatric drug treatment, a clinical guidance for TDM use is provided by the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP).[4] This information is of high value and is summarized by a four-tier system:

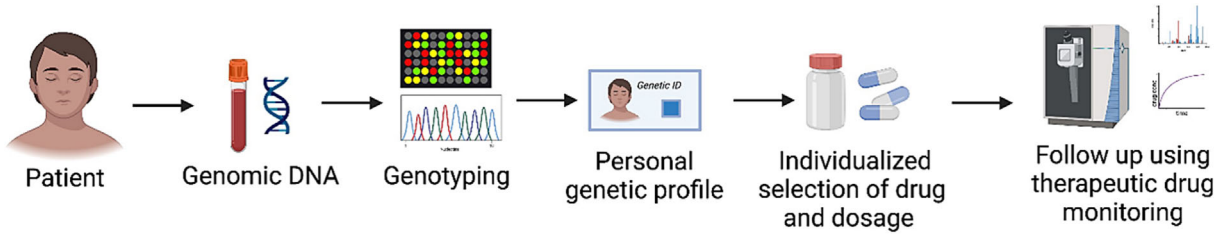

FIGURE 1 Integrating pharmacogenomics and TDM to personalize drug therapy (from Jukic et al.[3]). Pharmacogenomics is used to identify the important genetic variations in the patient that affect drug response and adverse effects. This information is taken into account when prescribing the type and dose of drug to be used. Follow-up analyses with TDM allow validation and adjustment of the baseline conditions used.

Level 1: Highly recommended.
Level 2: Recommended.
Level 3: Useful.
Level 4: Potentially useful.

Among different drugs, five antidepressants and eight antipsychotics are classified as level 1 drugs, while 15 antidepressants and 10 antipsychotics fall into level 2.[3] A point that should be mentioned regarding TDM of central nervous system (CNS) dugs is potential variability in blood–brain barrier (BBB) transport which may cause discordance between concentration measured in plasma and the site of pharmacological action in the brain. Especially variability in phenotype of efflux transporters such as P-glycoprotein (Pgp; **ABCB1**) and breast cancer resistance protein (BCRP; **ABCG2**) are considered important for pharmacokinetic variability beyond factors causing individual differences in plasma concentrations, which may then reduce the predictive power of TDM.

2 | ENZYME ACTIVITY MONITORING BY MEASUREMENT OF ENDOGENOUS/DIETARY COMPOUNDS

More recently, efforts have been made to predict hepatic ADME gene expression, possibly including CYP enzyme activity, based on the determination of the metabolism of endogenous/dietary compounds. One example is 4β-hydroxycholesterol as a possible predictor of the rate of hepatic **CYP3A4**/5-dependent metabolism. While 4β-hydroxycholesterol is sensitive in measuring CYP3A induction, the ability to capture CYP3A inhibition has been less successful.[12] The latter may be due to the long elimination half-life of 4β-hydroxycholesterol, which overall is unlikely to become a biomarker in future precision medicine.

Recently, analyses of **solanidine**, a potato compound with very specific **CYP2D6**-mediated metabolism, using Orbitrap detection of TDM samples, have been shown to provide novel information about the CYP2D6 phenotype.[13–15] For example, measurements of solanidine and its metabolites were recently showed to predict genetically determined CYP2D6 poor metabolizers (PM) with an accuracy of 100%,[9] and significantly predict intra-*CYP2D6* genotype variability in CYP2D6 metabolism of **risperidone**, a commonly used antipsychotic drug.[15] This opens up a new dimension for prediction of patient phenotype by revealing enzyme activities, which has usually required pharmacogenetic analyses, along with the direct concentration measurements of the drug of interest. Implementation of solanidine analysis as part of TDM will provide information on patients' CYP2D6 phenotype, including status as a potential CYP2D6 PM,[15] and capture non-genetic variability including drug–drug interactions. Analysis of solanidine and metabolites will be most appropriate using high-resolution MS detectors for exact mass determination, but ordinary MS instruments, e.g. triple quadrupole mass spectrometers, can be used since solanidine is available commercially for preparation of reference standards.

3 | LIQUID BIOPSIES FOR PREDICTION OF HEPATIC DRUG METABOLISM

Liquid biopsies involve the analysis of various biomarkers such as circulating tumour DNA (ctDNA), circulating tumour cells, miRNAs and proteins in blood or other body fluids.[16] The analysis of these components has so far mainly been used for early cancer detection and for continuous monitoring of a patient's disease status during treatment and many of them have received regulatory approval and are used in clinical practice for specific cancers.[12] Thus, liquid biopsies can help to identify specific mutations or changes that are vulnerable to certain drugs or therapies. They allow oncologists to select the most appropriate targeted therapies or immunotherapies for individual patients. However, their widespread acceptance and reimbursement by health systems may vary by region and cancer type.

Recent identification of extracellular vesicles (EVs) as carriers of tissue components in blood, such as cfRNA and proteins and peptides,[17–22] has opened a field in which attempts have been made to quantify the expression of mRNA, proteins and catalytic activity in the vesicles corresponding to various liver enzymes and transporters[19] (see Figure 2). A recent review summarized the results obtained regarding the use of EVs for analyses of CYP expression and induction in animals and humans.[19] The authors conclude that there is evidence for a correlation between the presence of specific enzymes and transporters in EVs and the tissue they originate from but claim that it is important to clarify the degree to which the regulation of enzymes and transporters in tissues is reflected in the cargo of EVs. Rowland et al.[23] identified peptides and cfRNAs originating from several different ADME genes and from samples of six different healthy Caucasian males that described a correlation of CYP3A4 protein and mRNA with **midazolam** clearance. The number of subjects might, however, be too small to draw conclusions regarding the significance of this finding. This method has subsequently been proposed to challenge TDM as a method to continuously monitor the capacity of hepatic metabolism of different drugs during medical treatment. For example, Achour et al.[24] in 2021 reported that exosomes isolated from human plasma contain functional proteins and mRNA for several cytochrome P450 and UDP-glucuronosyltransferase enzymes when they compared exosomal RNA expression with the liver protein expression for 97 liver-enriched genes. They obtained a high correlation for 12 key drug-metabolizing enzymes and four drug transporters. Furthermore, they indicate that exosome-derived mRNA and protein biomarkers track the induction of CYP3A4 expression by **rifampicin** and that exosome-derived CYP3A and UGT proteins are metabolically active under ex vivo conditions, as shown by results from 29 matched samples. In a follow-up study, Achour et al.[25] used liquid biopsies and presented a correlation between plasma exosomal cfRNA expression and hepatic protein levels of eight cytochrome P450 enzymes (CYPs) and four UDP-glucuronosyltransferases (UGTs) in a cohort of 30 acutely ill patients with cardiovascular disease in a hospital setting. After accounting for exosomal excretion, expression in the liquid biopsy appeared to correlate with the activity phenotype for CYP1A2, CYP2B6, CYP2C9, CYP3A and P-gp ($r = .44–0.70$, $P \leq .05$) in patients receiving the Geneva Cocktail, a mixture of investigational drugs used to measure CYP activities.[25]

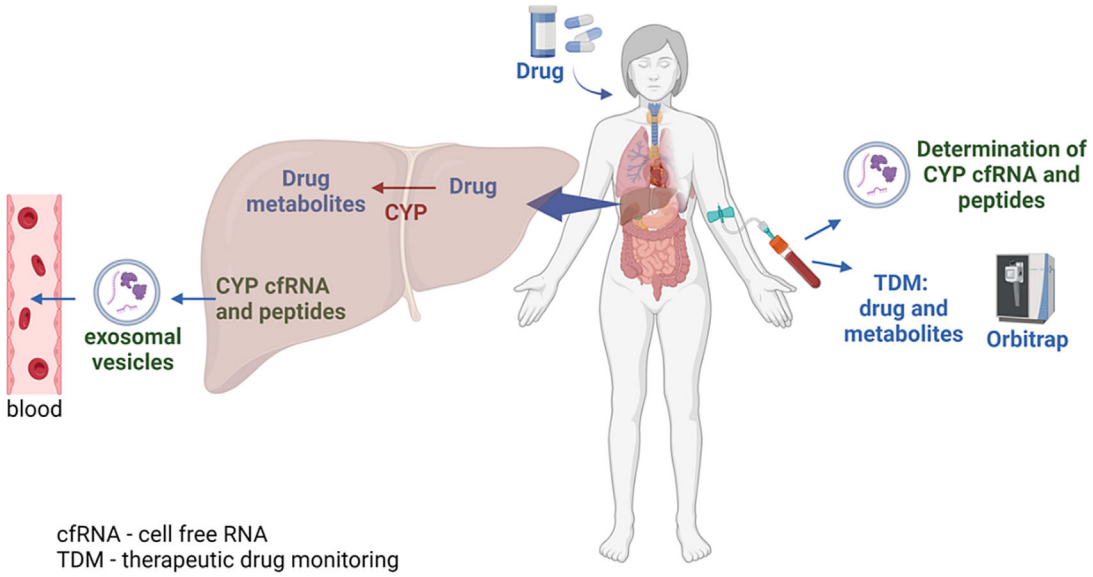

FIGURE 2 Illustration of the relationship between hepatic metabolism of drugs and the use of blood EV components from blood to quantify RNA and protein expression and enzyme catalytic activities in order to predict the hepatic situation in vivo. The oral drugs are metabolized by CYPs in the liver. The cfRNA and peptides related to these CYPs are exported in exosomal vesicles and their composition and abundancies as well as the drug and metabolite levels can be determined after a blood samples is taken (figure produced using Biorender.com).

The results are interesting, even though the number of subjects studied is small and the data would have to be reproduced. The variability in the expression of different ADME genes in the liver is very large and a solid documentation would therefore require very large cohorts up to at least 1,000 subjects. We examined whether a correlation between mRNA expression and catalytic activity of eight different CYP enzymes could be obtained using data from 96 different human livers[26] (Figure 3). However, the observed mean Pearson correlation of these analyses was .285. The relationship for CYP2D6 and CYP3A4 was the highest (0.33–0.34) and the weakest for CYP1A2 and CYP2B6 (0.18–0.21). The reason for this lack of correspondence may lie in the versatile extent of post-translational regulation of different liver enzymes, in which the RNA product originating from a gene can be translated and modified to exert different effects in different organelles and thus only a part of the transcribed mRNA used for production of catalytically active enzyme. Rowland et al.[27] have commented on this study and added the information that CYP proteins can be quantified in plasma extracellular vesicles and that CYP3A4 protein activity can be quantified in plasma extracellular vesicles using midazolam as a probe substrate. Achour et al.[28] also responded and add that the expression of cfRNA secreted in exosomes represents the accumulated expression of RNA over longer periods of time and is much less sensitive to temporal variations due to the longer half-life of exosomes.

A major question to consider regarding the difficulties in predicting hepatic drug metabolism by analyses of exosomal cfRNA corresponding to the different enzymes is the extensive post-translational events that the different enzymes undergo in the liver as summarized in Figure 4 and by Aguiar et al.[29] One can summarize the most important factors questioning a correlation between the expression of EV cfRNA, protein and activity of corresponding hepatic enzymes in vivo as:

- the determined cfRNA could originate from different tissues or from other genes (e.g. pseudogenes);
- the autophagosomal lysosomal pathway involves long-term cellular expression of an inactive enzyme;
- substrates stabilize enzyme expression in many cases, while phosphorylation of, for example, CYP3A4 and CYP2E1 triggers rapid degradation;
- the presence of dietary and other inhibitors in patients affects the rate of catalytic activity;
- the hepatic metabolism of drugs in vivo is the subject of important drug–drug interactions;
- the electron transfer to the enzymes, the binding of drugs and stability of the enzymes in the hepatocytes are influenced by phosphorylation, ubiquitination, acetylation and glycosylation of the proteins (Figure 4).

Taken together, it is evident that before liquid biopsies and EVs can be used to predict the rate of *in vivo*- related hepatic metabolism of drugs, further replication by other research teams and validation of the data are required.

4 | PREDICTION OF DRUG METABOLISM AND ADVERSE DRUG EVENTS USING PHARMACOGENOMICS

The interindividual differences in drug metabolism and susceptibility for adverse drug reactions are extensive. The exact contribution of different factors is difficult to estimate but drug–drug interactions, pathophysiological factors, particularly concerning the liver and kidneys, and genetic factors are among the most important.[30]

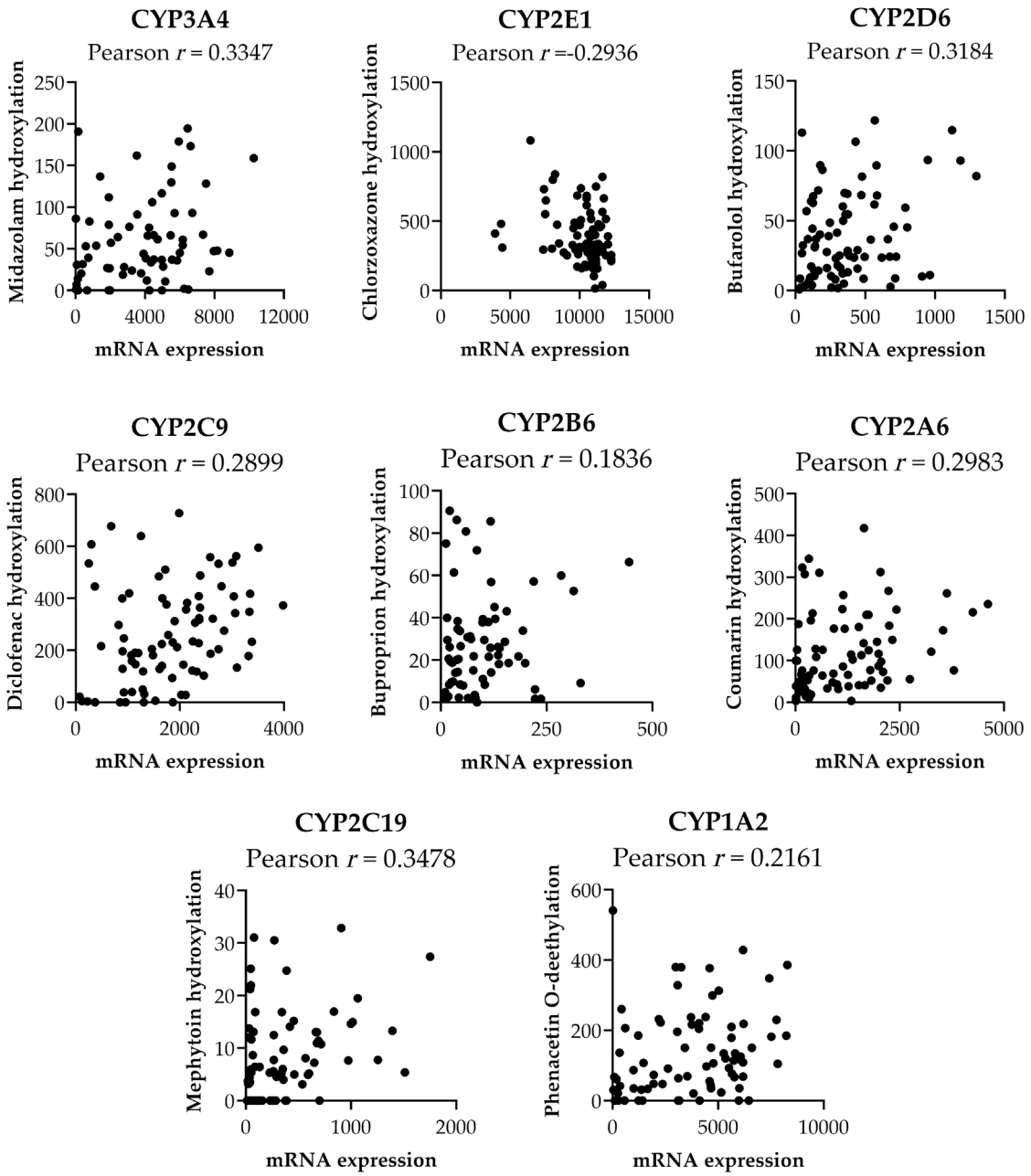

FIGURE 3 Analyses of the relationship between CYP-catalysed metabolism of drugs and the corresponding mRNA expression in 96 different human livers. Data and picture from Pridgeon et al.[26]

4.1 | Missing heritability

While the impact of the known common allelic variants of different *CYP* genes on pharmacokinetic parameters is of immense clinical importance and affects substantial subpopulations, it must be recognized that there are yet unidentified genetic factors that have a significant impact on drug pharmacokinetics. Twin studies have proven invaluable in assessing the genetic component that contributes to interindividual variations in drug metabolism.[31] Regarding the heritability of CYP-mediated drug metabolism, these studies have shown that pharmacokinetic parameters related to the CYP2D6 substrate metoprolol and CYP2C9 substrate torsemide have high heritability. Correlation coefficients range from .9 to .95 in monozygotic twins and from .3 to .4 in dizygotic twins, giving an h_2SNP > of .8.[32] At the same time, it is evident that the majority of heritable factors are unknown. Twin studies (Figure 5) indicate that, for the examples given above, only about 40% of the inheritance in drug metabolism is explained by the currently known genetic variants of *CYP2D6* and *CYP2C9*.

Several potential factors could explain the gaps in pharmacogenomic knowledge of drug pharmacokinetics[33]:

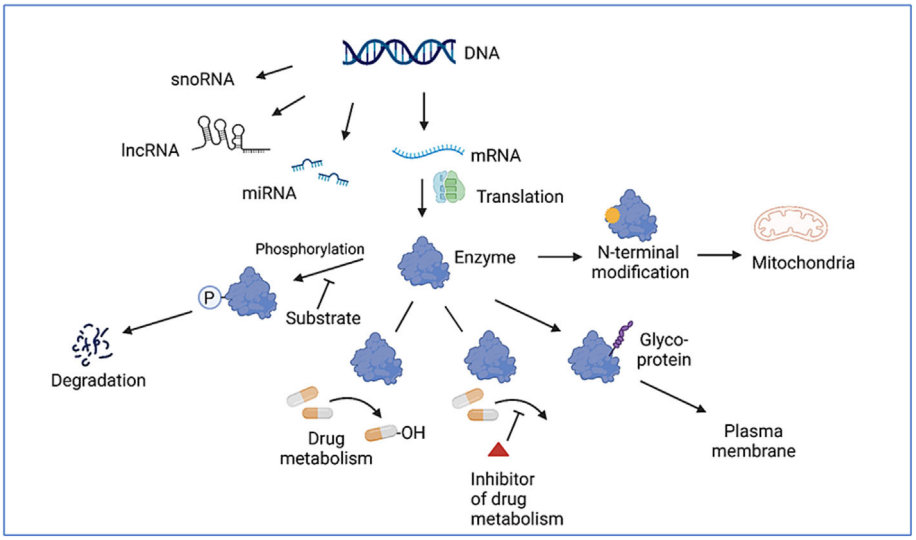

FIGURE 4 Post-transcriptional events among gene products encoded by different ADME genes in human liver. *N*-terminal modifications, phosphorylations and glycosylations determine the fate of the gene product involving transport to different hepatocyte compartments as well as the rate and intracellular localization of degradation (generated by Biorender.com).

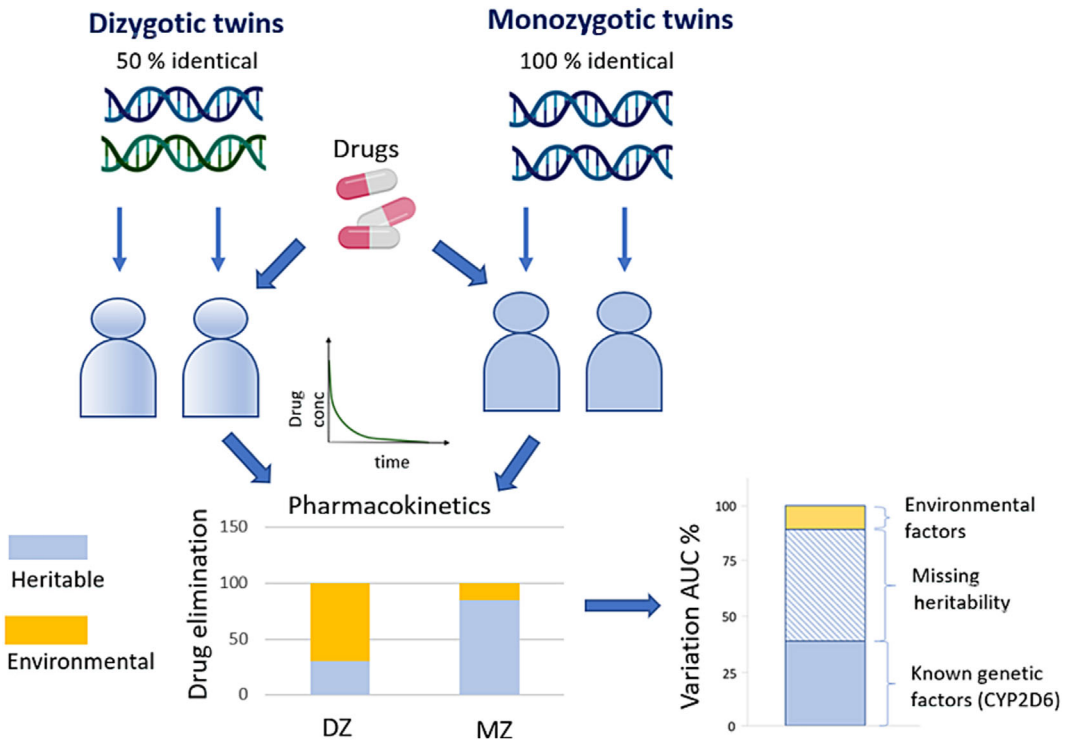

FIGURE 5 Missing heritability for interindividual variation in pharmacokinetics as revealed by twin studies. In many cases the majority of the origin of the heritable dependent variation is unknown.[3] The data are mainly obtained from Matthaei et al.[32]

1. The contribution of rare genetic variants which account for about 4%–6% of the missing heritability.
2. Incomplete next-generation sequencing at genetically complex loci requiring long-read sequencing or specialized bioinformatics tools.
3. The need to fully know the functionally distinct haplotypes of alleles that all carry a genetic variant classified in allelic nomenclature.
4. The fact that a particular enzyme variant may have altered specificity for different substrates compared to the normal variant.
5. The global inheritance of genetic variants that indirectly affect the level of enzyme expression, mainly located in the same genetic area as the gene in question.
6. The direct regulation of ADME genes by polymorphic nuclear factors such as NFIB which is important for the expression of CYP2D6.
7. The high influence of structural variants (SVs) among ADME genes may account for up to 22% of the interindividual variability in drug metabolism and response.

8. The lack of clean pharmacokinetic (metabolic) phenotypes where covariates are adjusted for, which enable discovery of novel pharmacogenetic variants.

Among the most challenging and yet open aspects of the missing heritability is the role of regulatory regions far away from the gene in question. Results by Khor et al.[34] using genome-wide association studies (GWAS)-based analyses of 497 patients and 149 phenotyped liver samples for the metabolism of tamoxifen, revealed that the extended CYP2D6 locus at 22q13 is the principal genetic determinant of endoxifen plasma concentration but that long-distance haplotypes connecting CYP2D6 with adjacent regulatory sites account for the unexplained portion of genetic variability.

Another example is the contribution of structural variation (SV), i.e. variations of a region of DNA approximately 1 kb and larger in size which can include inversions and balanced translocations or genomic imbalances (insertions and deletions). In a recent study by Tremmel et al.,[35] a comprehensive investigation of genetic structural variability within 908 pharmacogenes (344 ADME genes and 564 drug targets) was conducted. For this analysis, publicly available whole-genome sequencing data from a cohort of 10,847 unrelated individuals were used. The study uncovered a total of 14,984 distinct SVs spanning a broad size spectrum from 50 bp to 106 mb. On average, each individual had 10.3 SVs with potential functional consequences affecting the coding regions of ADME genes and another 1.5 SVs affecting drug targets. In addition, the authors identified 1,276 non-coding SVs that had overlaps with gene regulatory elements. These results suggest that non-coding SVs make a significant contribution, possibly accounting for about 22% of the total number of genetic variants of importance. However, future validations in the clinical setting are needed to verify to what extent these SVs influence drug metabolism and response in vivo.

The long distance-based regulatory regions are usually in the same chromosome but their location is most often completely unrelated to the gene in question. Thus, examination of single nucleotide polymorphisms (SNPs) associated with limb malformations identified within the introns of the *LMBR1* gene were found to only affect the expression of the sonic hedgehog gene (Shh), which is located nearly 2 Mb away.[36,37] Furthermore, in another mice study it was found that the CRISP CAS-mediated removal of a 20 kb segment from this first intron in the Fat mass and obesity-associated (*FTO*) gene had no discernible impact on *FTO* gene expression. Instead, it led to alterations in the expression of unrelated genes, *IRX3* and *IRX5*, situated hundreds of thousands of base pairs away from the *FTO* gene.[38] The complexity of understanding the genetic variants involved in controlling ADME gene expression is illustrated by the recent study of the number of SNPs that have a statistically significant effect on height in different individuals.[39] The authors compiled GWAS data from 5.4 million individuals of diverse ancestry, which revealed that 12,111 independent SNPs are significantly associated with height and account for almost all of the SNP-based common heritability. These SNPs are clustered within 7, 209 non-overlapping genomic segments with an average size of about 90 kb, covering about 21% of the genome. Although the complexity of the genetic basis for height differences must be higher than for ADME gene variation encompassing a much lower set of genes, this example illustrates the principle of the broad contribution of genes to a specific phenotype. Thus, we believe that translating these complex genetic relationships into clinical practice for real-world precision medicine will constitute a big challenge.

4.2 | Epigenetic aspects

More than 60 human ADME genes are known to be subject to epigenetic control.[40,41] Epigenetic changes have been associated with abnormalities in gene expression of drug-metabolizing enzymes in human cancers, supporting that epigenetic changes may influence the expression of genes involved in pharmacokinetics, pharmacodynamics and toxicity.[40–44] However, the extent to which epigenetic factors actually are responsible for heritable factors involved in the control of expression of various ADME genes, if any, is still unknown. Furthermore, clinically based determination of epigenetic modifications is very difficult since surrogate tissues like blood do not provide relevant information. More research is needed to link action of exogenous factors to epigenetic alterations in precision medicine.

5 | IMPLEMENTATION OF PHARMACOGENOMICS INTO THE CLINICS

Research in the field of pharmacogenetics has been conducted since about 1984. The first discoveries were the description of functional mutations in the *NAT2* gene in the 1959s[45] and in 1988 the first defective *CYP2D6* variant was described.[46] The real surge of interest in this field occurred in the early twenty-first century after the first description of the sequence of the human genome. From then on, the development has, however, been rather slow. For the first 20 years, however, scientific work was done without knowledge of the complexity of the human genome and the complex regulation of gene expression, and clinical work often involved studies of less well-characterized patients and evaluations of genetic variants with silent mutations or mutations of little functional significance, and indeed the interethnic differences were not pronounced. Among the most important discoveries used in clinics to date are the polymorphisms of *TPMT* and *DPYD* and insights into the importance of *HLA* alleles in the development of idiosyncratic drug side effects.[33] In addition, the genotyping of the highly oncogenic *BRCA1* and *BRCA2* genes can improve the outcome of olaparib and bevacizumab maintenance in high-grade ovarian cancer treatment.[47] Furthermore, the polymorphism of *CYP2C19* and *CYP2D6* plays important roles for the pharmacokinetics of several different drugs (see Section 3 in Ref. 48). Still missing in this field is, though, the actual genetic background for much of the inherited genetic variability, as described above. Genetic variants of somatic genomes are indeed useful tools for cancer treatment, but this part falls only within the realm of precision medicine, where pathologists play a central role in interpretations and treatment pathways.

6 | THE TOOLS FOR THE CLINICAL IMPLEMENTATION

The clinical implementation of pharmacogenomics poses a major problem, as previously described.[49] To date, there are at least 19 different clinical pharmacogenomics (PGx) initiatives that report successful integration of clinical services in various settings, including academic health centres and community practices.[50] These programmes have uncovered relevant pharmacogenetic variations that are ready for practical integration and can change drug prescribing practices. As Krebs and Milani[51] noted, there is an urgent need for greater standardization of the various genetic variant interrogation initiatives. One possible means to ensure consistent translation of these variants into metabolizing phenotypes is to set a minimum standard for variant screening to determine alleles and provide simple guidance on the use of translation tables.

Economic and efficacy evaluations have provided evidence for the substantial benefits of genotype-guided treatments, such as those described for the treatment of major depressive disorder (MDD).[52] Using a cohort of 194 149 adults (aged 19–99 years) recruited over 20 years with major depression who were eligible for pharmacological treatment, Ghanbarian et al. found that PGx-guided treatment resulted in 37% fewer treatment-resistant patients and 1,869 fewer deaths and 21,346 fewer hospital admissions over 20 years, resulting in a substantial $ 4926 reduction in costs per patient. Although the contribution of placebo effects *vs.* true genetic prediction is unclear, the data indeed suggest an opportunity to achieve major patient and societal benefits through preventive genotyping for the treatment of MDD.

Multiple ongoing studies exploring the application of PGx are currently in progress. Collectively, these dynamic initiatives have effectively transformed several challenges associated with PGx implementation into actionable solutions, bringing us closer to realizing the potential of pharmacogenomics. From a technical perspective, integrating pharmacogenomic information into electronic health records is feasible, but it is essential that healthcare providers not only have access to this data, but also have the ability to interpret the information correctly for relevant clinical decisions on drug prescribing for improved treatment outcomes.

When it comes to performing genetic analysis in the clinical setting, collaboration with accredited genetic analysis centres is essential, but this aspect is still underdeveloped in many countries. There is also an urgent need to address the question of who bears the costs associated with genotyping. Few studies have examined the cost–benefit aspect, and there is a clear need for more cost-effectiveness studies, ideally conducted in different health systems.

Perhaps the best tool for implementing pharmacogenomics in the clinic is pharmacogenomic labelling, such as that summarized by the Food and Drug Administration (FDA)[48] on the drug labels. The measures include obligatory or strongly recommended genetic analyses prior to administration. However, their implementation in clinical routine is not yet widespread.

The actual genetic effects for interindividual differences in drug metabolism and response are for many drugs relatively small compared with the influence of interacting co-medications, lifestyle (e.g. smoking) and pathophysiological aspects. Moreover, prospective studies are challenging because of the difficulties of not conducting open-label studies in this area and because of the high placebo effects of patients' being aware of receiving personalized treatment, regardless of group allocation/randomization. A recent experience is the open label PREPARE study,[53] which included nearly 7,000 subjects where patients in the study arm who were treated based on their pharmacogenomic profile in 12 different key genes ($n = 186$) had 30% fewer adverse events, while the control group in the study arm ($n = 456$), which was not initially genotyped and received standard drug treatment, also had a 30% decrease in adverse effects.[54-57] The responsible authors for the PREPARE study have in a comment pointed out a case mix between the different clinical centre as a possible factor supporting a conclusion of a true effect of genotyping causing a decrease in the number of adverse drug reactions registered.[58] Anyway, this study has provided a lot of knowledge to take into account during planning of future large clinical pharmacogenomic trials.

Clearly, the field of pharmacogenomics would gain much from future very large randomized prospective studies with closed labelling. The genetic variations responsible for interindividual differences in drug response are very gene- and substrate-specific, so such studies would be limited to a small number of drugs. However, the costs would be enormous, making funding by the general public very difficult. Trials like the very successful one with abacavir have almost entirely been financed by the company in question. A more pragmatic approach would be to accept that most drug effects are dose-dependent, hence using exact data on genotype-*vs*-exposure effects as the basis for recommendation on genotype-guided dosing to achieve clinical benefits. If implementation of pharmacogenetics in the clinical setting is accepted on these premises, initiatives such as the Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines can be utilized for precision dosing.

7 | CONCLUSIONS

According to the authors, the results of liquid biopsy-based determination of hepatic drug metabolism need to be repeated in larger cohorts before conclusions can be drawn about the feasibility of this method in the clinic. The more traditional TDM analyses have been greatly improved by the new, more versatile and sensitive mass spectrometers suitable for such analyses. In addition, the discovery of endogenous or dietary components as markers for CYP metabolic phenotypes opens the way for new, simpler and cheaper methods for accurate CYP phenotype analyses. For clinical implementation today, the most feasible analytical method involves ADME-specific arrays where the most important genetic variants can be determined in special hospital or commercial settings. With regard to the future application of pharmacogenomics in the clinic, more emphasis needs to be placed on the identification of missing heritabilities and on larger studies with well-characterized patients, as well as on the complete control of confounding factors such as diet, co-medication and physical

health. Indeed, with lower prices for whole genome sequencing, this method combined with AI-driven computational analyses will soon be developed into a clinically useful instrument.

In addition, the causes of the lack of heritability also need to be clarified. The complexity of the regulation of ADME gene expression described above suggests that future large studies based on long-read NGS of complete genomes combined with phenotypic characterization of patients receiving different drugs are needed to identify the missing heritability. With the subsequent continuous integration of these data into AI-based analysis systems, it would be likely that a reasonable and satisfactory picture would emerge, allowing the construction of clinically very useful algorithms for significantly improving the quality of genetically based prediction of drug metabolism and response.

7.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY, and are permanently archived in the Concise Guide to PHARMACOLOGY 2023/24.[59,60]

AUTHOR CONTRIBUTIONS

Both authors contributed equally to the manuscript.

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CONFLICT OF INTEREST STATEMENT

Magnus Ingelman-Sundberg is a co-founder and co-owner of HepaPredict AB. Espen Molden declares no conflicts of interest.

DATA AVAILABILITY STATEMENT

This is a review, and we do not refer to data other than literature.

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REFERENCES

1. Rothschild AJ, Parikh SV, Hain D, et al. Clinical validation of combinatorial pharmacogenomic testing and single-gene guidelines in predicting psychotropic medication blood levels and clinical outcomes in patients with depression. *Psychiatry Res*. 2021;296:113649. doi:10.1016/j.psychres.2020.113649
2. Radosavljevic M, Svob Strac D, Jancic J, Samardzic J. The role of pharmacogenetics in personalizing the antidepressant and anxiolytic therapy. *Genes (Basel)*. 2023;14(5):1095. doi:10.3390/genes14051095
3. Jukic M, Milosavljević F, Molden E, Ingelman-Sundberg M. Pharmacogenomics in treatment of depression and psychosis: an update. *Trends Pharmacol Sci*. 2022;43(12):1055-1069. doi:10.1016/j.tips.2022.09.011
4. Schoretsanitis G, Kane JM, Correll CU, et al. Blood levels to optimize antipsychotic treatment in clinical practice: a joint consensus statement of the American Society of Clinical Psychopharmacology and the therapeutic drug monitoring task force of the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie. *J Clin Psychiatry*. 2020;81(3):19cs13169. doi:10.4088/JCP.19cs13169
5. Al-Roubaie Z, Guadagno E, Ramanakumar AV, et al. Clinical utility of therapeutic drug monitoring of antiepileptic drugs: systematic review. *Neurol Clin Pract*. 2020;10(4):344-355. doi:10.1212/CPJ.0000000000000722
6. Syversen SW, Jørgensen KK, Goll GL, et al. Effect of therapeutic drug monitoring vs standard therapy during maintenance infliximab therapy on disease control in patients with immune-mediated inflammatory diseases: a randomized clinical trial. *JAMA*. 2021;326(23):2375-2384. doi:10.1001/jama.2021.21316
7. Pedersen L, Szecsi PB, Johansen PB, et al. Evaluation of therapeutic drug monitoring in the clinical management of patients with rheumatic diseases: data from a retrospective single-center cohort study. *Biol Theory*. 2020;14:115-125. doi:10.2147/BTT.S262511
8. Schagen MR, Volarevic H, Francke MI, et al. Individualized dosing algorithms for tacrolimus in kidney transplant recipients: current status and unmet needs. *Expert Opin Drug Metab Toxicol*. 2023;19(7):429-445. doi:10.1080/17425255.2023.2250251
9. Hagel S, Bach F, Brenner T, et al. Effect of therapeutic drug monitoring-based dose optimization of piperacillin/tazobactam on sepsis-related organ dysfunction in patients with sepsis: a randomized controlled trial. *Intensive Care Med*. 2022;48(3):311-321. doi:10.1007/s00134-021-06609-6
10. Mabilat C, Gros MF, Nicolau D, et al. Diagnostic and medical needs for therapeutic drug monitoring of antibiotics. *Eur J Clin Microbiol Infect Dis*. 2020;39(5):791-797. doi:10.1007/s10096-019-03769-8
11. Hiemke C, Bergemann N, Clement HW, et al. Consensus guidelines for therapeutic drug monitoring in neuropsychopharmacology: update 2017. *Pharmacopsychiatry*. 2018;51(1–2):9-62. doi:10.1055/s-0043-116492
12. Tomalik-Scharte D, Lütjohann D, Doroshyenko O, et al. Plasma 4beta-hydroxycholesterol: an endogenous CYP3A metric? *Clin Pharmacol Ther*. 2009;86(2):147-153. doi:10.1177/0091270009360041
13. Magliocco G, Desmeules J, Matthey A, et al. Metabolomics reveals biomarkers in human urine and plasma to predict cytochrome P450 2D6 (CYP2D6) activity. *Br J Pharmacol*. 2021;178(23):4708-4725. doi:10.1111/bph.15651
14. Wollmann BM, Størset E, Kringen MK, Molden E, Smith RL. Prediction of CYP2D6 poor metabolizers by measurements of solanidine and metabolites—a study in 839 patients with known CYP2D6 genotype. *Eur J Clin Pharmacol*. 2023;79(4):523-531. doi:10.1007/s00228-023-03462-y
15. Wollmann BM, Smith RL, Kringen MK, Ingelman-Sundberg M, Molden E, Størset E. Evidence for solanidine as a dietary CYP2D6 biomarker: significant correlation with risperidone metabolism. *Br J Clin Pharmacol*. 2023;90(3):740-747. doi:10.1111/bcp.15721
16. Armakolas A, Kotsari M, Koskinas J. Liquid biopsies, novel approaches and future directions. *Cancers (Basel)*. 2023;15(5):1579. doi:10.3390/cancers15051579

17. Doyle LM, Wang MZ. Overview of extracellular vesicles, their origin, composition, purpose, and methods for exosome isolation and analysis. *Cells*. 2019;8(7):727. doi:10.3390/cells8070727
18. Habtemariam HD, Guchelaar HJ. The potential application of extracellular vesicles from liquid biopsies for determination of pharmacogene expression. *Pharmaceuticals (Basel)*. 2022;15(2):252. doi:10.3390/ph15020252
19. Gagliardi A, Bajraktari-Sylejmani G, Barocelli E, et al. Extracellular vesicles as surrogates for drug metabolism and clearance: promise vs reality. *Life (Basel)*. 2023;13(8):1745. doi:10.3390/life13081745
20. Lee Y, Kim JH. The emerging roles of extracellular vesicles as intercellular messengers in liver physiology and pathology. *Clin Mol Hepatol*. 2022;28(4):706-724. doi:10.3350/cmh.2021.0390
21. Jackson KD, Achour B, Lee J, et al. Novel approaches to characterize individual drug metabolism and advance precision medicine. *Drug Metab Dispos*. 2023;51(10):1238-1253.
22. Rodrigues AD, van Dyk M, Sorich MJ, et al. Exploring the use of serum-derived small extracellular vesicles as liquid biopsy to study the induction of hepatic cytochromes P450 and organic anion transporting polypeptides. *Clin Pharmacol Ther*. 2021;110(1):248-258. doi:10.1002/cpt.2244
23. Rowland A, Ruanglertboon W, van Dyk M, et al. Plasma extracellular nanovesicle (exosome)-derived biomarkers for drug metabolism pathways: a novel approach to characterize variability in drug exposure. *Br J Clin Pharmacol*. 2019;85(1):216-226. doi:10.1111/bcp.13793
24. Achour B, Al-Majdoub ZM, Grybos-Gajniak A, et al. Liquid biopsy enables quantification of the abundance and interindividual variability of hepatic enzymes and transporters. *Clin Pharmacol Ther*. 2021;109(1):222-232. doi:10.1002/cpt.2102
25. Achour B, Gosselin P, Terrier J, et al. Liquid biopsy for patient characterization in cardiovascular disease: verification against markers of cytochrome P450 and P-glycoprotein activities. *Clin Pharmacol Ther*. 2022;111(6):1268-1277. doi:10.1002/cpt.2576
26. Pridgeon CS, Johansson I, Ingelman-Sundberg M. Liquid biopsies or therapeutic drug monitoring for CYP activity profile determination. *Clin Pharmacol Ther*. 2022;112(5):1000-1003. doi:10.1002/cpt.2695
27. Rowland A, Useckaite Z, Sorich MJ. The broader context of liquid biopsy in absorption, distribution, metabolism, and elimination. *Clin Pharmacol Ther*. 2022;112(6):1154. doi:10.1002/cpt.2734
28. Achour B, Rostami-Hodjegan A. Is liquid biopsy only restricted to diagnostics or can it go beyond the confines of genotyping and phenotyping for quantitative pharmacology? *Clin Pharmacol Ther*. 2022;112(6):1152-1153. doi:10.1002/cpt.2732
29. Aguiar M, Masse R, Gibbs BF. Regulation of cytochrome P450 by posttranslational modification. *Drug Metab Rev*. 2005;37(2):379-404. doi:10.1081/dmr-46136
30. Ingelman-Sundberg M. Pharmacogenetics: an opportunity for a safer and more efficient pharmacotherapy. *J Intern Med*. 2001;250(3):186-200. doi:10.1046/j.1365-2796.2001.00879.x
31. Lauschke VM, Ingelman-Sundberg M. Prediction of drug response and adverse drug reactions: from twin studies to next generation sequencing. *Eur J Pharm Sci*. 2019;130:65-77. doi:10.1016/j.ejps.2019.01.024
32. Matthaei J, Brockmöller J, Tzvetkov MV, et al. Heritability of metoprolol and torsemide pharmacokinetics. *Clin Pharmacol Ther*. 2015;98(6):611-621. doi:10.1002/cpt.258
33. Lauschke VM, Zhou Y, Ingelman-Sundberg M. Pharmacogenomics beyond single common genetic variants: the way forward. *Annu Rev Pharmacol Toxicol*. 2023;64:33-51. doi:10.1146/annurev-pharmtox-051921-091209
34. Khor CC, Winter S, Sutiman N, et al. Cross-ancestry genome-wide association study defines the extended CYP2D6 locus as the principal genetic determinant of endoxifen plasma concentrations. *Clin Pharmacol Ther*. 2023;113(3):712-723. doi:10.1002/cpt.2846
35. Tremmel R, Zhou Y, Schwab M, Lauschke VM. Structural variation of the coding and non-coding human pharmacogenome. *NPJ Genom Med*. 2023;8(1):24. doi:10.1038/s41525-023-00371-y
36. Lettice LA, Heaney SJ, Purdie TA, et al. A long-range Shh enhancer regulates expression in the developing limb and fin and is associated with preaxial polydactyly. *Hum Mol Genet*. 2003;12(14):1725-1735. doi:10.1093/hmg/ddg180
37. Kane L, Williamson I, Flyamer IM, et al. Cohesin is required for long-range enhancer action at the Shh locus. *Nat Struct Mol Biol*. 2022;29(9):891-897. doi:10.1038/s41594-022-00821-8
38. Sobreira DR, Joslin AC, Zhang Q, et al. Extensive pleiotropism and allelic heterogeneity mediate metabolic effects of IRX3 and IRX5. *Science*. 2021;372(6546):1085-1091. doi:10.1126/science.abf1008
39. Yengo L, Vedantam S, Maroul E, et al. A saturated map of common genetic variants associated with human height. *Nature*. 2022;610(7933):704-712. doi:10.1038/s41586-022-05275-y
40. Zhong XB, Leeder JS. Epigenetic regulation of ADME-related genes: focus on drug metabolism and transport. *Drug Metab Dispos*. 2013;41(10):1721-1724. doi:10.1124/dmd.113.053942
41. Jin J, Zhong XB. Epigenetic mechanisms contribute to intraindividual variations of drug metabolism mediated by cytochrome P450 enzymes. *Drug Metab Dispos*. 2023;51(6):672-684. doi:10.1124/dmd.122.001007
42. Fisel P, Schaeffeler E, Schwab M. DNA methylation of ADME genes. *Clin Pharmacol Ther*. 2016;99(5):512-527. doi:10.1002/cpt.343
43. Zhao M, Ma J, Li M, et al. Cytochrome P450 enzymes and drug metabolism in humans. *Int J Mol Sci*. 2021;22(23):12808. doi:10.3390/ijms222312808
44. Wang J, Lushan Y, Jiang H, et al. Epigenetic regulation of differentially expressed drug-metabolizing enzymes in cancer. *Drug Metab Dispos*. 2020;48(9):759-768. doi:10.1124/dmd.120.000008
45. Knight RA, Selin MJ, Harris HW. Genetic factors influencing isoniazid blood levels in humans. *Trans Conf Chemother Tuberc*. 1959;8:52-56.
46. Gonzalez FJ, Skoda RC, Kimura S, et al. Characterization of the common genetic defect in humans deficient in debrisoquine metabolism. *Nature*. 1988;331(6155):442-446. doi:10.1038/331442a0
47. Labidi-Galy SI, Rodrigues M, Sandoval JL, et al. Association of location of BRCA1 and BRCA2 mutations with benefit from olaparib and bevacizumab maintenance in high-grade ovarian cancer: phase III PAOLA-1/ENGOT-ov25 trial subgroup exploratory analysis. *Ann Oncol*. 2023;34(2):152-162. doi:10.1016/j.annonc.2022.11.003
48. US Food and Drug Administration (FDA). Table of pharmacogenetic associations. https://www.fda.gov/medical-devices/precision-medicine/table-pharmacogenetic-associations. Accessed November 24, 2023.
49. Chang WC, Tanoshima R, Ross CJD, Carleton BC. Challenges and opportunities in implementing pharmacogenetic testing in clinical settings. *Annu Rev Pharmacol Toxicol*. 2021;61(1):65-84. doi:10.1146/annurev-pharmtox-030920-025745
50. Luczak T, Brown SJ, Armbruster D, Hundertmark M, Brown J, Stenehjem D. Strategies and settings of clinical pharmacogenetic implementation: a scoping review of pharmacogenetics programs. *Pharmacogenomics*. 2021;22(6):345-364. doi:10.2217/pgs-2020-0181
51. Krebs K, Milani L. Translating pharmacogenomics into clinical decisions: do not let the perfect be the enemy of the good. *Hum Genomics*. 2019;13(1):39. doi:10.1186/s40246-019-0229-z
52. Ghanbarian S, Wong GWK, Bunka M, et al. Cost-effectiveness of pharmacogenomic-guided treatment for major depression. *Cmaj*. 2023;195(44):E1499-E1508. doi:10.1503/cmaj.221785
53. Swen JJ, van der Wouden CH, Manson LE, et al. Ubiquitous pharmacogenomics consortium. *Lancet*. 2023;401(10374):347-356. doi:10.1016/S0140-6736(22)01841-4

54. Peñas-LLedó E, LLerena A. Clinical use of pre-emptive pharmacogenetic programmes. *Lancet*. 2023;401(10374):320-321. doi:10.1016/S0140-6736(22)02461-8
55. Rogers A, Mackenzie IS, MacDonald TM, Doney ASF. The PREPARE study: benefits of pharmacogenetic testing are unclear. *Lancet*. 2023;401(10391):1850. doi:10.1016/S0140-6736(23)00853-X
56. Curtis D. The PREPARE study: benefits of pharmacogenetic testing are unclear. *Lancet*. 2023;401(10391):1850-1851. doi:10.1016/S0140-6736(23)00854-1
57. Damkier P, Andersen MA. Temporary like Achilles: pre-emptive germline pharmacogenetic testing. *Basic Clin Pharmacol Toxicol*. 2023;133(4):397-399. doi:10.1111/bcpt.13934
58. Swen JJ, Manson LEN, Böhringer S, Pirmohamed M, Guchelaar HJ. The PREPARE study: benefits of pharmacogenetic testing are unclear—authors' reply. *Lancet*. 2023;401(10391):1851-1852. doi:10.1016/S0140-6736(23)00852-8
59. Alexander SPH, Fabbro D, Kelly E, et al. The concise guide to PHARMACOLOGY 2023/24: enzymes. *Br J Pharmacol*. 2023;180(S2):S289-S373. doi:10.1111/bph.16181
60. Alexander SPH, Fabbro D, Kelly E, et al. The concise guide to PHARMACOLOGY 2023/24: transporters. *Br J Pharmacol*. 2023;180(S2):S374-S469. doi:10.1111/bph.16182

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