

Role of DPYD and MTHFR Gene Polymorphism on 5-Fluorouracil Pharmacokinetics and Treatment-Related Toxicity in Breast Cancer Patients: A Step Towards Tailored Medicine

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Research Article

Keywords: Breast Cancer, Pharmacogenomics, Pharmacokinetics, DPYD, MTHFR, 5-Fluorouracil, Toxicity, Personalized Medicine

Posted Date: October 4th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11038770/v1>

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Additional Declarations: No competing interests reported.

Abstract

Background

The cytotoxic drug fluorouracil (5-FU) has long been used to treat breast cancers. This study aims to investigate the association of clinical and pharmacogenetic factors with the pharmacokinetic profile of 5-FU, drug exposure and toxicity profile in breast cancer patients.

Methodology:

The 200 breast cancer patients taking 5-FU infusion were taken from the Nishtar Hospital's Oncology Department, Multan. Mann-Whitney U tests and multivariate linear regression models were used to evaluate the effect of *DPYD* and *MTHFR* gene polymorphism on the pharmacokinetics of 5-FU and drug-induced toxicity.

Results

Participants who grew up in an urban area had significantly higher AUC_{0-t} (195.08 $\mu\text{g}\cdot\text{h}/\text{mL}$ vs. 43.17 $\mu\text{g}\cdot\text{h}/\text{mL}$; $p < 0.001$). Genetic variants are significantly associated with pharmacokinetic parameters. The pharmacokinetic evaluation revealed a mean half-life of 0.25 hours, a clearance rate of 26.37 $\text{mg}/(\mu\text{g}/\text{mL})/\text{h}$, a C_{max} of 28.08 $\mu\text{g}/\text{mL}$, and an AUC_{0-t} of 55.33 $\mu\text{g}/\text{mL}\cdot\text{h}$. Body mass index showed significant associations with T_{max} , C_{max} , and AUC_{0-t} ($p < 0.05$). Gastrointestinal toxicity, nausea, vomiting, and anemia were associated with genotype-specific dose variability.

Conclusion

These findings provide strong data-driven evidence to support the integration of clinical characteristics and genetic profiling into personalized dosing and monitoring strategies to optimize efficacy while minimizing adverse effects in breast cancer management.

INTRODUCTION

Breast cancer, the most commonly diagnosed malignancy worldwide, approximately 2.3 million women are diagnosed with breast cancer each year, and continues to be a major cause of cancer-related death for women. The death rate is disproportionately high in areas lacking structured screening programs (Sung et al., 2021). With age-standardized rates of 34.6 per 100,000 women, almost twice the world norm, and more than 70% of cases identified at advanced stages, Pakistan has an unacceptably high prevalence of breast cancer (Raza et al., 2020). Due to a lack of targeted medicines, financial hurdles, and a limited medical facility, chemotherapy is a crucial strategy for this delayed presentation. 5-fluorouracil (5-FU), a pyrimidine derivative analog that inhibits thymidylate synthase (TYMS) to impair DNA synthesis, is still a mainstay of chemotherapy regimens for breast cancer, especially when combined with anthracyclines and taxanes (García-Alfonso et al., 2022). Its limited therapeutic index, however, limits its clinical usefulness: up to 30% of patients have significant toxicity (such as myelosuppression, gastrointestinal mucositis, or hand-foot syndrome), which frequently calls for dosage adjustments or therapy cessation (Deenen et al., 2021). The necessity of pharmacogenomic-guided precision dosage is highlighted by the substantial correlation between these adverse effects and inter-individual variability in 5-FU pharmacokinetics, which is mostly caused by polymorphisms in genes controlling drug metabolism and folate pathways (Amstutz & Offer, 2023).

Pharmacogenomic apprehensions (discoveries) have revolutionized oncology by providing customized dose methods that reduce toxicity while maintaining efficacy. Dihydropyrimidine dehydrogenase (DPD), the rate-limiting enzyme encoded by *DPYD*, is essential to 5-FU metabolism because it catabolizes more than 80% of given 5-FU into inactive metabolites (Lunenburg et al., 2020). Clinically actionable variants that cause partial or complete DPD deficiency include rs3918290 G > A (*DPYD**2A), a splice-site mutation that causes exon 14 deletion, and rs1801160 C > T (*DPYD**13), a missense variant that impairs enzyme stability. These variants can cause life-threatening toxicity and long-term widespread susceptibility (Meulendijks et al., 2020). Heterozygous *DPYD*2A carriers, for instance, show a 50–70% decrease in enzyme activity, which is associated with a 4-fold higher risk of grade ≥ 3 adverse outcomes and a 10% mortality rate in extreme cases (Del Re et al., 2023). In order to personalize fluoropyrimidine dosage, international consortia, such as the Clinical Pharmacogenetics Implementation Consortium (CPIC), now support preemptive *DPYD* genotyping. This approach has been demonstrated to lower toxicity-related morbidity by 30% in European populations. (Caudle et al., 2020).

Simultaneously, the *MTHFR* gene encodes methylenetetrahydrofolate reductase (MTHFR), which modulates folate metabolism, a route essential to 5-FU's mode of action. Nucleotide synthesis and methylation activities depend on the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is catalyzed by the MTHFR enzyme. The regulatory domain of *MTHFR* consists of the rs1801131 T > G (A1298C) polymorphism, which decreases enzyme activity by 30–40%. This changes intracellular folate pools and may compromise the anticancer efficacy of 5-FU or increase toxicity by impairing DNA repair (Kawakami et al., 2021). Data from South Asian cohorts, where allele frequencies surpass 20% and dietary folate intake is below optimum, remain sparse, despite meta-analyses in East Asian and Caucasian populations indicating that *MTHFR*1298C carriers have greater rates of gastrointestinal toxicity (Yang et al., 2021). Although there is evidence that genetic variation and environmental factors (such as consanguinity and malnutrition) may increase the clinical impact of these variants, this gap is especially evident in Pakistan, a region with a high prevalence of breast cancer but underrepresentation in pharmacogenomic studies.

The ultimate objective is to assist in advancing the development of tailored medicine approaches to the treatment of breast cancer, which may enhance therapeutic results and lessen adverse drug effects. In our study, we examine how 5-FU pharmacokinetics and toxicity are affected by the *DPYD* rs3918290 G

> A, rs1801160 C > T, and *MTHFR* rs1801131 T > G polymorphisms in a prospective cohort of patients with breast cancer from Southern Punjab, Pakistan. We correlate genetic variants with drug bioavailability, clearance rates, and treatment-related adverse events graded according to CTCAE v5.0 criteria using high-performance liquid chromatography (HPLC) for pharmacokinetic profiling of 5-FU and its metabolites. The findings of our study provide a foundation to enhance precision oncology in underprivileged populations by establishing population-specific biomarkers for 5-FU dose and highlighting the interaction between genetic susceptibility and spatial epidemiological determinants. With the potential to lower morbidity, medical expenses, and gaps in cancer outcomes, the present study proposes the integration of genotype-guided chemotherapy by addressing a critical gap in South Asian pharmacogenomic data.

This study aims to explore the relation of clinical and pharmacogenetic factors, particularly *DPYD* and *MTHFR* genes polymorphism, with the pharmacokinetic profile of 5-fluorouracil (5-FU) in breast cancer patients. Moreover, elucidate genotype-dependent pharmacokinetic variability, drug exposure and toxicity patterns to support the pharmacogenomic profiling in clinical settings to enhance dose management, optimize therapeutic outcomes and personalized dosing.

MATERIALS AND METHODS

STUDY APPROVAL

The study was approved by the Institutional Review Boards of the Institute of Molecular Biology and Biotechnology at Bahauddin Zakariya University, Multan, and the Oncology Department at Nishtar Medical University, Multan. The breast cancer patients taking 5-Fluorouracil infusion were asked to provide a sample voluntarily after informed consent. Individuals suffering from any other type of cancer were excluded from this study. The data of 200 breast cancer patients, along with whole blood, were taken from the Nishtar Hospital's Oncology Department, Multan.

CHEMOTHERAPY REGIMEN AND DOSE

This study includes patients receiving FAC regimen. Every 21 days, each cycle was repeated. On the first day of each cycle, 750 mg/m² of 5-Fu was infused intravenously. Depending on the patient's tolerability and the tumor response, each patient received a different number of cycles.

SAMPLE COLLECTION

The participants of the study were requested to provide information as per the consent form and the questionnaire at the Oncology Department of Nishtar Medical Hospital, Multan. The information about their sex, age, occupation, family history, disease stage, biophysical traits, and toxic profile (like gastrointestinal toxicity, anemia, hair loss, nausea, vomiting, and pain) was collected from patients under the supervision of an oncologist. The venous blood samples of patients were collected during the administration of the drug at three-time intervals. The average dose given to the patients was 750mg. Blood was collected from patients at three different time intervals based on the infusion rate of the drug.

- The first sample was taken after passing the half-life of the 5-FU drug i.e approximately within 15–20 minutes
- The second sample was taken at Constant Steady State Concentration (CPSS) level.
- The third sample was taken at the excretion time of the drug from the body in some cases.
- The last sample was taken post-infusion after 15–20 minutes of the infusion of the 5-FU.

Blood samples were collected during infusion and processed promptly. To reduce the degradation of the 5-Fu, the plasma was separated within a short time and kept at low temperatures until analysis.

Extracting genomic DNA from blood

The obtained blood samples from patients were kept at 4°C until further processing, and genomic DNA was extracted using the organic extraction method (Barker 1998) (Bartlett & White 2003, Sheikh et al 2005). The extraction steps were performed using strict quality control measures. The quality and quantity of extracted DNA were estimated using horizontal gel electrophoresis and nanodrop.

Amplification and genotyping using TETRA-ARMS PCR

UCSC In-Silico PCR (<http://genome.ucsc.edu>) was used to verify the specificity of the tetra arm primers, which were generated using the PRIMER1 tool (<http://primer1.soton.ac.uk/primer1.html>). (Ye et al. 2012). (Ahlawat et al. 2014). The targeted DNA in the *MTHFR* and *DPYD* genes was amplified using the TETRA-ARMS-PCR method. (De Oliveira and Medrano, 2014). A Bio-Rad thermal cycler was used to amplify each SNP. The sequences of primers and conditions for PCR are provided in the Supplementary-Tables 1, 2, and 3, respectively

Analysis of 5-Fluorouracil in plasma samples

The analysis of drug in the patient's blood samples was performed using high-performance liquid chromatography (HPLC) using protocols developed by Hanif et al. in 2018 (Haneef et al. 2018)(Padya et al. 2022).

Statistical analysis

Allele frequencies were analyzed to measure the Hardy-Weinberg Equilibrium. The "PK Solver" add-in of MS Excel was used for pharmacokinetic analysis, including AUC (Area under Curve), MRT (Mean Residence Time), Maximum time (Tmax) to absorb maximum amount of drug, Maximum concentration absorbed Cmax, Half-life (t1/2), etc. The Mann-Whitney U test was used to compare the pharmacokinetic variables along with different demographic, clinical, and genetic factors. The Multivariate Linear Regression Model (MLRM) was used to model the pharmacokinetic variables for different clinical and genetic factors. The Omnibus test was used to evaluate the goodness of the fit of the model for predicting the response of the drug.

Table 1
Frequency Distribution of Patients Risk and Clinical Factors

Variables	Categories	N (%)
Childhood Residence	Rural	184 (92%)
	Urban	16 (8%)
Use of Birth Control Pill	No	72 (36%)
	Yes	128 (64%)
No of Children	<=2	56 (28%)
	3–4	96 (48%)
	>=5	48 (24%)
Agricultural Pesticide Exposure	Yes	164 (82%)
	No	36 (18%)
Family History of Disease	No	80 (40%)
	Yes	120 (60%)
Chemotherapy Cycle	1st	44 (22%)
	2nd	76 (38%)
	3rd	76 (38%)
	4th	4 (2%)
Breast Cancer Stage	2.00	100 (50%)
	3.00	100 (50%)
MTHFR (rs1801131) T > G	GG	52 (26%)
	GT	116 (58%)
	TT	32 (16%)
DPYD (rs3918290) G > A	GA	18 (9%)
	GG	182 (91%)
DPYD (rs1801160) C > T	CC	188 (94%)
	CT	12 (6%)

RESULTS

Patient Demographics and Clinical Characteristics

The results of demographic and clinical parameters were shown in Table 1, while the descriptive analysis of continuous variables (such as age, dosage, and pharmacokinetics) was shown in Table 2. The majority of patients enrolled in this this were found to be at the advanced stages of cancer. 200 patients enrolled in the cohort were allocated equally between stage II and stage III breast cancer patients. Participants had an average body mass index (BMI) of 24.04 (SD ± 3.82), a mean body surface area (BSA) of 1.52 m² (SD ± 0.09), and an average age of 43.82 years (SD ± 7.21). According to sociodemographic data, 86% of the patients reported working on farms, and 92% of them had grown up in a rural area. Sixty percent of patients reported having a family history of breast cancer, and sixty-four percent of participants admitted to using birth control pills or drinking alcohol. There was variation in reproductive history, with 24% having five or more children and 48% having three to four. Crucially, a large number of these clinical and demographic characteristics demonstrated strong correlations with 5-fluorouracil's (5-FU) pharmacokinetic behavior. For instance, patients who grew up in an urban area had significantly higher AUC_{0-t} (195.08 µg•h/mL, SD ± 150.44 vs. 43.17 µg•h/mL, SD ± 35.27; p < 0.001), lower clearance (12.17 mg•h/µg/mL, SD ± 13.63 vs. 27.6 mg•h/µg/mL, SD ± 28.0; p < 0.001), and Cmax (89.06 µg/mL, SD ± 67.53 vs. 22.78 µg/mL, SD ± 18.82; p < 0.001). With significantly higher AUC_{0-t} (128.3 µg•h/mL, SD ± 130.53 vs. 39.31 µg•h/mL, SD ± 21.69; p = 0.001), lower clearance (17.47 mg•h/µg/mL, SD ± 15.76 vs. 28.32 mg•h/µg/mL, SD ± 29.06; p = 0.003), and shorter MRT (0.31 h, SD ± 0.09 vs. 0.37 h, SD ± 0.16; p = 0.005).

Table 2
Deceptive Analysis of Patients clinical and Pharmacokinetic factors

Variables	Mean (SD)	Median (Q3-Q1)
Age	43.82 (7.21)	44.5 (50 – 40)
BMI	24.04 (3.82)	23.8 (25-21.9)
BSA (in m ²)	1.52 (0.09)	1.5 (1.5–1.5)
Got Married at Age	26.48 (5.86)	27 (30 – 22)
Dose (in mg)	769 (63.32)	750 (750–750)
t _{1/2} (h)	0.25 (0.1)	0.25 (0.31 – 0.19)
V (mg)/(μg/ml)	11.09 (25.06)	6.28 (10.24–4.14)
CL (mg)/(μg/ml)/h	26.37 (27.44)	18.77 (31.61–11.66)
T _{max} (h)	2.06 (0.26)	2 (2–2)
C _{max} (μg/ml)	28.08 (31.53)	19.16 (31.85–11.86)
AUC 0-t (μg/ml*h)	55.33 (67.5)	39.41 (57.91–22.7)
MRT (h)	0.36 (0.15)	0.36 (0.44 – 0.27)

Distribution of DPYD and MTHFR Variants by Genotype

Three single nucleotide polymorphisms (SNPs) that are pharmacogenetically significant were assessed in this study: *MTHFR* rs1801131 (T > G), *DPYD* rs1801160 (C > T), and *DPYD* rs3918290 (G > A). Only 6% of the patients had heterozygous CT, according to genotypic analysis, whereas 94% of the patients had the wild-type CC genotype for *DPYD* rs1801160. 91% of individuals with *DPYD* rs3918290 were GG heterozygotes, while 9% were GA heterozygotes. The diversity of *MTHFR* rs1801131, on the other hand, was higher: 58% of participants were GT, 26% GG, and 16% TT. Figures 1–3 display the results of the PCR-based genotyping, which exhibit distinct banding patterns that match the anticipated amplicon sizes.

5-FU's Pharmacokinetic Properties

PK Solver was used to calculate the parameters for the HPLC-based plasma analysis used for the pharmacokinetic profile of 5-FU. The mean peak plasma concentration (C_{max}) was 28.08 μg/mL (SD ± 31.5), and the mean AUC_{0–t} was 55.33 μg·h/mL (SD ± 67.5). C_{max} (T_{max}) took an average of 2.06 hours (SD ± 0.26) to reach. Significant inter-individual variability was seen in the clearance (CL), which had a wide range with a mean value of 26.37 mg·h/μg/mL (SD ± 27.44) and the mean residence time (MRT) of 0.36 hours (SD ± 0.15). Both genetic and demographic factors have a major impact on these PK disparities.

Relationships between 5-FU Pharmacokinetics and Genotypes

Using the Mann–Whitney U test, a non-parametric technique appropriate for comparing two independent groups when the data are not normally distributed, the impact of individual genotypes on PK parameters was statistically evaluated. AUC_{0–t} (121.01 μg·h/mL, SD ± 126.97; p = 0.174) and C_{max} (62.46 μg /mL, SD ± 63.96; p = 0.174) were significantly higher in carriers of the *DPYD* rs1801160 CT genotype, and their MRT was shorter (0.28 h, SD ± 0.13 vs. 0.37 h, SD ± 0.15; p = 0.036) than those with the CC genotype. Likewise, *DPYD* rs3918290 GA carriers showed changed drug kinetics, such as shorter MRT (0.32 h, SD ± 0.09 vs. 0.37 h, SD ± 0.15; p = 0.212) and decreased clearance (15.8 mg·h/μg/mL, SD ± 7.12 vs. 27.41 mg·h/μg/mL, SD ± 28.47; p = 0.019). When compared to GG homozygotes, GT carriers of *MTHFR* rs1801131 exhibited longer T_{max} (2.09 h, SD ± 0.27 vs. 2.02 h, SD ± 0.3; p = 0.033) and increased AUC_{0–t} (67.33 μg·h/mL, SD ± 85.24 vs. 37.53 μg·h/mL, SD ± 17.62; p = 0.377). These findings may indicate that altered folate metabolism is a contributing factor to prolonged drug retention. Table 3 provides a summary of these variations.

Impact of Clinical and Demographic Factors on 5-FU Disposition:

5-FU metabolism was independently impacted by demographic variables. Urban-raised patients had substantially higher C_{max} and AUC_{0–t} (195.08 μg·h/mL, SD ± 150.44 vs. 43.17 μg·h/mL, SD ± 35.27; p < 0.001). In comparison to their rural counterparts, they had significantly lower clearance (12.17 mg·h/μg/mL, SD ± 13.63 vs. 27.6 mg·h/μg/mL, SD ± 28.0; p < 0.001) and increased clearance (89.06 μg/mL, SD ± 67.53 vs. 22.78 μg/mL, SD ± 18.82; p < 0.001). Furthermore, compared to women who were not exposed to pesticides showed shorter MRT (0.31 h, SD ± 0.09; p = 0.005), worse clearance (17.47 mg·h/μg/mL, SD ± 15.76; p = 0.003), and higher systemic exposure (AUC_{0–t} = 128.3 μg·h/mL, SD ± 130.53; p = 0.001). Drug disposition was also affected by cancer stage and chemotherapy cycle; for example, patients with stage III disease (T_{max} = 2.10 h, SD ± 0.32; p = 0.043) and those in later chemotherapy cycles (2nd cycle: T_{max} = 2.11 h, SD ± 0.31; p = 0.279) displayed delayed absorption and higher AUC. Drug clearance and MRT were negatively correlated with age and BMI, suggesting either an overall decline in metabolic capacity with aging or an increase in adiposity

Multidimensional Evaluation of Pharmacokinetic Predictors:

Multivariate linear regression model was used to further analyze the simultaneous contributions of several factors. By controlling for the presence of other predictors, this statistical method calculates the independent impact of each predictor on the result variable. To measure the influence of genetic polymorphisms, demographic characteristics, and clinical variables on PK endpoints including CL, T_{max}, C_{max}, and AUC_{0–t}, regression coefficients (B),

standard errors (SE), and p-values were computed (Table 4). Increased AUC_{0-t} ($B = 113.8$, $SE = 20.9$; $p < 0.001$) and C_{max} ($B = 47.35$, $SE = 9.99$; $p < 0.001$) were substantially correlated with urban upbringing. Higher AUC_{0-t} ($B = 42.7$, $SE = 11.2$; $p < 0.001$) and shorter MRT ($B = -0.10$, $SE = 0.04$; $p = 0.008$) were linked to being a housewife. Increased systemic exposure was independently predicted by later chemotherapy cycles (second cycle: $B = 0.22$, $SE = 0.04$; $p < 0.001$) and a higher cancer stage (Stage III: $B = 26.62$, $SE = 7.00$; $p < 0.001$).

Table 3
Variation in Pharmacokinetic Parameters due to Clinical and Genetic factors

Variables	Categories	Dose (in mg)	t1/2 (h)	V (mg)/(μg/ml)	CL (mg)/(μg/ml)/h	Tmax (h)	Cmax (μg/ml)	AUC 0-t (μg/ml*h)	MRT (h)
		Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Childhood Residence	Rural	761.96 (52.46)	0.25 (0.11)	11.68 (26)	27.6 (28)	2.04 (0.25)	22.78 (18.82)	43.17 (35.27)	0.37 (0.15)
	Urban	850 (109.54)	0.21 (0.05)	4.33 (5.25)	12.17 (13.63)	2.25 (0.26)	89.06 (67.53)	195.08 (150.44)	0.31 (0.08)
	P-value	0.000	0.044	0.000	0.000	0.000	0.000	0.000	0.044
Agricultural Pesticide Exposure	Yes	763.41 (55.41)	0.26 (0.11)	12.38 (27.44)	28.32 (29.06)	2.05 (0.27)	20.67 (11.3)	39.31 (21.69)	0.37 (0.16)
	No	794.44 (87.65)	0.21 (0.06)	5.19 (4.55)	17.47 (15.76)	2.11 (0.21)	61.8 (60.27)	128.3 (130.53)	0.31 (0.09)
	P-value	0.045	0.005	0.000	0.003	0.098	0.002	0.001	0.005
Family History of Disease	No	737.5 (31.32)	0.22 (0.07)	8.15 (5.97)	28.87 (26.87)	2.08 (0.18)	24.76 (29.76)	48.66 (58.95)	0.31 (0.11)
	Yes	790 (70.29)	0.27 (0.11)	13.05 (31.88)	24.7 (27.79)	2.05 (0.3)	30.29 (32.6)	59.77 (72.55)	0.4 (0.17)
	P-value	0.000	0.000	0.936	0.031	0.294	0.031	0.073	0.000
Chemotherapy Cycle	1st	740.91 (29.08)	0.29 (0.18)	21.21 (51.7)	29.69 (42.34)	2 (0.22)	32.1 (31.78)	60.08 (59.89)	0.41 (0.26)
	2nd	784.21 (65.43)	0.25 (0.07)	7.53 (4.54)	21.5 (13.58)	2.11 (0.31)	34.88 (42.45)	71.18 (94.39)	0.36 (0.1)
	3rd	771.05 (71.77)	0.23 (0.07)	8.84 (5.87)	29.16 (27.31)	2.05 (0.22)	19.75 (10.85)	38.38 (22.64)	0.34 (0.1)
	4th	750 (0)	0.24 (0)	10.24 (0)	29.43 (0)	2 (0)	12.7 (0)	23.77 (0)	0.35 (0)
	P-value	0.005	0.689	0.008	0.078	0.279	0.059	0.136	0.689
Breast Cancer Stage	2	776 (79.29)	0.26 (0.07)	8.69 (5.56)	25.44 (23.91)	2.02 (0.17)	21.26 (10.4)	39.37 (18.45)	0.38 (0.1)
	3	762 (40.9)	0.24 (0.13)	13.49 (34.92)	27.3 (30.65)	2.1 (0.32)	34.89 (42.39)	71.29 (91.13)	0.35 (0.19)
	P-value	0.345	0.000	0.058	0.571	0.043	0.740	0.469	0.000
MTHFR (rs1801131) T > G	GG	788.46 (77.09)	0.26 (0.06)	7.96 (3.1)	23.35 (11.44)	2.02 (0.3)	20.41 (10.16)	37.53 (17.62)	0.37 (0.09)
	GT	768.97 (59.58)	0.25 (0.12)	12.98 (32.48)	25.74 (28.46)	2.09 (0.27)	33.22 (39.64)	67.33 (85.24)	0.37 (0.18)
	TT	737.5 (33.6)	0.24 (0.08)	9.31 (8.24)	33.56 (39.27)	2 (0)	21.91 (12.15)	40.74 (22.33)	0.34 (0.12)
	P-value	0.002	0.072	0.185	0.639	0.033	0.650	0.377	0.072
DPYD (rs3918290) G > A	GA	805.56 (66.17)	0.22 (0.07)	5.04 (2.78)	15.8 (7.12)	2.11 (0.21)	27.26 (9.79)	55.51 (24.33)	0.32 (0.09)
	GG	765.38 (62.05)	0.25 (0.11)	11.69 (26.18)	27.41 (28.47)	2.05 (0.26)	28.16 (32.93)	55.31 (70.39)	0.37 (0.15)
	P-value	0.002	0.212	0.002	0.019	0.267	0.028	0.012	0.212
DPYD (rs1801160) C > T	CC	770.21 (65.13)	0.26 (0.1)	11.13 (25.68)	24.98 (23.29)	2.06 (0.27)	25.88 (27.15)	51.13 (60.05)	0.37 (0.15)
	CT	750 (0)	0.19 (0.09)	10.48 (11.98)	48.12 (62.05)	2 (0)	62.46 (63.96)	121.01 (126.97)	0.28 (0.13)
	P-value	0.394	0.036	0.344	0.174	0.393	0.174	0.174	0.036
Combination of Polymorphism of genes	GGGACC	750 (0)	0.31 (0)	5.05 (0)	11.19 (0)	2 (0)	33.13 (0)	59.85 (0)	0.45 (0)
	GGGGCC	790 (78.25)	0.26 (0.07)	8.08 (3.1)	23.84 (11.4)	2.02 (0.3)	19.9 (10.02)	36.64 (17.38)	0.37 (0.09)
	GT GGCC	750 (0)	0.2 (0)	13.81 (0)	47.29 (0)	1.5 (0)	9.11 (0)	12.9 (0)	0.29 (0)

GTGACC	821.43 (67.12)	0.21 (0.06)	5.28 (3.1)	17.11 (7.62)	2.14 (0.23)	25.57 (10.56)	53.77 (27.54)	0.31 (0.09)
GTGGCC	762.77 (57.24)	0.27 (0.13)	14.63 (35.84)	27.09 (30.61)	2.12 (0.26)	30.55 (36.51)	62.23 (81.13)	0.38 (0.19)
GTGGCT	750 (0)	0.13 (0)	0.46 (0)	2.56 (0)	2 (0)	146.67 (0)	289.06 (0)	0.18 (0)
TTGACC	750 (0)	0.21 (0)	3.34 (0)	11.27 (0)	2 (0)	33.24 (0)	63.33 (0)	0.3 (0)
TTGGCC	731.82 (39.48)	0.24 (0.08)	7.61 (5.72)	22.01 (11.61)	2 (0)	21.45 (9.36)	40.05 (17.49)	0.35 (0.12)
TTGGCT	750 (0)	0.23 (0.09)	15.48 (11.81)	70.9 (65.34)	2 (0)	20.36 (18.73)	36.98 (33.58)	0.33 (0.13)
P-value	0.000	0.012	0.000	0.001	0.000	0.002	0.000	0.012

p-values were calculated using Man-Witny U and Kruskal-Wilks Test

Table 4
Relation Between Pharmacokinetic Parameters with Demographic and Genetic Factors using Multivariate Linear Regression Model

Dependent Variable:		t1/2 (h)		V (mg)/(µg/ml)		CL (mg)/(µg/ml)/h		Tmax (h)		Cmax (µg/ml)		AUC 0-t (µg/ml*h)		MRT (h)	
Variables	Categories	B (SE)	P-Value	B (SE)	P-Value	B (SE)	P-Value	B (SE)	P-Value	B (SE)	P-Value	B (SE)	P-Value	B (SE)	P-Value
Constant		0.73 (0.16)	0.00	-14.3 (43.6)	0.74	-78.1 (44.6)	0.08	1.38 (0.34)	0.00	181 (34.9)	0.00	363.3 (72.9)	0.00	1.06 (0.23)	0
Age		0 (0)	0.20	-0.07 (0.36)	0.84	-0.46 (0.36)	0.21	0.01 (0)	0.04	0.56 (0.28)	0.05	0.94 (0.59)	0.12	0 (0)	0
BMI		0 (0)	0.06	-0.28 (0.64)	0.66	0.28 (0.66)	0.67	-0.02 (0)	0.00	-1.15 (0.52)	0.03	-2.74 (1.07)	0.01	-0.01 (0)	0
BSA (in m²)		-0.02 (0.16)	0.92	31.7 (42.4)	0.46	34.8 (43.4)	0.42	-0.35 (0.33)	0.28	112.3 (33.9)	0.00	-284 (70.8)	0.00	-0.02 (0.23)	0
Marital Age		0 (0)	0.90	0.72 (0.38)	0.06	0.99 (0.39)	0.01	0.01 (0)	0.00	-0.65 (0.31)	0.04	-0.83 (0.64)	0.20	0 (0)	0
Dose (in mg)		0 (0)	0.09	-0.02 (0.06)	0.75	0.05 (0.06)	0.45	0 (0)	0.02	0.04 (0.05)	0.43	0.19 (0.1)	0.07	0 (0)	0
Childhood Residency	[Rural]														
	[Urban]	0.12 (0.05)	0.01	-5.7 (12.5)	0.65	-29.1 (12.8)	0.02	0.21 (0.1)	0.03	47.35 (9.99)	0.00	113.8 (20.9)	0.00	0.17 (0.07)	0
Agricultural Pesticide Exposure	[Farm worker]														
	[Housewife]	-0.07 (0.02)	0.01	-6.68 (6.69)	0.32	0.37 (6.85)	0.96	0.1 (0.05)	0.05	20.16 (5.36)	0.00	42.7 (11.2)	0.00	-0.1 (0.04)	0
Chemotherapy cycle	[1st]														
	[2nd]	-0.07 (0.02)	0.00	-16.29 (5.64)	0.00	-0.67 (5.77)	0.91	0.22 (0.04)	0.00	1.37 (4.51)	0.76	12.45 (9.43)	0.19	-0.1 (0.03)	0
	[3rd]	-0.07 (0.02)	0.00	-11.84 (5.06)	0.02	3.99 (5.18)	0.44	0.09 (0.04)	0.02	-9.4 (4.05)	0.02	-13.1 (8.45)	0.12	-0.1 (0.03)	0
	[4th]	-0.13 (0.06)	0.02	-6.3 (15.6)	0.69	25.1 (16)	0.12	0.14 (0.12)	0.26	-36 (12.5)	0.00	-56.9 (26.1)	0.03	-0.19 (0.08)	0
Breast Cancer Stage	[2]														
	[3]	-0.05 (0.02)	0.00	2.14 (4.19)	0.61	7.11 (4.29)	0.10	0.17 (0.03)	0.00	9.21 (3.35)	0.01	26.62 (7)	0.00	-0.07 (0.02)	0
Family History of Disease	[No]														
	[Yes]	0.1 (0.02)	0.00	6.16 (4.48)	0.17	-11.25 (4.59)	0.02	-0.13 (0.03)	0.00	17.07 (3.59)	0.00	28.44 (7.49)	0.00	0.14 (0.02)	0
Combination of Polymorphism of genes	GGGACC														
	GGGGCC	-0.07 (0.07)	0.33	-12.2 (19)	0.52	-0.9 (19.5)	0.96	0.03 (0.15)	0.82	-12 (15.2)	0.43	-26.3 (31.8)	0.41	-0.1 (0.1)	0
	GTGGCC	-0.17 (0.08)	0.05	-5.9 (22.2)	0.79	32.3 (22.8)	0.16	-0.66 (0.17)	0.00	-45.9 (17.8)	0.01	-97.3 (37.2)	0.01	-0.24 (0.12)	0
	GTGACC	-0.11 (0.07)	0.15	-17 (20.1)	0.40	-10.6 (20.6)	0.61	0.15 (0.16)	0.33	0.7 (16.1)	0.97	9 (33.6)	0.79	-0.16 (0.11)	0
	GTGGCC	-0.08 (0.07)	0.25	-6.2 (18.9)	0.74	6.4 (19.3)	0.74	0.09 (0.15)	0.56	-13.8 (15.1)	0.36	-27.6 (31.6)	0.38	-0.12 (0.1)	0
	GTGGCT	-0.19 (0.09)	0.04	3.3 (23.6)	0.89	8.6 (24.2)	0.72	-0.5 (0.18)	0.01	40.6 (18.9)	0.03	51.7 (39.4)	0.19	-0.27 (0.13)	0
	TTGACC	-0.12 (0.09)	0.18	-11.8 (25.2)	0.64	-9.9 (25.8)	0.70	0 (0.19)	1.00	11.3 (20.1)	0.58	25.8 (42.1)	0.54	-0.18 (0.14)	0
	TTGGCC	-0.1 (0.07)	0.19	-12.6 (19.8)	0.53	0.6 (20.3)	0.98	0.14 (0.15)	0.35	-14.4 (15.9)	0.37	-26.8 (33.2)	0.42	-0.14 (0.11)	0
	TTGGCT	-0.16 (0.08)	0.04	-4 (20.3)	0.84	58.5 (20.8)	0.01	0.28 (0.16)	0.08	-10.2 (16.3)	0.53	-9.5 (34)	0.78	-0.23 (0.11)	0

Table 5
Goodness of Fit of Multivariate linear regression model

Dependent Variable	t1/2 (h)	V (mg)/(μg/ml)	CL (mg)/(μg/ml)/h	T max (h)	C max (μg/ml)	AUC 0-t (μg/ml*h)	MRT (h)
Omnibus Test	117.76	52.18	64.09	124.01	218.31	226.73	117.76
Log Likelihood	227.55	-901.43	-913.61	49.42	-864.35	-1012.36	154.25
Akaike's Information Criterion (AIC)	-409.11	1848.86	1873.22	-52.84	1774.69	2070.72	-262.5
Bayesian Information Criterion (BIC)	-333.25	1924.72	1949.08	23.02	1850.56	2146.58	-186.64

Pharmacokinetic Impacts of Gene-Gene Interactions:

Combinations of genotypes from many loci demonstrated intriguing trends in modifying drug kinetics. Compared to wild-type carriers (GG-GG-CC: 39.31 μg·h/mL, SD ± 21.69; $p < 0.001$), patients with the GT-GG-CT combination showed a more than fivefold increase in systemic 5-FU exposure, with an AUC_{0-t} of 289.06 μg·h/mL (SD ± 135.99). In this group, there was a substantial decrease in clearance (2.56 mg·h/μg/mL, SD ± 0.87 vs. 28.32 mg·h/μg/mL, SD ± 29.06; $p < 0.001$) and a significant increase in C_{max} values (146.67 μg/mL, SD ± 65.13 vs. 22.78 μg/mL, SD ± 18.82; $p < 0.001$). With an AUC_{0-t} of 91.11 μg·h/mL (SD ± 38.06), C_{max} of 40.44 μg/mL (SD ± 13.32), and decreased clearance (13.6 mg·h/μg/mL, SD ± 3.5; $p < 0.05$), GT-GA-CC, another high-risk genotype, also led to increased drug exposure. According to these results, which are graphically depicted in Fig. 4, some genotype combinations may have additive or synergistic effects on 5-FU metabolism, significantly changing pharmacokinetic results and emphasizing the potential use of combinatorial genotyping in tailored treatment.

The Toxicity Profile and Genetic Factors:

Six frequently reported 5-FU-related side events- alopecia, vomiting, nausea, pain, gastrointestinal (GI) toxicity, and anemia- were used to assess the toxicity profile. The results are presented in Table 6. Regardless of metabolic status, alopecia was reported universally (100%) across all genotype groups. Other toxicities, on the other hand, were associated with poor pharmacokinetics and differed considerably by genetic background. 66.7% of individuals with the *DPYD* rs1801160 CT genotype reported vomiting, 100% reported nausea and gastrointestinal damage, and all showed anemia symptoms. Despite receiving an average 5-FU dose of 750 mg, these individuals showed higher exposure and markedly decreased clearance (AUC_{0-t} = 121.01 μg·h/mL, SD ± 126.97). On the other hand, patients with the *MTHFR* rs1801131 GG genotype had the lowest rates of anemia (30.77%) and vomiting (30.77%). They also had better clearance rates and were able to tolerate higher dosages (mean dose = 788.46 mg, SD ± 77.09). The *DPYD* rs3918290 GG group showed consistently low rates of anemia (34.07%), nausea (51.65%), and vomiting (15.38%), which were consistent with enhanced clearance (mean CL = 27.8 mg·h/μg/mL, SD ± 28.46) and moderate AUC_{0-t} values (mean = 53.31 μg·h/mL). On the other hand, while receiving greater doses (mean = 805.56 mg, SD ± 66.17), *DPYD* rs3918290 GA heterozygotes had very low rates of vomiting (11.11%) and anemia (22.22%) but somewhat heightened nausea (66.67%). These findings are explained in detail in Supplementary-Table 4.

Combining pharmacokinetic and toxicity profiles showed a higher percentage of increased side effects and decreased metabolism. Patients with the *DPYD* rs1801160 CT and *DPYD* rs3918290 GA genotypes in particular had greater rates of nausea, vomiting, GI symptoms, and anemia, as well as raised AUC_{0-t} and decreased clearance. For example, CT carriers showed a strong toxicity risk associated with impaired metabolism, with a mean AUC_{0-t} 121.01 μg·h/mL, 66.7% reporting GI toxicity, and 100% having anemia. On the other hand, genotypes linked to more effective 5-FU clearance, namely *MTHFR* rs1801131 GG and *DPYD* rs3918290 GG, were linked to lower systemic exposure and noticeably less toxicity. With vomiting and anemia rates around 35%, these individuals maintained mean AUC_{0-t} levels below 55 μg·h/mL, suggesting a lower toxicity profile. These results highlight how pharmacogenetic screening can be used to detect high-risk people before 5-FU is administered and to optimize dosage in order to reduce side effects. Though it should be noted that not every unfavorable outcome was exclusive to a particular genotype. All subgroups experienced alopecia, indicating that some toxicities might be more associated with systemic exposure or other unknown factors, such as treatment duration or epigenetic variation. However, the robust correlation between pharmacokinetics and toxicity found here highlights how crucial it is to incorporate genetic screening into standard oncologic treatment. With considerably higher C_{max} (B = 40.6, SE = 18.9; $p = 0.033$) and AUC_{0-t} (B = 51.7, SE = 39.4; $p = 0.192$) and a considerable decrease in clearance (B = 8.6, SE = 24.2; $p = 0.007$), the GT-GG-CT genotype was the most detrimental genetic combination in terms of pharmacokinetics. The Omnibus test (Table 5) verified the robustness of the model across all PK parameters ($\chi^2 = 226.73$; $p < 0.001$).

Table 6
Toxicity profiling of 5-FU drug in breast cancer patients

Toxicity Profile	Categories	Statistics	Dose (mg)						
			<i>MTHFR</i> rs1801131 T > G			<i>DPYD</i> rs3918290G > A		<i>DPYD</i> rs1801160C > T	
			GG	GT	TT	GA	GG	CC	CT
Hair Loss	Yes	N (%)	52 (100%)	116 (100%)	32 (100%)	18 (100%)	182 (100%)	188 (100%)	12 (100%)
		Mean (SD)	788.46 (77.09)	768.97 (59.58)	737.5 (33.6)	805.56 (66.17)	765.38 (62.05)	770.21 (65.13)	750 (0)
Vomiting	No	N (%)	36 (69.23%)	70 (60.34%)	18 (56.25%)	16 (88.89%)	108 (59.34%)	120 (63.83%)	4 (33.33%)
		Mean (SD)	805.56 (87.65)	764.29 (60.88)	750 (0)	812.5 (67.08)	768.52 (67.18)	775 (69.51)	750 (0)
	Yes	N (%)	16 (30.77%)	46 (39.66%)	14 (43.75%)	2 (11.11%)	74 (40.66%)	68 (36.17%)	8 (66.67%)
		Mean (SD)	750 (0)	776.09 (57.48)	721.43 (46.88)	750 (0)	760.81 (53.81)	761.76 (56.06)	750 (0)
Nausea	No	N (%)	28 (53.85%)	54 (46.55%)	14 (43.75%)	12 (66.67%)	84 (46.15%)	92 (48.94%)	4 (33.33%)
		Mean (SD)	810.71 (92.65)	762.96 (63.83)	750 (0)	833.33 (65.13)	766.67 (70)	776.09 (73.93)	750 (0)
	Yes	N (%)	24 (46.15%)	62 (53.45%)	18 (56.25%)	6 (33.33%)	98 (53.85%)	96 (51.06%)	8 (66.67%)
		Mean (SD)	762.5 (42.35)	774.19 (55.62)	727.78 (42.78)	750 (0)	764.29 (54.68)	764.58 (55.21)	750 (0)
Pain	No	N (%)	52 (100%)	108 (93.1%)	28 (87.5%)	18 (100%)	170 (93.41%)	176 (93.62%)	12 (100%)
		Mean (SD)	788.46 (77.09)	770.37 (61.54)	750 (0)	805.56 (66.17)	768.82 (61.53)	773.86 (64.58)	750 (0)
	Yes	N (%)	0 (0%)	8 (6.9%)	4 (12.5%)	0 (0%)	12 (6.59%)	12 (6.38%)	0 (0%)
		Mean (SD)	0 (0)	750 (0)	650 (0)	0 (0)	716.67 (49.24)	716.67 (49.24)	0 (0)
Gastrointestinal toxicity	No	N (%)	30 (57.69%)	68 (58.62%)	14 (43.75%)	10 (55.56%)	102 (56.04%)	108 (57.45%)	4 (33.33%)
		Mean (SD)	783.33 (57.74)	770.59 (54.8)	750 (0)	850 (57.74)	763.73 (45.68)	772.22 (53.54)	750 (0)
	Yes	N (%)	22 (42.31%)	48 (41.38%)	18 (56.25%)	8 (44.44%)	80 (43.96%)	80 (42.55%)	8 (66.67%)
		Mean (SD)	795.45 (98.69)	766.67 (66.31)	727.78 (42.78)	750 (0)	767.5 (78.39)	767.5 (78.39)	750 (0)
Anemia	No	N (%)	38 (73.08%)	80 (68.97%)	26 (81.25%)	10 (55.56%)	134 (73.63%)	132 (70.21%)	12 (100%)
		Mean (SD)	794.74 (83.66)	766.25 (52.03)	734.62 (36.79)	810 (77.46)	764.93 (60.71)	769.7 (65.31)	750 (0)
	Yes	N (%)	14 (26.92%)	36 (31.03%)	6 (18.75%)	8 (44.44%)	48 (26.37%)	56 (29.79%)	0 (0%)
		Mean (SD)	771.43 (54.47)	775 (74.16)	750 (0)	800 (53.45)	766.67 (66.31)	771.43 (65.27)	0 (0)

DISCUSSION

This research offers important new information on how genetic variants interact with non-genetic demographic and clinical factors to influence the pharmacokinetics (PK) and toxicity profile of 5-fluorouracil (5-FU) in patients with breast cancer. In particular, we show that changes in the *DPYD* and *MTHFR* genes, along with lifestyle and environmental factors such as early living conditions and occupational exposures, have a substantial impact on drug metabolism, systemic exposure, and side effects. These results represent a major step toward tailored chemotherapy and increased therapeutic precision by highlighting the need to incorporate pharmacogenetic and a thorough patient profile into clinical decision-making frameworks.

Clinical and Demographic Factors' Effect on 5-FU Pharmacokinetics:

The significant impact of the patient's living surroundings and environment on 5-FU pharmacokinetics is one of the study's important aspects. Environmental exposure to agricultural pesticides has been associated with genetic damage and altered enzyme function, which may affect the toxicity and disposition of drugs. In order to investigate its possible role in inter-individual variability in 5-FU PK, pesticide exposure was included as a covariate, given the significant prevalence of farm labor among women in this study sample. People who grew up in cities had far higher area under the curve (AUC_{0-t}) and peak plasma concentrations (C_{max}) as well as lower clearance rates than people who grew up in rural areas. These findings most likely reflect the complex, multifaceted effects of urban living, such as changed food habits, increased exposure to industrial pollutants, and lifestyle choices that may have an impact on the activity of the hepatic enzymes involved in the metabolism of 5-FU (Wang et al., 2021). For instance, variations in sedentary behavior or nutritional inadequacies may alter the expression or functionality of important metabolic enzymes. On the other hand, patients from rural regions showed improved 5-FU clearance, especially those who had been exposed to pesticides at work. This implies that hepatic enzymes, particularly cytochrome P450 isoforms, are induced by long-term exposure to xenobiotics, including organophosphates, and that these enzymes speed up drug biotransformation and clearance (Smith et al., 2015; Zhang et al., 2019). Although systemic drug buildup may be lessened by this enzyme activation, dose modifications may be necessary to preserve therapeutic efficacy.

Pharmacokinetic variability was also influenced by occupational roles; housewives, for example, had lower clearance and higher systemic 5-FU exposure than agricultural laborers. These discrepancies could result from variations in endogenous hormone levels, dietary practices, or physical activity, all of which have an effect on pharmacokinetic processes. Furthermore, pharmacokinetic parameters like T_{max} and AUC_{0-t} were independently influenced by disease-related factors like cancer stage and the number of chemotherapy cycles given. This indicates the dynamic interplay between tumor burden, physiological status, and drug disposition. When taken as a whole, these findings support a patient-specific, nuanced approach to dosage that takes changing clinical and lifestyle circumstances into account during treatment (Garcia et al., 2022).

DPYD and MTHFR Genetic Variants as the Main Factors Affecting 5-FU Metabolism

By verifying that functional polymorphisms in *DPYD*, specifically rs1801160 (C > T) and rs3918290 (G > A), are important drivers of 5-FU pharmacokinetics and toxicity, our results support and expand on previous pharmacogenomic studies (Lunenborg et al., 2020; Meulendijks et al., 2019). The CT genotype at rs1801160 was associated with a significantly greater incidence of toxicities, including nausea, vomiting, gastrointestinal disturbances, and hematologic adverse effects, as well as a significantly higher systemic exposure to 5-FU (elevated AUC_{0-t} and C_{max}). This is consistent with the biological function of *DPYD*-encoded dihydropyrimidine dehydrogenase (DPD) as the primary catalyst in 5-FU degradation. Drug buildup and subsequent toxicity result from significantly decreased DPD activity, even in heterozygous carriers of loss-of-function alleles.

Reduced drug clearance and increased systemic exposure were also associated with the rs3918290 variation, a splice-site mutation that interferes with normal mRNA processing of *DPYD* in carriers of the GA genotype. The therapeutic Pharmacogenetics Implementation Consortium (CPIC) guidelines, which advocate pre-treatment genotyping to guide dose individualization and avoid severe toxicity risks, have incorporated this mutation, highlighting its therapeutic significance (Caudle et al., 2020). 5-FU pharmacokinetics were likewise impacted by *MTHFR* polymorphisms, albeit less significantly. Notably, among GT carriers, rs1801131 (T > G) was linked to delayed T_{max} and a tendency toward higher AUC_{0-t} . Because of *MTHFR*'s essential function in folate metabolism and DNA methylation pathways that are essential for nucleotide synthesis and repair, thymidylate synthase inhibition may be amplified by reduced enzyme performance, which could increase 5-FU cytotoxicity. These findings are in line with earlier research that linked *MTHFR* variations to changes in the toxicity and response to fluoropyrimidine treatment. (Rosmarin et al., 2018; Liu et al., 2021).

Interactions between genes and the environment: A Framework with Multiple Factors:

The identification of combination genetic influences and how they interact with environmental exposures on 5-FU disposition is a noteworthy contribution of this work. Individuals with particular multi-locus genotypes, including GT-GG-CT and GT-GA-CC, had much higher systemic drug exposure and increased toxicity. Particularly, the GTGGCT haplotype was linked to an AUC increase of over five times when compared to wild-type genotypes, suggesting that polymorphisms may have additive or synergistic effects that affect metabolic capacity over time. In clinical pharmacology, this intricacy highlights the shortcomings of single-gene analysis and the necessity of integrative genomic profiling. (Takahashi et al., 2022). Furthermore, multivariate regression analysis showed that pharmacokinetic results are independently and jointly predicted by genetic polymorphisms and non-genetic variables. This lends credence to a thorough, multivariate model of 5-FU metabolism wherein clinical status, environmental factors, and genetic composition interact to influence drug exposure and clearance, which in turn affects therapeutic efficacy and safety. To improve individualized treatment and fine-tune dosing algorithms, such integrative prediction models are crucial (Wang et al., 2020). The clearance values of 5-fluorouracil observed in this study were lower than those reported in certain previous publications. The variation could be due to infusion-based administration, the distinct characteristics of Southern Punjabi breast cancer patients, and physical alterations driven on by the disease that impact drug metabolism. Differences in reported clearance values may also be caused by variations in study design, sampling strategy, and analytical techniques between research studies. Although 5-FU is known to be chemically unstable, rapid plasma separation and low-temperature storage were adopted to minimize degradation.

Clinical Toxicity and Consequences for Tailored Chemotherapy:

Differential toxicity profiles demonstrated the clinical significance of pharmacokinetic heterogeneity. While baldness was common, carriers of genotypes with decreased metabolism, particularly rs1801160 CT heterozygotes, were far more likely to experience additional toxicities, such as tiredness, anemia, and gastrointestinal disorders. Crucially, even though this genotype only accounted for 6% of the population, its disproportionate effect on adverse events and drug exposure makes routine pharmacogenomic screening to identify high-risk patients before starting chemotherapy strongly recommended (Amstutz et al., 2018). On the other hand, good clearance and decreased toxicity were connected to genotypes linked to preserved or improved drug metabolism, such as *DPYD* rs3918290 GG and *MTHFR* GG, indicating that such individuals may safely tolerate regular 5-FU dose regimens. These results support the idea that genotype-guided dosage methods can lower treatment-related morbidity and enhance therapeutic indices (Lunenborg et al., 2020).

Limitations and Prospects:

The use of a well-characterized clinical cohort, thorough PK evaluations, and the concurrent integration of genetic and environmental components within a strong statistical framework are what set this analysis apart. Our findings' validity and generalizability are reinforced by the use of both regression-based and non-parametric approaches. However, there are a few limits that need to be noted. Extrapolation to more genetically varied cohorts, which might show different allele frequencies and gene-environment interactions, may be limited by the study population's relative ethnic homogeneity. Other genetic contributors, such as *TYMS*, *CYP2C9*, and *UGT1A1* variants, along with epigenetic and transcriptomic factors, may further influence pharmacokinetics and warrant further investigation, even though *DPYD* and *MTHFR* polymorphisms are known determinants of 5-FU response (Lunenborg et al., 2020; Wang et al., 2021). Furthermore, our research was limited to pharmacokinetic and toxicological endpoints; in order to completely clarify the clinical significance of these genetic and environmental factors, it will be essential to include pharmacodynamic measurements as well as long-term clinical outcomes, including tumor response and survival.

CONCLUSION

Contextualized within a framework of demographic, clinical, and environmental factors, our results have strong evidence that *DPYD* and *MTHFR* genetic variants significantly influence the toxicity profiles and 5-FU pharmacokinetics in patients with breast cancer. This work demonstrates the possibility of personalized medicine in oncology and supports the therapeutic efficacy of pre-treatment genotyping, especially for high-risk variants as *DPYD* rs1801160 CT. To advance precision chemotherapy and enhance patient outcomes, pharmacogenomic screening must be extended to a wider range of patients, and multidimensional clinical endpoints must be included.

Declarations

Ethics approval and consent to participate.

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of Institute of Molecular Biology and Biotechnology, Bahauddin Zakariya University Multan, Pakistan.

Consent for publication.

Not Applicable.

Author Contributions

M.S. and S.Z. with R.S.S contributed to the conceptual study design and development of the research methodology. M.S collect the clinical samples. M.S., A.B.A., S.Z., and S.Z., contribute in experimental work and data curation. H.K. performed all statistical data analysis and visualization. M.S., A.I.M.B, M.S., N.A., and M.H. contributed to pharmacokinetic analysis, including HPLC-based experiments. M.S., S.Z., and H.K. contributed to writing and revision of the draft. R.S.S. supervised the study and reviewed the draft. All authors read and approved the final manuscript.

Acknowledgement

NA

Funding

The authors did not receive any funding or support for this study.

Competing interests

The authors declared no financial and non-financial competing interests.

Availability of data and materials

The datasets used in this study can be asked from the corresponding author upon a reasonable request.

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Figures

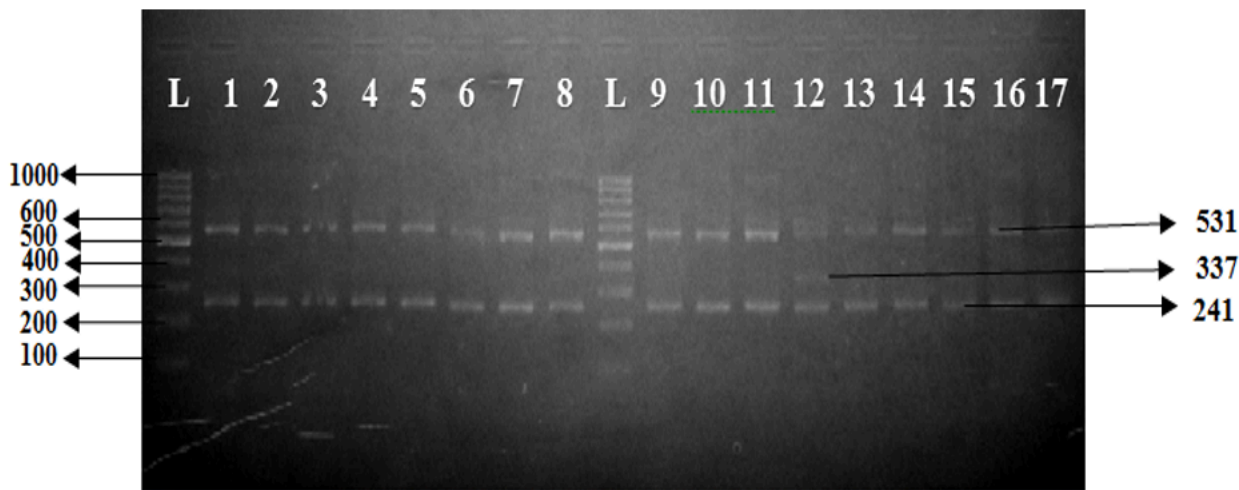


Figure 1

PCR Electrophorized Gel for DPD Gene variant rs3918290 G>A: DNA samples from breast cancer patients were subjected to tetra-arm PCR (1–17). The bands are apparent in the gel image: 531bp (outer band), the homozygous mutant G/G band was of 241bp, and 337bp (homozygous wild A/A). Lanes 1–11 and 13–17 display the homozygous G/G genotype, however, lane 12 displays a heterozygous G/A genotype. 100bp molecular ladder represented by L

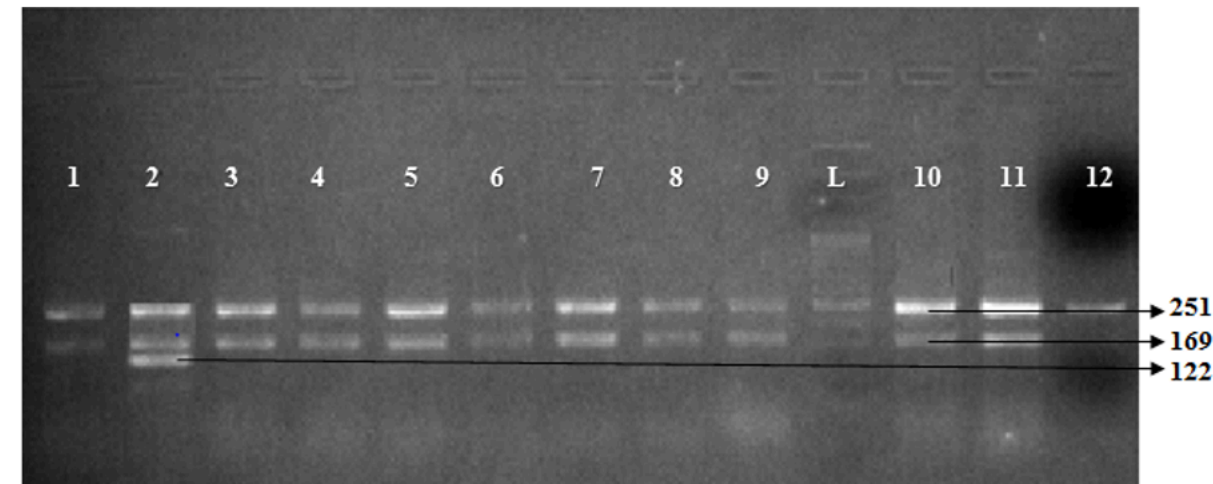


Figure 2

PCR Electrophorized Gel for DPD Gene variant rs1801160 C>T: DNA samples from breast cancer patients were subjected to tetra-arm PCR (1–12). The bands are apparent in the gel image: 251bp (outer band), the homozygous mutant C/C band was of 169bp, and 337bp (homozygous wild T/T). Lanes 1,3–09 and 12 display the homozygous C/C genotype, however, lane 2,11 displays a heterozygous C/T genotype. 100bp molecular ladder represented by L

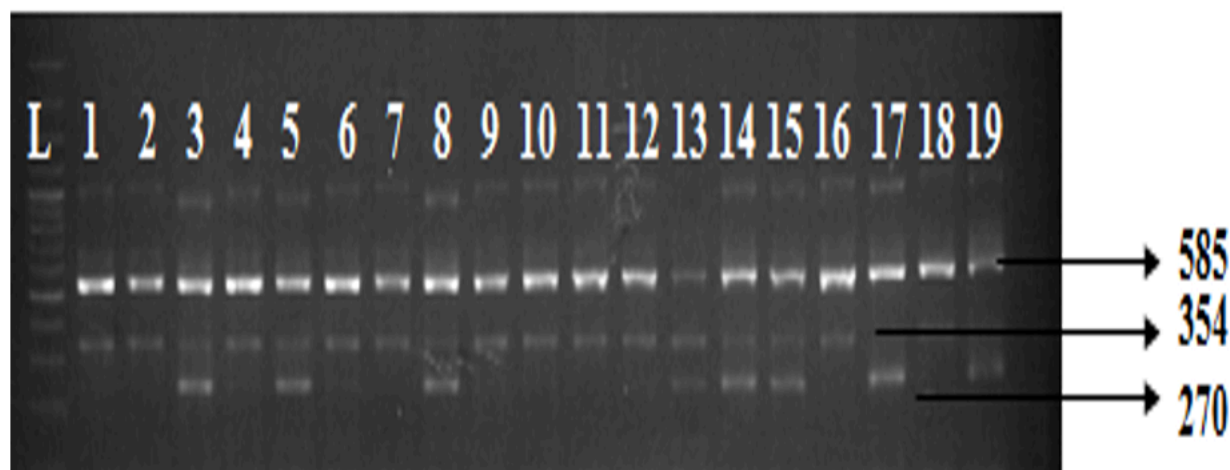


Figure 3

PCR Electrophoresis Gel for MTHFR gene variant rs1801131 T>G: DNA samples from breast cancer patients were subjected to tetra-arm PCR (1–19). The bands are apparent in the gel image: 585bp (outer band), the homozygous mutant T/T band was 354 bp, and 240bp (homozygous wild type G/G). In starting well, 100bp molecular ladder represented by L, Lane (3,5,8,13,14,15,17) represented Heterozygous T/G genotype while Lane (1,2,4,6,7,9,10,11,12,16,18) represented homozygous mutant TT genotype and lane 19 shows homozygous wild GG genotype.

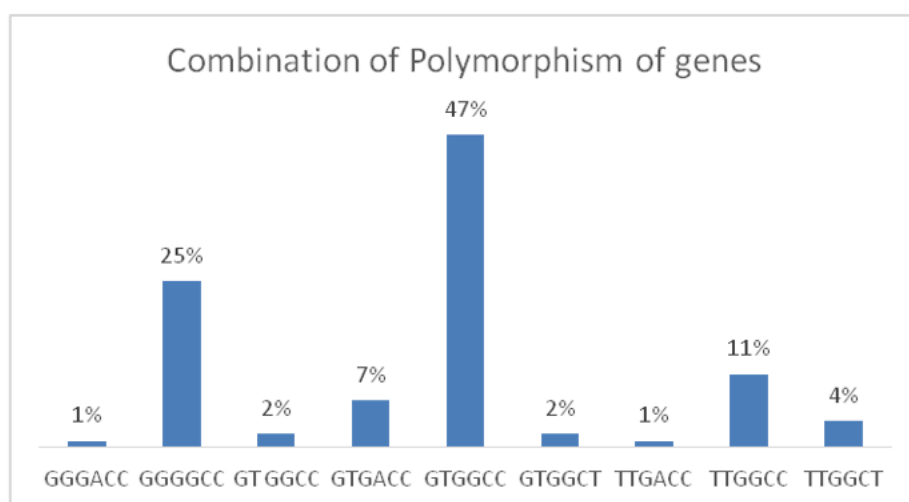


Figure 4

Frequency distribution of the Combination of polymorphisms of the studied genes in breast cancer patients

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