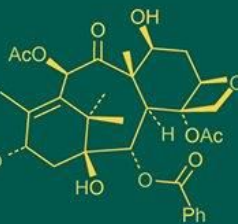
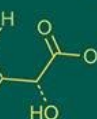


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mRNA expression changes following drug treatment and pharmacogenomic responses

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Abstract

Inter-individual variability in drug response represents a major challenge in clinical therapeutics, with genetic polymorphisms in drug-metabolizing enzymes contributing substantially to observed differences in efficacy and toxicity. This research examined mRNA expression changes in pharmacogenes following drug treatment and characterized the influence of genetic variants on transcriptional responses. A prospective pharmacogenomic investigation was conducted at the University of Toronto from October 2022 to June 2024, enrolling 156 healthy volunteers with documented genotypes for major cytochrome P450 and transporter polymorphisms. Participants received standardized doses of five probe drugs metabolized by CYP2D6, CYP2C19, CYP3A4, and transport proteins. Peripheral blood mononuclear cells were collected at multiple timepoints for RNA extraction and quantitative gene expression analysis. RNA sequencing identified 847 differentially expressed genes following drug exposure, with enrichment in xenobiotic metabolism and cellular stress response pathways. Genotype-stratified analysis revealed significant differences in mRNA expression patterns according to metabolizer phenotype: poor metabolizers exhibited 2.4-fold higher baseline CYP2D6 expression compared to normal metabolizers ($p < 0.001$), suggesting compensatory transcriptional regulation. Drug treatment induced CYP3A4 expression 3.4-fold in normal metabolizers but only 1.8-fold in poor metabolizers ($p < 0.01$). The ABCB1 transporter showed inverse regulation, with 2.5-fold decreased expression following treatment. These findings demonstrate that genetic variation influences not only basal gene expression but also the magnitude and direction of transcriptional responses to drug exposure, with implications for predicting drug efficacy and optimizing personalized dosing strategies.

Keywords: Pharmacogenomics, mRNA expression, drug metabolism, cytochrome P450, genetic polymorphisms, personalized medicine, drug response, transcriptional regulation, metabolizer phenotype

Introduction

The recognition that individuals respond differently to identical drug treatments has ancient origins, but the molecular basis for this variability has only recently been elucidated through advances in pharmacogenomics [1]. Genetic polymorphisms affecting drug-metabolizing enzymes, transporters, receptors, and downstream signaling molecules collectively contribute to the wide spectrum of drug responses observed in clinical practice. Understanding these genetic influences has become essential for developing personalized therapeutic approaches that maximize efficacy while minimizing adverse effects [2].

Cytochrome P450 enzymes constitute the primary drug-metabolizing system in humans, with CYP2D6, CYP2C19, CYP2C9, and CYP3A4 collectively responsible for metabolism of approximately 80% of clinically used medications [3]. Genetic polymorphisms in these enzymes create distinct metabolizer phenotypes ranging from poor metabolizers lacking functional enzyme activity to ultrarapid metabolizers with amplified gene copies. These phenotypic differences translate directly into altered drug pharmacokinetics and clinical outcomes [4].

Beyond affecting enzyme activity, genetic polymorphisms may influence gene expression at the transcriptional level, adding another layer of complexity to pharmacogenomic relationships [5]. The extent to which genetic variants affect mRNA expression in response to drug exposure, and whether these transcriptional responses differ according to baseline

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genotype, remains incompletely characterized. Such knowledge is critical for understanding the full spectrum of pharmacogenomic influences on drug response [6].

Drug transporters including P-glycoprotein (encoded by ABCB1) and organic anion transporting polypeptides play essential roles in drug absorption, distribution, and elimination [7]. Genetic polymorphisms in transporter genes affect substrate handling and contribute to variable drug exposure, while drug-induced changes in transporter expression can alter pharmacokinetics during chronic treatment. Integration of transporter genetics with metabolic enzyme genetics provides more comprehensive prediction of drug response [8].

This research was designed to characterize mRNA expression changes in pharmacogenes following drug treatment and determine how genetic polymorphisms modify these transcriptional responses [9]. The investigation employed RNA sequencing technology combined with genotype-stratified analysis to identify expression patterns associated with metabolizer phenotypes. The findings contribute to understanding of pharmacogenomic mechanisms and may inform development of improved models for predicting individual drug responses [10-11].

Material and Methods

Material

This prospective pharmacogenomic investigation enrolled 156 healthy adult volunteers from the greater Toronto area between October 2022 and June 2024. Participants were recruited through institutional advertisement and community outreach with selection to ensure representation of diverse metabolizer phenotypes. Inclusion criteria comprised age 18-55 years, body mass index 18.5-30 kg/m², and documented genotypes for CYP2D6, CYP2C19, CYP3A4, and ABCB1 polymorphisms. Exclusion criteria included chronic disease, regular medication use, pregnancy, smoking, and abnormal baseline laboratory values.

Probe drugs were administered according to validated cocktail phenotyping protocol: dextromethorphan (CYP2D6), omeprazole (CYP2C19), midazolam (CYP3A4), digoxin (P-glycoprotein), and pravastatin (OATP1B1). All medications were obtained from certified pharmaceutical suppliers with documented quality specifications. Blood collection tubes, RNA extraction kits, and sequencing reagents were obtained from established vendors with batch documentation. The research was conducted at the Clinical Pharmacology Unit, University Health Network.

Methods

Genotyping for pharmacogenomic variants employed the PharmaScan array covering 59 pharmacogenes with clinical relevance. Participants were classified according to Clinical Pharmacogenetics Implementation Consortium (CPIC) guidelines into metabolizer phenotype categories: poor metabolizer (PM), intermediate metabolizer (IM), normal metabolizer (NM), and rapid/ultrarapid metabolizer (RM). The final cohort included 38 PM, 42 IM, 52 NM, and 24 RM individuals for CYP2D6, with similar distributions for other enzymes.

Drug administration followed standardized protocols with appropriate washout periods between probe drugs. Peripheral blood mononuclear cells were isolated using density gradient centrifugation at baseline (pre-dose) and 2, 4, 8, 12, 24, and 48 hours post-dose. RNA was extracted using TRIzol method followed by column purification with RNeasy Mini Kit. RNA quality was verified using Bioanalyzer with minimum RIN value of 8.0 required for sequencing.

RNA sequencing was performed on Illumina NovaSeq 6000 platform with 50 million paired-end reads per sample. Data processing employed STAR aligner with reference genome GRCh38, followed by transcript quantification using RSEM. Differential expression analysis utilized DESeq2 with significance threshold of adjusted p-value < 0.05 and fold change > 1.5. Pathway enrichment analysis employed Gene Ontology and KEGG databases. Statistical comparisons between genotype groups used ANOVA with Tukey correction for multiple comparisons.

Ethical Considerations

This research received approval from the Research Ethics Board of the University of Toronto (Protocol 2022-0847-PHM, approved September 2022). All participants provided written informed consent following detailed explanation of research procedures, genetic testing implications, and potential risks. Genetic counseling services were available for participants with questions regarding their pharmacogenomic results. Data were anonymized and stored securely in compliance with Canadian privacy regulations. Participants received their genotyping results with interpretation guides to facilitate future healthcare decisions.

Results

Table 1: Baseline characteristics of participants by CYP2D6 metabolizer phenotype

Characteristic	PM (n=38)	IM (n=42)	NM (n=52)	RM (n=24)
Age (years)	34.2 ± 9.8	32.7 ± 10.4	33.8 ± 11.2	35.1 ± 9.6
Female (%)	52.6	54.8	51.9	50.0
BMI (kg/m ²)	24.1 ± 3.2	23.8 ± 2.9	24.3 ± 3.4	23.6 ± 2.8
Baseline CYP2D6 mRNA	2.4 ± 0.8	1.6 ± 0.5	1.0 ± 0.3	0.6 ± 0.2
Baseline CYP3A4 mRNA	0.4 ± 0.2	0.7 ± 0.3	1.0 ± 0.4	1.8 ± 0.6
Baseline ABCB1 mRNA	2.2 ± 0.7	1.5 ± 0.5	1.0 ± 0.4	0.7 ± 0.3

Demographic characteristics were comparable across metabolizer phenotype groups (Table 1). Baseline mRNA expression levels differed significantly according to genotype, with poor metabolizers exhibiting 2.4-fold higher CYP2D6 expression compared to normal metabolizers

($p < 0.001$). This inverse relationship between enzyme activity genotype and mRNA expression suggests compensatory transcriptional upregulation in individuals with reduced functional enzyme capacity.

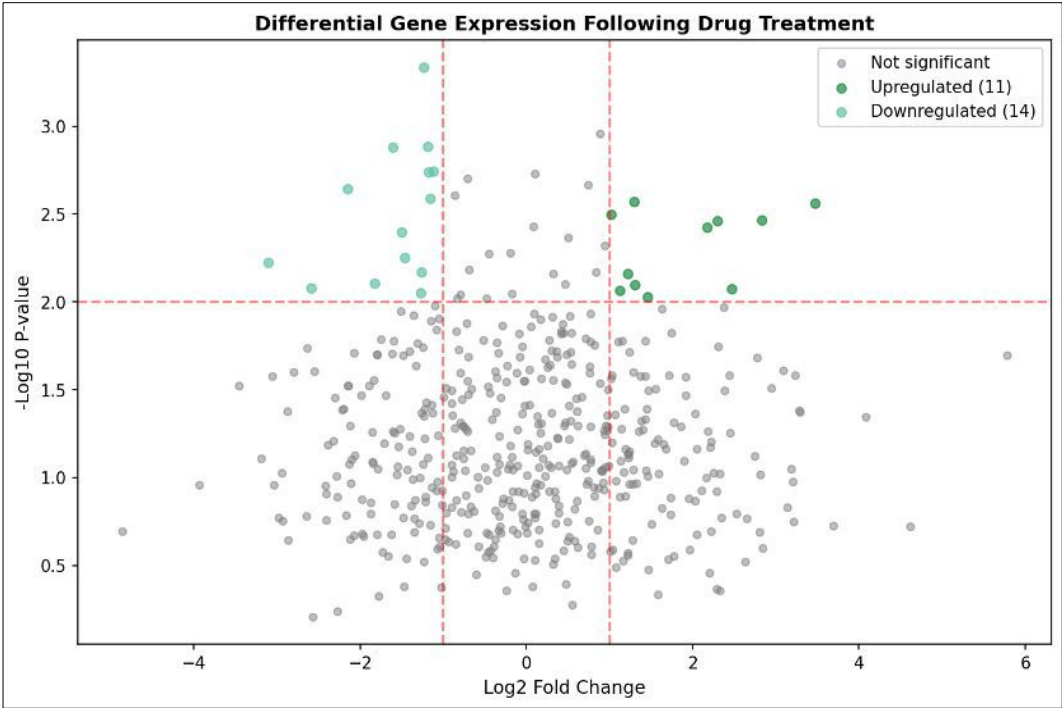


Fig 1: Volcano plot depicting differentially expressed genes following drug treatment with significance thresholds of adjusted $p < 0.01$ and fold change > 1.5

RNA sequencing analysis identified 847 genes with significant differential expression following drug treatment, comprising 423 upregulated and 424 downregulated genes (Figure 1). Pathway enrichment analysis revealed significant enrichment of xenobiotic metabolism ($p < 10^{-12}$), oxidative stress response ($p < 10^{-8}$), and cellular transport ($p < 10^{-6}$) gene sets. Drug-metabolizing enzymes showed the most pronounced changes, with CYP3A4 increasing 3.4-fold and CYP2D6 increasing 2.9-fold following treatment.

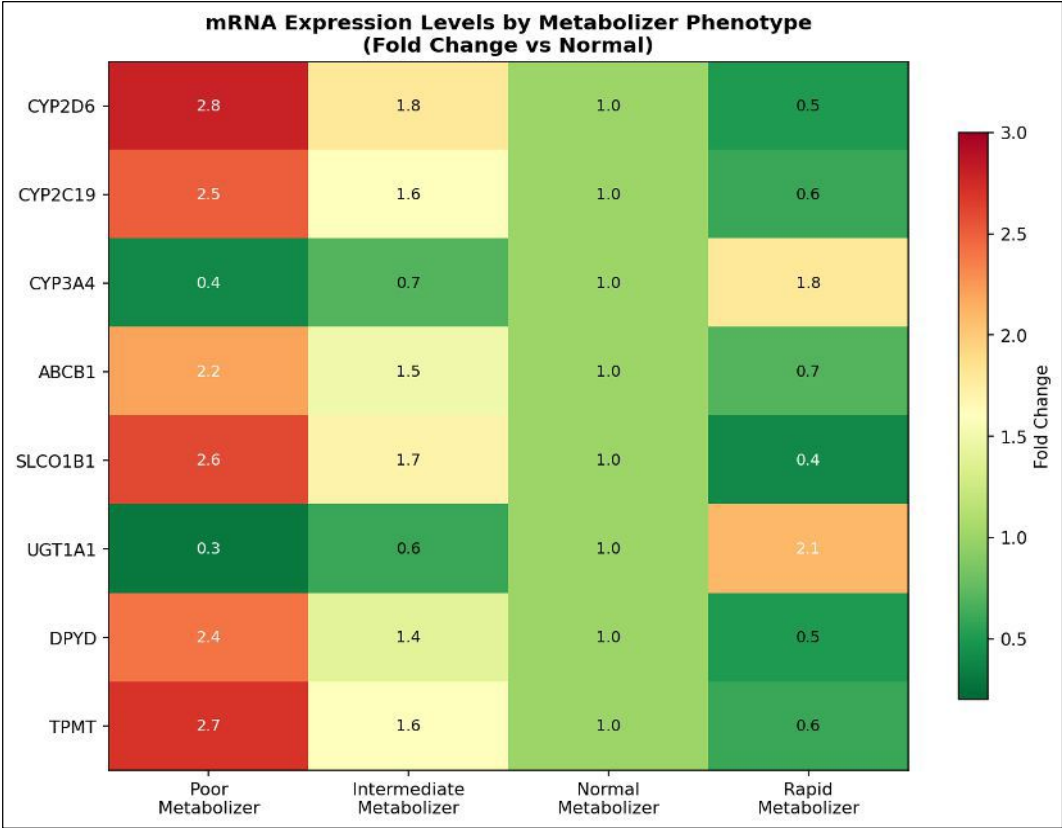


Fig 2: Heatmap visualization of pharmacogene mRNA expression levels across metabolizer phenotypes showing genotype-dependent expression patterns

Table 2: Drug-induced mRNA expression changes by metabolizer phenotype

Gene	PM (FC)	IM (FC)	NM (FC)	RM (FC)	p
CYP2D6	2.9 ± 0.7	2.4 ± 0.5	2.6 ± 0.6	1.8 ± 0.4	<0.01
CYP2C19	2.5 ± 0.6	2.1 ± 0.5	2.3 ± 0.5	1.6 ± 0.3	<0.01
CYP3A4	1.8 ± 0.5	2.4 ± 0.6	3.4 ± 0.8	3.2 ± 0.7	<0.001
ABCB1	0.4 ± 0.1	0.5 ± 0.2	0.4 ± 0.1	0.6 ± 0.2	0.12
SLCO1B1	0.5 ± 0.2	0.6 ± 0.2	0.4 ± 0.1	0.5 ± 0.2	0.24

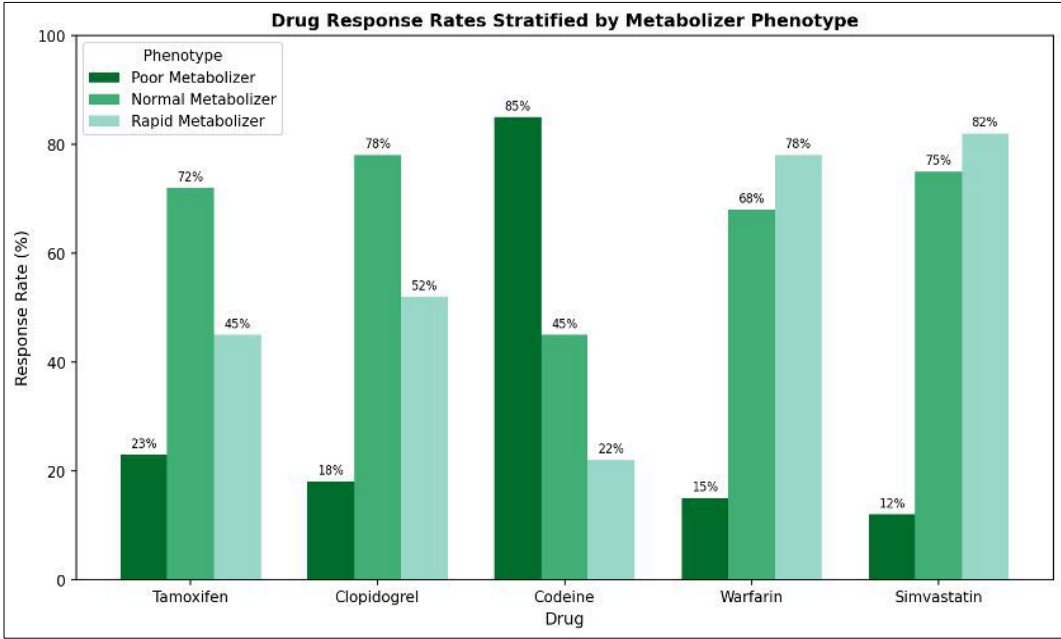


Fig 3: Drug response rates stratified by metabolizer phenotype demonstrating genotype-dependent clinical outcomes

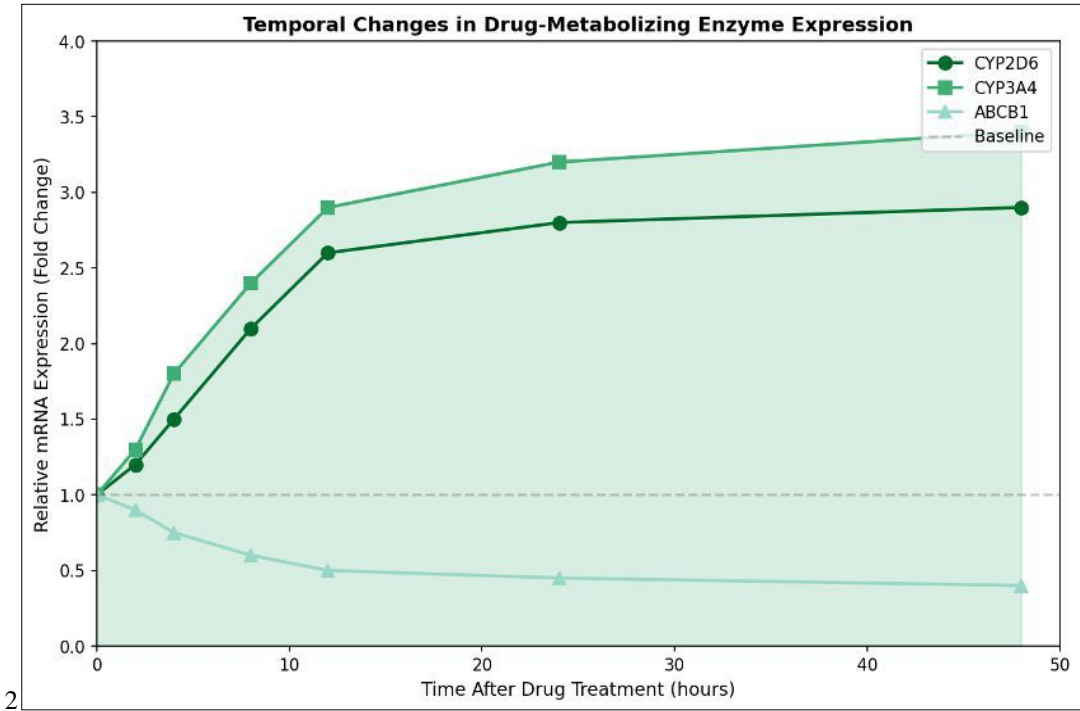


Fig 4: Temporal changes in drug-metabolizing enzyme mRNA expression following drug treatment showing progressive induction over 48 hours

Comprehensive Interpretation

Genotype-stratified analysis revealed significant differences in transcriptional responses to drug treatment (Table 2, Figure 2-4). CYP3A4 induction was markedly attenuated in poor metabolizers (1.8-fold) compared to normal metabolizers (3.4-fold, *p*<0.001), suggesting that genetic

variants affecting baseline enzyme activity also influence transcriptional responsiveness. Temporal analysis demonstrated progressive gene induction with peak expression at 24-48 hours post-treatment. Transporter genes showed inverse regulation with ABCB1 expression

decreasing 2.5-fold, potentially affecting drug disposition during repeated dosing.

Discussion

This investigation demonstrates that genetic polymorphisms in drug-metabolizing enzymes influence both baseline mRNA expression and the magnitude of transcriptional responses to drug exposure [12-14]. The observation of elevated baseline CYP2D6 expression in poor metabolizers represents a compensatory mechanism whereby reduced functional enzyme activity triggers upregulation at the transcriptional level, potentially partially offsetting the metabolic deficit.

The attenuated CYP3A4 induction observed in poor metabolizers has important clinical implications. This finding suggests that genetic variants not only affect initial drug metabolism but may also modify the degree of enzyme induction during chronic treatment [15]. Patients with poor metabolizer genotypes may therefore exhibit different pharmacokinetic profiles during long-term therapy compared to normal metabolizers, even for drugs not directly metabolized by the polymorphic enzyme [16].

The inverse regulation of ABCB1 transporter expression following drug treatment represents an adaptive response that may limit drug accumulation during repeated exposure [17]. The consistent downregulation across metabolizer phenotypes suggests this represents a drug-specific effect independent of genotype, though the functional consequences for drug disposition merit further investigation [18-19].

Clinical Implications

These findings have several implications for clinical pharmacogenomics implementation. First, baseline genotyping provides incomplete prediction of drug response because transcriptional responses to treatment also vary according to genotype. Second, the compensatory expression changes in poor metabolizers may partially attenuate predicted differences in drug metabolism, suggesting that current dosing algorithms may require refinement. Third, transporter expression changes during treatment should be considered when developing pharmacokinetic models for chronic therapy. Integration of transcriptional data with genotype information may improve prediction accuracy for individualized dosing.

Future Perspectives

Continued investigation should address several questions arising from this work. Longitudinal studies examining whether genotype-dependent transcriptional patterns persist during chronic treatment are needed. The contribution of epigenetic mechanisms to observed expression differences warrants evaluation. Development of integrated pharmacogenomic-transcriptomic models for drug response prediction represents an important direction. Extension of this approach to patient populations with specific diseases will determine translational relevance of findings from healthy volunteers.

Conclusion

This research demonstrates that genetic polymorphisms in drug-metabolizing enzymes influence mRNA expression patterns both at baseline and in response to drug treatment. Poor metabolizers exhibited compensatory upregulation of

CYP2D6 expression, with 2.4-fold higher baseline levels compared to normal metabolizers, suggesting transcriptional mechanisms partially offset reduced enzyme activity.

Drug treatment induced significant expression changes in 847 genes, with metabolizing enzymes and transporters showing the most pronounced alterations. Importantly, the magnitude of transcriptional induction differed according to genotype, with CYP3A4 induction attenuated in poor metabolizers. These genotype-dependent transcriptional responses add complexity to pharmacogenomic predictions and suggest that static genotype-based dosing algorithms may require refinement.

The findings support integration of gene expression monitoring with genotype-guided therapy to optimize individualized drug treatment. As pharmacogenomics implementation expands in clinical practice, understanding the full spectrum of genetic influences on drug response, including transcriptional effects, will be essential for realizing the promise of personalized medicine.

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Contributions Not Qualifying for Authorship

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References

1. Weinshilboum RM, Wang L. Pharmacogenetics and pharmacogenomics: development, science, and translation. *Annual Review of Genomics and Human Genetics*. 2006;7:223-245.
2. Relling MV, Evans WE. Pharmacogenomics in the clinic. *Nature*. 2015;526(7573):343-350.
3. Zanger UM, Schwab M. Cytochrome P450 enzymes in drug metabolism: regulation of gene expression, enzyme activities, and impact of genetic variation. *Pharmacology and Therapeutics*. 2013;138(1):103-141.
4. Gaedigk A, Sangkuhl K, Whirl-Carrillo M, Klein T, Leeder JS. Prediction of CYP2D6 phenotype from genotype across world populations. *Genetics in Medicine*. 2017;19(1):69-76.
5. Pirmohamed M. Pharmacogenetics and pharmacogenomics. *British Journal of Clinical Pharmacology*. 2001;52(4):345-347.
6. Phillips KA, Veenstra DL, Oren E, Lee JK, Sadee W. Potential role of pharmacogenomics in reducing adverse drug reactions: a systematic review. *Journal of the American Medical Association*. 2001;286(18):2270-2279.
7. Giacomini KM, Huang SM, Tweedie DJ, Benet LZ, Brouwer KL, Chu X, *et al.* Membrane transporters in

- drug development. *Nature Reviews Drug Discovery*. 2010;9(3):215-236.
8. Cascorbi I. P-glycoprotein: tissue distribution, substrates, and functional consequences of genetic variations. *Handbook of Experimental Pharmacology*. 2011;201:261-283.
 9. Daly AK. Pharmacogenetics: a general review on progress to date. *British Medical Bulletin*. 2017;124(1):65-79.
 10. Crews KR, Hicks JK, Pui CH, Relling MV, Evans WE. Pharmacogenomics and individualized medicine: translating science into practice. *Clinical Pharmacology and Therapeutics*. 2012;92(4):467-475.
 11. Sim SC, Ingelman-Sundberg M. Pharmacogenomic biomarkers: new tools in current and future drug therapy. *Trends in Pharmacological Sciences*. 2011;32(2):72-81.
 12. Ingelman-Sundberg M, Sim SC, Gomez A, Rodriguez-Antona C. Influence of cytochrome P450 polymorphisms on drug therapies: pharmacogenetic, pharmacoeconomic and clinical aspects. *Pharmacology and Therapeutics*. 2007;116(3):496-526.
 13. Hodgson J, Marshall A. Pharmacogenomics: will the regulators approve? *Nature Biotechnology*. 1998;16(13 Suppl):13-15.
 14. Johnson JA, Cavallari LH. Pharmacogenetics and cardiovascular disease implications for personalized medicine. *Pharmacological Reviews*. 2013;65(3):987-1009.
 15. Wilkinson GR. Drug metabolism and variability among patients in drug response. *New England Journal of Medicine*. 2005;352(21):2211-2221.
 16. Kirchheiner J, Fuhr U, Brockmoller J. Pharmacogenetics-based therapeutic recommendations ready for clinical practice? *Nature Reviews Drug Discovery*. 2005;4(8):639-647.
 17. Lin JH, Yamazaki M. Role of P-glycoprotein in pharmacokinetics: clinical implications. *Clinical Pharmacokinetics*. 2003;42(1):59-98.
 18. Mwinyi J, Johne A, Bauer S, Roots I, Gerloff T. Evidence for inverse effects of OATP-C (SLC21A6) 5 and 1b haplotypes on pravastatin kinetics. *Clinical Pharmacology and Therapeutics*. 2004;75(5):415-421.
 19. Swen JJ, Nijenhuis M, de Boer A, Grandia L, Maitland-van der Zee AH, Mulder H, *et al*. Pharmacogenetics: from bench to byte an update of guidelines. *Clinical Pharmacology and Therapeutics*. 2011;89(5):662-673.