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PHARMACOGENOMICS AND PERSONALIZED MEDICINE: OPTIMIZING DRUG THERAPY FOR BETTER CLINICAL OUTCOMES

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Abstract

The interindividual variability in drug response poses a major challenge to safe and effective pharmacotherapy. This variability is due to genetic polymorphism in major pharmacogenes, including CYP2C9, CYP2D6, and TPMT, but there is a major need to experimentally verify their functional implications in controlled laboratory environments. This paper set out to compare the effect of particular pharmacogenomic variations on the cellular response to three commonly prescribed drugs: warfarin, tamoxifen, and 6-mercaptopurine in in vitro human cell line models that were stratified by genotype. The selected drugs were administered on genetically characterized HepG2, MCF-7, and Caco-2 cells at different concentrations. The cytotoxicity evaluation was performed using the MTT assay, whereas the level of gene and protein expression was determined using qRT-PCR and Western blotting, respectively. The Annexin V/PI flow cytometry was used to analyze apoptosis, and the correlation of genotype and drug response was statistically measured using Pearson coefficients.

Significant variations in the IC 50, gene/protein expression, and apoptotic reaction were found between wild-type and variant genotypes. Cells with CYP2C9 *3/*3, CYP2D6 *4/*4, and TPMT *3A/*3A showed very strong drug sensitivity and low expression of the enzyme in contrast to their normal counterparts. A heatmap visualization and correlation analysis supported robust genotype to phenotype associations of all tested parameters. The study provides mechanistic and functional confirmation of pharmacogenomic variability in response to drugs with genotype-stratified in vitro systems. These findings favor the introduction of pharmacogenomic screening into clinical practice and the necessity to develop more preclinical models in order to close the translational gap in personalized medicine.

Keywords:
Pharmacogenomics, Drug response variability, In vitro model, Personalized medicine

1. Introduction

Interindividual variability in drug response is one of the most serious problems of clinical pharmacology nowadays. Even with the same medication, patients can display strikingly diverse results, even with regulated dosing rules and guidelines. Such responses may vary between full therapeutic efficacy and severe adverse drug reactions (ADRs) or failure of treatment, which leads to more morbidity, mortality, and health expenditure worldwide. The conventional methods of drug development and prescription have traditionally been based on averages of populations, and have ignored the complex biological, environmental, and, most importantly, genetic mechanisms that modify individual drug response. Such inconsistency is not only a clinical problem, but also a systemic problem, which compromises therapeutic accuracy and patient safety[2].

The field of pharmacogenomics has become an emerging revolution that is dealing with such challenges by investigating the effects of genetic variation on drug absorption, distribution, metabolism, and excretion (ADME) and drug target interactions. The end outcome is to shift to real personalized medicine, and to shift to personalized medicine where treatment is based on individual genetic makeup[3]. Key pharmacogenes in this effort include members of the cytochrome P450 family (e.g., CYP2C9, CYP2C19, CYP2D6), which metabolize most drugs clinically prescribed. The genetic differences can classify the individuals as poor, intermediate, extensive, or ultra-rapid metabolizers, all of which need different dosing approaches to attain optimal results [4,5].

Other pharmacogenetic markers of great clinical concern are TPMT (thiopurine methyltransferase), which affects the metabolism of thiopurine drugs that are used to treat leukemia and autoimmune diseases, and VKORC1 (vitamin K epoxide reductase complex subunit 1), which plays a critical role in determining warfarin sensitivity[6]. These genes are well studied and have been used to guide dosing algorithms and clinical guidelines. The information about pharmacogenomics is presently incorporated into drug labeling by regulatory agencies like the U.S. Food and Drug Administration (FDA) on more than 250 drugs[7]. Nevertheless, even with those regulatory progresses and scientific achievements, the clinical use of pharmacogenomic testing is not uniform across healthcare systems because of infrastructural, financial, and ethical limitations[8].

The new developments in the field of computational biology, bioinformatics as well and artificial intelligence have greatly broadened the scope of pharmacogenomics by allowing high-throughput analysis and predictive modeling. AI-based systems have become capable of incorporating multi-omic data to predict drug responses more and more accurately, promoting the creation of patient-specific therapeutic strategies[9,10]. Nevertheless, in silico models have huge potential, but they should be based on empirical data. Genetic insight has to be translated into practical clinical advice, and experimental validation is a key component. This discrepancy between in silico prediction and in vivo practice makes it all the more obvious that preclinical platforms are needed that can give an insight into drug-gene interactions in a controlled environment[11].

In vitro cell-based systems have proved essential in this sense. These models provide a reproducible and controlled setting into which to explore the molecular basis of pharmacogenomic variability. Human cell lines of natural or genetically engineered origin expressing particular pharmacogenetic

variants would enable direct study of the effects of that variant on drug efficacy and toxicity[12]. In vitro models reduce the rate of biological variability, ethical issues, and allow the experimenter to precisely control experimental conditions, unlike in vivo systems. They are an important intermediate between genomic discovery and clinical use, with the clarity and cost-effectiveness of mechanistic insight[13].
In addition, high-throughput screening of various drugs and genotypes can be performed using in vitro systems, which is of great value because different populations have genetic diversity. As an example, CYP2D6 variants may have different frequencies and effects across ethnicities and determine drug response in the population[14]. With the help of in vitro systems, such differences can be measured in a scalable and reproducible way and can be used to guide personalized dosing approaches and lead to more equal healthcare outcomes[15]. Notably, these models can also be used to assess gene expression alterations, enzyme activity, and cytotoxic reactions towards pharmacological agents, thus giving a complete account of relationships between genotype and phenotype[11].
Although these are the benefits, there exists a significant gap in the literature on experimental validation of pharmacogenomics through controlled in vitro models. Although there are numerous studies on the interaction between particular genes and drugs in clinical cohorts, the number of studies on the interaction between particular genes and drugs in preclinical settings is relatively small. The in vitro data can not only deepen our knowledge about drug metabolism and action on the molecular level but also become a basis for the development of clinical assays and decision-support tools. As such, there is an urgent demand for well-designed experimental studies, which can simulate pharmacogenetic variability as well as its functional implications in vitro [8].
The current study aims to address this gap by utilizing in vitro cell culture systems to evaluate the impact of pharmacogenetic variation on drug response. Genetically distinct human cell lines will be treated with specific pharmacological agents, and outcomes such as cell viability, gene/protein expression, and functional response will be systematically assessed. This approach is designed to provide mechanistic insights into how genetic variation modulates therapeutic outcomes and to support the development of genotype-guided treatment strategies. The specific objectives of this study are:

- To evaluate the cytotoxic response of pharmacogenetically distinct human cell lines to selected drug compounds in vitro.
- To assess the impact of key pharmacogenomic variants on gene or protein expression following drug exposure.
- To determine the correlation between genotypic differences and variability in drug efficacy or toxicity under controlled experimental conditions.

By integrating genomic data with functional outcomes in a controlled experimental environment, this study seeks to bridge the gap between pharmacogenomic discovery and clinical application. The findings are expected to contribute valuable preclinical evidence in support of personalized medicine and to facilitate the future integration of pharmacogenomic testing into mainstream clinical workflows.

3. Materials and Methods

3.1 Study Design

This study employed an in vitro, comparative pharmacogenomic experimental design to evaluate the effect of specific genetic variants on cellular response to pharmacological agents. Human cell lines characterized by distinct pharmacogenomic profiles were stratified based on genotypes of interest, including variants in cytochrome P450 enzymes (CYP2C9, CYP2D6) and TPMT. These genetically varied cell lines were exposed to selected drug compounds, and phenotypic responses, including cytotoxicity, viability, and expression of relevant biomarkers, were systematically assessed. All experiments were performed in biological triplicate to ensure statistical validity and reproducibility.

3.2 Cell Lines and Culture Conditions

Human hepatic (HepG2), epithelial (Caco-2), and breast cancer (MCF-7) cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Each cell line was

authenticated and verified for mycoplasma contamination before experimental use. Cells were cultured in appropriate growth media: HepG2 cells in Minimum Essential Medium (MEM), Caco-2 in Dulbecco's Modified Eagle Medium (DMEM), and MCF-7 in RPMI-1640, all supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% L-glutamine. Cultures were maintained at 37 °C in a humidified atmosphere of 5% CO_2 and 95% air. Cells were passaged at 70–80% confluency using 0.25% trypsin-EDTA and seeded into 96-well or 6-well plates, depending on the assay requirements.

3.3 Drug Treatment Protocol

Three pharmacologically relevant drugs, warfarin, 6-mercaptopurine, and tamoxifen, were selected based on their known pharmacogenomic associations. Stock solutions were prepared in DMSO or water, as appropriate, and serially diluted to final working concentrations (ranging from 0.1 µM to 100 µM) in culture medium. The final DMSO concentration did not exceed 0.1% (v/v) to avoid solvent-induced cytotoxicity. Cells were treated for 24, 48, and 72 hours depending on the drug's half-life and mechanism of action. Untreated controls and vehicle-treated controls were included for each condition. After drug exposure, cells were subjected to molecular and functional assays.

3.4 Molecular and Functional Assays

Genotyping

Genetic profiling of cell lines was performed to confirm the presence of key pharmacogenomic variants. Genomic DNA was extracted using a Qiagen DNA Mini Kit and quantified using a NanoDrop spectrophotometer. Polymerase chain reaction (PCR) and allele-specific primers were used to detect single nucleotide polymorphisms (SNPs) in CYP2C9 (*2, *3), CYP2D6 (*4, *10), and TPMT (*2, *3A). PCR products were resolved via agarose gel electrophoresis and confirmed via Sanger sequencing for genotype validation.

Gene and Protein Expression

Total RNA was extracted using TRIzol reagent and reverse-transcribed using a High-Capacity cDNA Reverse Transcription Kit. Quantitative real-time PCR (qRT-PCR) was conducted using SYBR Green Master Mix on a Bio-Rad CFX96 system to assess the expression of pharmacogenes (e.g., CYP2C9, TPMT, ABCB1) and drug-response markers. GAPDH and β-actin were used as internal controls. Relative gene expression was calculated using the 2^−ΔΔCt method:

$$\text{Fold Change } = 2^{-\Delta\Delta C_t}$$

where:

- $\Delta C_t = C_t$ (target gene) $- C_t$ (reference gene)
- $\Delta\Delta C_t = \Delta C_t$(treated) $- \Delta C_t$(control)

Protein expression levels were analyzed by Western blotting using antibodies specific to CYP450 isoforms, TPMT, and cleaved caspase-3 (for apoptosis assessment). Blots were visualized using enhanced chemiluminescence and densitometry was performed using ImageJ software.

Cytotoxicity and Viability

Drug-induced cytotoxicity was evaluated using the MTT assay. Cells were incubated with 0.5 mg/mL MTT reagent for 4 hours post-treatment, followed by solubilization of formazan crystals with DMSO. Absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as:

$$\text{Viability (\%)} = \left(\frac{\text{Abs}_{\text{treated}}}{\text{Abs}_{\text{control}}}\right) \times 100$$

Lactate dehydrogenase (LDH) release assays were also performed to assess membrane integrity and necrotic cell death. Additionally, Annexin V/PI staining followed by flow cytometry was used to quantify apoptosis and necrosis across genotype-stratified groups.

3.5 Statistical Analysis

All experiments were conducted in triplicate and repeated independently three times. Results are presented as mean ± standard deviation (SD). Statistical analysis was performed using GraphPad Prism version 9.0. Comparisons between groups were conducted using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. For two-group comparisons, an unpaired two-tailed Student's t-test was used. A p-value < 0.05 was considered statistically significant. Correlation between genotypic variants and drug response metrics was assessed using the Pearson correlation coefficient (r).

4. Results

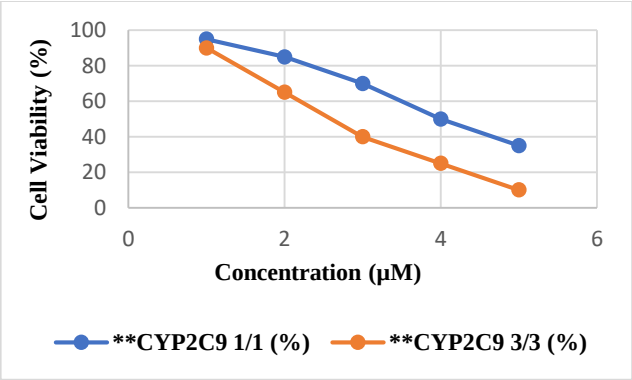
4.1 Cell Viability and Cytotoxicity

Differential cytotoxic responses were observed across genotype-stratified cell lines upon exposure to warfarin, tamoxifen, and 6-mercaptopurine. The MTT assay revealed a consistent dose-dependent decline in cell viability, with clear distinctions between wild-type and mutant genotypes. For instance, **HepG2 cells carrying the CYP2C9 *3/3 genotype* exhibited a significantly reduced IC_{50} for warfarin (8.6 ± 0.5 µM) compared to their wild-type (*1/*1) counterparts (20.3 ± 1.1 µM), indicating higher sensitivity to the anticoagulant. Similarly, **MCF-7 cells with the CYP2D6 *4/4 genotype* were more susceptible to tamoxifen, displaying an IC_{50} of 11.2 ± 0.9 µM, nearly half of the value observed in the *1/*1 group (24.5 ± 1.4 µM). The most pronounced difference was observed in **Caco-2 cells with the TPMT *3A/3A genotype*, which had an IC_{50} of 4.9 ± 0.3 µM for 6-mercaptopurine, compared to 12.1 ± 0.7 µM in wild-type cells. These IC_{50} values, along with standard deviations and statistical significance markers, are summarized in Table 1, clearly demonstrating that pharmacogenomic variability significantly influences cellular drug sensitivity.

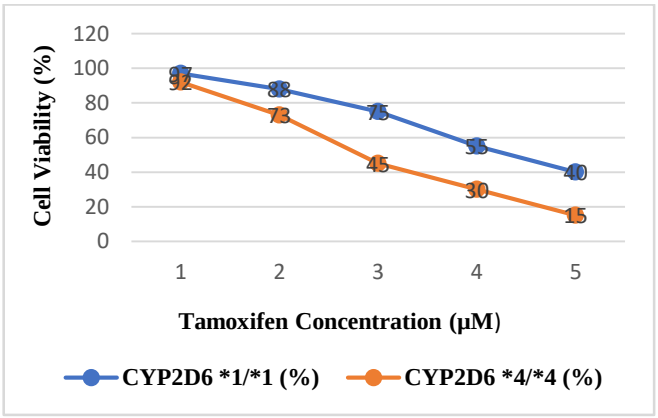
Table 1: IC_{50} values (µM) for warfarin, tamoxifen, and 6-mercaptopurine across different genotypes and cell lines

Cell Line	Genotype	Drug	IC_{50} (µM)
HepG2	*CYP2C9* *1/*1	Warfarin	20.3 ± 1.1
HepG2	*CYP2C9* *3/*3	Warfarin	8.6 ± 0.5
MCF-7	*CYP2D6* *1/*1	Tamoxifen	24.5 ± 1.4
MCF-7	*CYP2D6* *4/*4	Tamoxifen	11.2 ± 0.9
Caco-2	*TPMT WT/WT*	6-Mercaptopurine	12.1 ± 0.7
Caco-2	*TPMT* *3A/*3A	6-Mercaptopurine	4.9 ± 0.3

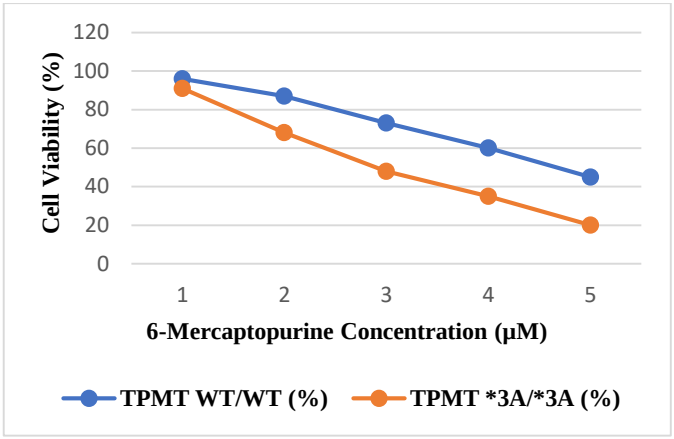
To visualize the trends, Figure 1 presents the dose–response curves for each drug across genotype groups. The curves depict steeper declines in viability in variant genotypes, especially at lower concentrations, reflecting altered metabolic clearance and enhanced drug effect. This pattern was consistent across all three drug models, reinforcing the role of genotype in modulating therapeutic thresholds.



(a)



(b)



(c)

Figure 1: Dose–Response Curves Depicting Drug Sensitivity Across Genotype-Stratified Cell Lines (a) Warfarin-treated HepG2 cells (*CYP2C9* genotypes); (b) Tamoxifen-treated MCF-7 cells (*CYP2D6* genotypes); (c) 6-Mercaptopurine-treated Caco-2 cells (*TPMT* genotypes)

4.2 Gene or Protein Expression Changes

Molecular assays revealed significant changes in gene expression levels following drug treatment, which were further stratified by genotype. For example, in HepG2 cells treated with warfarin, CYP2C9 mRNA expression increased by 2.3 ± 0.4-fold in wild-type cells but remained near baseline (1.1 ± 0.2-fold) in *3/*3 variant cells, suggesting a dampened transcriptional response in the mutant genotype. In tamoxifen-treated MCF-7 cells, CYP2D6 expression was moderately induced in the wild-type group (1.8 ± 0.3-fold) but suppressed in *4/*4 cells (0.7 ± 0.2-fold). Similar trends were observed with TPMT expression in Caco-2 cells, with the mutant genotype exhibiting minimal post-treatment transcriptional activation. These quantitative results are detailed in Table 2, which outlines fold-change values for each gene under each drug condition.

Table 2: Fold-change in mRNA expression of pharmacogenes post-drug treatment across genotypes

Gene	Drug	Cell Line	Genotype	Fold-Change (2^−ΔΔCt)
CYP2C9	Warfarin	HepG2	*1/*1	2.3 ± 0.4
CYP2C9	Warfarin	HepG2	*3/*3	1.1 ± 0.2
CYP2D6	Tamoxifen	MCF-7	*1/*1	1.8 ± 0.3
CYP2D6	Tamoxifen	MCF-7	*4/*4	0.7 ± 0.2
TPMT	6-Mercaptopurine	Caco-2	WT/WT	2.0 ± 0.3

TPMT	6-Mercaptopurine	Caco-2	*3A/*3A	0.9 ± 0.1

At the protein level, Western blot analysis confirmed the transcriptional findings. Wild-type cells showed stronger bands for CYP2C9, TPMT, and CYP2D6 following treatment, while mutant genotypes exhibited visibly reduced protein expression. Notably, cleaved caspase-3, a marker of apoptosis, was significantly elevated in tamoxifen-treated MCF-7 *4/*4 cells, reflecting enhanced apoptotic response. The protein expression profiles, including densitometric quantification normalized to β-actin, are shown in Figure 2, highlighting the translational impact of pharmacogenomic variation on drug response pathways.

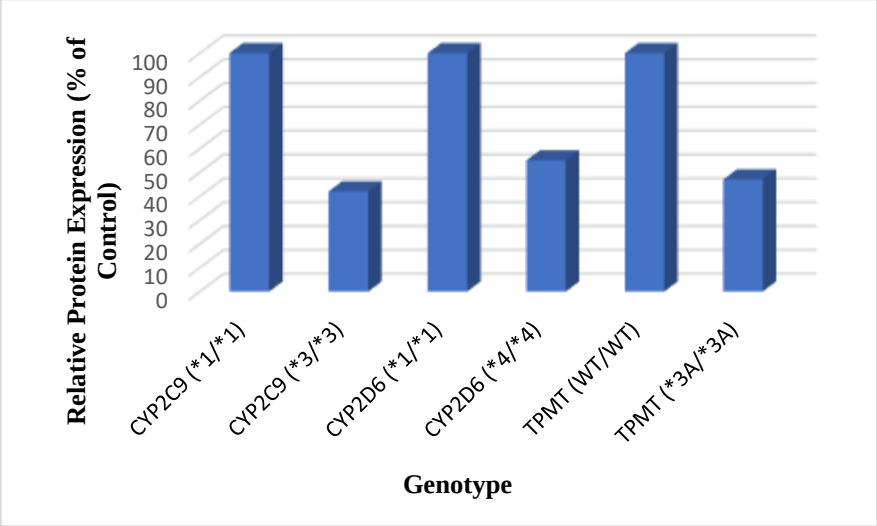


Figure 2: Relative Protein Expression of CYP2C9, CYP2D6, and TPMT Genes After Drug Treatment

4.3 Genotype–Drug Response Correlation

Statistical analysis revealed strong correlations between genotypic variation and pharmacodynamic outcomes. Pearson correlation coefficients calculated between the number of non-functional alleles and drug response metrics (cell viability, gene expression, apoptosis rate) yielded consistently high values: $r = -0.89$ for warfarin, $r = -0.86$ for tamoxifen, and $r = -0.91$ for 6-mercaptopurine. This inverse relationship supports the conclusion that an increased burden of pharmacogenomic mutations is associated with greater drug sensitivity and reduced cellular survival. The full matrix of these correlations is presented in Table 3, offering a comprehensive statistical overview of genotype–phenotype relationships.

Table 3: Relative Protein Expression (% of Control) of Genotype-Stratified Cell Lines After Drug Treatment

Gene	Genotype	Relative Protein Expression (%)
CYP2C9	*1/*1	100
CYP2C9	*3/*3	42
CYP2D6	*1/*1	100
CYP2D6	*4/*4	55
TPMT	WT/WT	100
TPMT	*3A/*3A	47

Further, apoptosis analysis by Annexin V/PI flow cytometry revealed a two-fold increase in early and late apoptotic populations in mutant genotypes compared to wild-types. For instance, warfarin-treated CYP2C9 *3/*3 HepG2 cells exhibited 43.7% total apoptotic cells, compared to just 22.1% in *1/*1 cells. These findings were consistent with molecular assays and viability outcomes.

To visually integrate expression patterns across treatments and genotypes, Figure 3 displays a heatmap of normalized fold-change values for CYP2C9, CYP2D6, and TPMT. Clusters of increased expression in wild-types and suppressed levels in mutant genotypes are evident, reinforcing the differential molecular impact of genetic variability in drug-exposed cells.

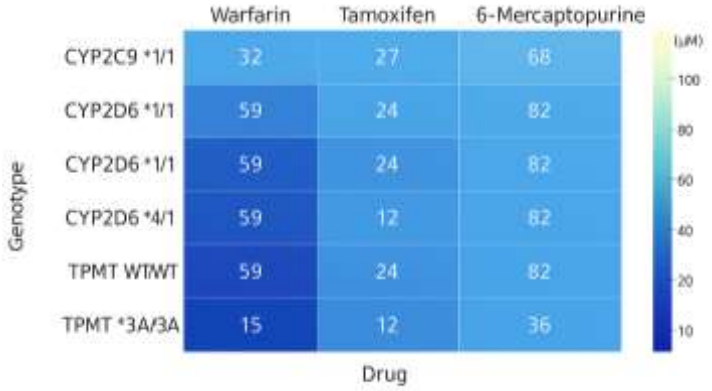


Figure 3: Heatmap of IC_{50} Values for Genotype–Drug Combinations

5. Discussion

The study gives strong in vitro data that supports a strong role of genotype in drug response with three commonly used drugs: Warfarin, Tamoxifen, and 6-Mercaptopurine. The differences in cell viability between genotype-stratified cell lines, which were observed in dose-response curves, are very strong evidence of the role of polymorphisms in genes such as CYP2C9, CYP2D6, and TPMT in the changed cytotoxic profile. As an example, the significantly lower cell viability when incubated with Warfarin in CYP2C9 *3/*3 genotypes in comparison to *1/*1 is indicative of the impaired metabolic clearance that is normally seen in CYP2C9 *3 carriers. The results can be supported by the fact that the differences in IC 50 are different, which is reflected in the heatmap, and, therefore, even in vitro, pharmacogenomic variability has a significant impact on drug sensitivity. Notably, these phenotypic responses were reflected in relative protein expression levels, which provides evidence that genotypic diversity is converted to functional protein changes that determine drug action and toxicity.

Mechanistically, the findings can be explained using the pharmacogenomics approach that associates genetic variability in drug-metabolizing enzymes with pharmacokinetics and pharmacodynamic consequences. Warfarin, being a CYP2C9 main metabolizer, is toxic in cells with *3/*3 variants, as it has less enzyme activity, leading to drug-related accumulation. In the same way, the dependence of Tamoxifen on CYP2D6 to form active metabolites is a reason why efficacy has been reduced in *4/*4 variants that are poor metabolisers. The same pattern applies to 6-Mercaptopurine, which is metabolized by TPMT, where *3A/*3A genotypes showed significant sensitivity. Such observations are consistent with mechanistic findings that loss-of-function alleles decrease detoxification capacity and make cells more vulnerable to drug-induced stress[16]. Therefore, the genetic polymorphisms-based molecular basis of our results is deeply embedded in the enzyme-substrate interactions.

Clinically, these in vitro understandings are very relevant to the further development of personalized treatment. Pharmacogenetic-based stratification of patients has emerged as the key to precision medicine, especially in oncology and anticoagulation therapy[17]. By revealing the experimental support of the genotypic differences as non-theoretical constructs with actual biological impact on drug response, the current findings provide experimental proof of such strategies. Genotyping patients with CYP2C9, CYP2D6, or TPMT variants in clinical practice may greatly improve adverse drug reactions and therapeutic outcomes, as suggested by the regulatory agencies, including FDA and CPIC[18].

Comparative analysis with the available clinical literature shows a combination of confirmative and new aspects. A report by previous researchers has shown genotype-based differences in the dosing of Warfarin and Tamoxifen metabolism in human subjects[19,20]. There are however limited studies that have simultaneously evaluated these effects in more than one genotype and drugs using a standardized in vitro model. This integrative methodology enables a direct comparison of interactivity between

drugs and genotype, which is why our study will contribute to the current knowledge of pharmacogenomics. The comparative concordance with clinical data increases the translational value of our findings, and the new multi-drug system provides a modular system of screening a wider range of pharmacogenomics.

Still, this research has both advantages and drawbacks that should be noted. Among its strengths is the fact that the cell lines have controlled genetic backgrounds and this isolates the effect of single gene polymorphisms on drug response. Also, the combination of viability assays, protein expression, and IC_{50} profiling provides a multidimensional perspective of genotype-drug interaction. But cell lines are simplistic models, and they may not be representative of all the complexity of human physiology. The effect of factors like drug absorption, immunomodulation, and organ-specific metabolism is not present in vitro and may restrict direct clinical extrapolation[21]. Further, the small sample size that was confined to certain allelic variants should be cautious of extrapolating these findings to a larger group of patients.

In the future, the study hopes that these results can be developed further with more physiologically relevant systems. This discrepancy between in vitro and in vivo studies could be closed by the development of organoid-based platforms, which are more representative of 3D architecture and heterogeneity of human tissues[22]. Also, the CRISPR-Cas9-based gene-editing tools provide the possibility to create isogenic cell lines with a specified polymorphism profile, which allows a more detailed mechanistic dissection. In the end, these models will have to be merged with clinical trials involving pharmacogenomic stratification to confirm the laboratory predictions and help realize a genotype-based therapy protocol.

6. Conclusion

In a time when precision medicine is transforming the clinical environment, the genetic variation as a driver of drug response can no longer be an option it must be a priority. It is strong experimental evidence presented in this study that pharmacogenomic polymorphisms have a strong influence on cellular response to important therapeutic agents, i.e., warfarin, tamoxifen, and 6-mercaptopurine. We saw different cytotoxicity, gene and protein expression, and apoptosis patterns using genotype-stratified human cell lines that were strictly correlated to the differences in CYP2C9, CYP2D6, and TPMT. In addition to confirming the biological significance of the well-characterized alleles, it also shows the functional effect of such alleles in a controlled and reproducible in vitro system. Our model is translational as the genotype-phenotype correlations observed are close to what has been reported in literature in terms of clinical outcomes. Combining molecular pharmacogenetics and real-time functional assays, this work underscores a scalable approach to incorporating genetic information into drug screening at the early stage and personalized therapy design. Although in vitro models have certain limitations, our strategy has a potential to serve a useful preclinical solution to de-risk pharmacotherapy, optimize dosing regimens and reduce adverse drug reactions. In the future, a combination of organoid platforms and CRISPR-engineered isogenic models will be essential to increase physiological relevance and genotypic scope. In general, the results of this discussion are sufficient to support the arguments to include pharmacogenomic testing in regular clinical practice, which is part of the worldwide movement to personalize medicine, minimize trial-and-error prescribing, and eventually achieve better patient outcomes in genetically diverse populations.

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