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Pharmacogenetic markers associated with drug metabolism in patients with oncological diseases

N. Miteva-Marcheva[1,2], H. Ivanov[1,2], A. Linev[1,2], M. Topalov[1], V. Popov[3,5], G. Raycheva[3,6], Z. Grudeva-Popova[3,4], V. Stoyanova[1,2]

[1] *Department of Pediatrics and Medical Genetics, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria*
[2] *Department of Medical Genetics, University Hospital "Sveti Georgi" – Plovdiv, Plovdiv, Bulgaria*
[3] *Department of Clinical Oncology, Faculty of Medicine, Medical University of Plovdiv, Plovdiv, Bulgaria*
[4] *Clinic of Clinical Hematology, University Hospital "Sveti Georgi" - Plovdiv, Plovdiv, Bulgaria*
[5] *Clinic of Radiation Oncology, University Hospital "Sveti Georgi" - Plovdiv, Plovdiv, Bulgaria*
[6] *Clinic of Medical Oncology, University Hospital "Sveti Georgi" - Plovdiv, Plovdiv, Bulgaria*

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Pharmacogenetics is the field of genetics that investigates how an individual's genetic variations can impact their metabolism and response to pharmaceutical agents, aiming to identify genetic markers that predict drug efficacy and safety. Personalized medicine is an approach that utilizes an individual's genetic, genomic, and clinical information to tailor medical treatments and interventions, with the goal of optimizing therapeutic outcomes and minimizing adverse effects. The objective of our investigation is to ascertain specific pharmacogenetic markers, particularly single nucleotide polymorphisms (SNPs), linked to the metabolism of chemotherapy agents within a cohort of cancer patients. The study consisted of 19 patients with colorectal cancer (CRC), 12 patients with non-small cell lung cancer (NSCLC) and 9 women with breast cancer (BC). Circulating tumor DNA (ctDNA) was extracted from blood plasma and sequenced. We identified 23 germline pharmacogenetic variants within 16 genes that are potentially associated with the metabolism of the chemotherapy drugs administered to the subjects, and all patients experienced varying degrees of adverse drug reactions. These pharmacogenetic markers can be employed for preemptive testing in cancer patients prior to initiating treatment regimens, facilitating the selection of the most optimal medication at the precise dosage with minimal associated side effects. Pharmacogenetic markers are the modern approach to individualize therapy with minimal risk of toxicity.

Keywords: pharmacogenetics, colorectal cancer, non-small cell lung carcinoma, breast cancer.

INTRODUCTION

In the ever-evolving landscape of oncological treatment, precision and efficacy are paramount for improved patient outcomes. Personalized medicine, a transformative approach, tailors interventions to individual genetic makeup, with pharmacogenetic markers playing a central role. These genetic variations significantly influence drug metabolism, response, and toxicity. Understanding these markers is crucial in oncology, where individual genetic profiles shape treatment success. This article explores the intersection of pharmacogenetics and oncological drug metabolism, unraveling genetic nuances to optimize treatment strategies, minimize adverse effects, and enhance care quality for oncology patients. The investigation's primary aim is to discern pharmacogenetic markers, especially single nucleotide polymorphisms (SNPs), intricately linked to the metabolic processes of chemotherapeutic agents in the demographic of cancer patients.

EXPERIMENTAL

We conducted a study involving 40 patients, consisting of 19 individuals diagnosed with colorectal cancer (three of them were subsequently excluded from the study due to the imperative requirement for exclusive radiotherapy), 12 with non-small cell lung carcinoma, and 9 women with breast cancer. The cell-free DNA was extracted from the blood samples using the QIAamp MinElute ccfDNA Midi

* To whom all correspondence should be sent:
E-mail: miteva_md@abv.bg

Kit, adhering to the established protocol outlined in BioChain's cfPure® Cell Free DNA Extraction Kit (1). To assess the quality and quantity of the extracted DNA, we employed Agarose Gel Electrophoresis and the Qubit DNA Assay Kit in a Qubit 3.0 Fluorimeter (Life Technologies, CA, USA). The subsequent step involved Next-generation sequencing (NGS) utilizing a targeted assay, NovoPMTM 2.0, designed to identify genomic alterations in a comprehensive panel of 484 genes. These genes were specifically selected based on their paramount relevance for the accurate diagnosis and treatment of solid tumors, aligning with current medical literature and clinical guidelines. Additionally, an in-house bioinformatic algorithm, developed by Novogene (Cambridge, United Kingdom), was applied to predict the origin of short variant mutations (germline or somatic) exclusively through the sequencing and analysis of tumor samples.

RESULTS

Our principal focus centered on the identification of germline mutations that may constitute a risk factor for heightened chemotherapy toxicity in cohorts of patients diagnosed with colorectal cancer (Table 1), non-small cell lung cancer (Table 2), and breast cancer (Table 3). Through our meticulous analysis, we successfully pinpointed 23 germline pharmacogenetic variants distributed across 16 genes, each implicated in precipitating adverse effects. These variants include MTHFR 1286A>C, MTHFR c.665C>T, DPYD c.2194G>A, DPYD 1627A>G, DPYD c.496A>G, DPYD c.85T>C, CYP1B1 c.1294G>C, XPC c.2815C>A, XPC c.1496C>T, ABCG2 c.421C>A, SLC22A2 c.808T>G, SOD2 c.47T>C, EGFR c.1562G>A, ABCB1 2677T>G, ABCC2 c.1249G>A, GSTP1 c.313A>G, ATM c.5557G>A, SLCO1B3 c.334T>G, SLCO1B3 c.699G>A, TP53 c.215C>G, XRCC1 1196A>G, ERCC2 c.2251A>C, and ERCC2 c.934G>A.

Noteworthy adverse effects from 1st to 5th degree according to Common Terminology Criteria for Adverse Effects (CTCAE) observed within our patient cohorts encompassed a spectrum of manifestations, including but not limited to anaemia, leukopenia, thrombocytopenia, gastrointestinal toxicity, hepatotoxicity, neurotoxicity, nephrotoxicity, cardiotoxicity, alopecia, rash, lymphangitis, and bone/joint pain.

Owing to the congruence in the administered chemotherapy regimens across the sampled patient cohort, a systematic regrouping was conducted, resulting in the categorization of individuals into six distinct groups. These groups encompassed patients subjected to specific therapeutic interventions: platinum-based chemotherapeutics (comprising 27 patients), pyrimidine analogues (involving 17 patients), EGFR inhibitors (encompassing 11 patients), taxanes (including 15 patients), anthracyclines (comprising 6 patients), and Cyclophosphamide (involving 6 patients).

In the cohort comprising the initial patient group subjected to platinum-based chemotherapeutics, our investigation revealed the presence of 11 distinct variants distributed across seven genes that exhibited significant associations with drug toxicity (Table 4). Notably, within this cohort, 11 individuals manifested heterozygosity for the MTHFR c.1286A>C variant, 14 individuals carried the MTHFR c.665C>T variant, and 17 individuals bore the XPC c.2815C>A variant, with near-universal prevalence observed for the XPC c.1496C>T variant, barring only two exceptions.

Furthermore, within this patient population, 8 out of 27 patients demonstrated carriage of the SLC22A2 c.808T>G variant, while for the ABCC2 c.1249G>A variant, all patients, save for two, exhibited carrier status. The GSTP1 c.313A>G variant was identified in 26 patients, underscoring its substantial representation.

Turning our attention to the SLCO1B3 gene, two distinct variants, namely c.334T>G and c.699G>A, were identified, with 23 and 26 patients exhibiting carrier status, respectively. Additionally, the ERCC2 gene featured prominently, with the c.2251A>C variant identified in 19 carriers and the c.934G>A variant in 22 carriers within the patient cohort.

Within the cohort of patients subjected to pyrimidine analogues, encompassing a total of 17 individuals, our comprehensive genetic analysis revealed the presence of 14 distinct variants distributed across 10 genes, all of which demonstrated significant associations with drug toxicity (Table 5). Notably, the MTHFR gene exhibited two discernible variants: the c.1286A>C variant was identified in 8 heterozygous patients, while the c.665C>T variant was observed in 8 patients.

Further genetic scrutiny of the DPYD gene uncovered a spectrum of 4 variants: c.2194G>A was detected in 7 patients, c.1627A>G in 4 patients, c.496A>G in 3 patients, and c.85C>T in 14 patients. Additionally, the CYP1B1 gene featured the c.1294G>C variant in 11 patients, whereas the ABCG2 gene harbored the c.421C>A vari-

Table 1. Colorectal Cancer Patients: Chemotherapeutics and Adverse Drug Reactions (ADRs) Graded from 1st to 5th Degree According to CTCAE

Patient	Age	Sex	Treatment	ADRs from 1st to 5th degree						
				anaemia	leukopenia	thrombocytopenia	hepatotoxicity	nephrotoxicity	neurotoxicity	GIT toxicity
1	48	male	Oxaliplatin, Leucovorin, 5-Fluorouracil, Irinotecan, Bevacizumab	1			3			
2	68	male	Oxaliplatin, Capecitabine, Irinotecan Bevacizumab	2			3			
3	70	male	Oxaliplatin, Leucovorin, 5-Fluorouracil, Irinotecan, Panitumumab	1			3	1		
4	65	male	Oxaliplatin, Leucovorin, 5-Fluorouracil, Irinotecan, Bevacizumab, Ramucirumab	2			1		2	
5	62	female	Oxaliplatin, Leucovorin, 5-Fluorouracil, Irinotecan, Bevacizumab, Ramucirumab	1						
6	67	male	Oxaliplatin, Leucovorin, 5-Fluorouracil, Panitumumab	2	1	1	1			
7	75	male	Oxaliplatin, Leucovorin, 5-Fluorouracil, Panitumumab	1			3			
8	73	female	Oxaliplatin, Leucovorin,, 5-Fluorouracil	1					2	
9	75	male	Oxaliplatin, Leucovorin,, 5-Fluorouracil, Panitumumab	1			3			
10	64	female	Oxaliplatin, Leucovorin, 5-Fluorouracil	1	2	2	1			
11	70	male	Oxaliplatin, Leucovorin, 5-Fluorouracil, Irinotecan, Capecitabine, Panitumumab, Cetuximab	1	3	3				
12	63	male	Oxaliplatin, Irinotecan, Capecitabine, Bevacizumab	1	3	3				
13	74	male	Oxaliplatin, Leucovorin, 5-Fluorouracil	2	3	3				
14	63	male	Oxaliplatin, Leucovorin, 5-Fluorouracil, Irinotecan, Panitumumab, Ramucirumab	3			3	1		2
15	46	female	Oxaliplatin, Leucovorin, 5-Fluorouracil	3	2	2	3			
16	61	female	Oxaliplatin, Leucovorin, 5-Fluorouracil, Irinotecan, Capecitabine, Panitumumab	2			1			

ant in 3 patients. The ABCB1 gene exhibited the c.2677T>G variant in 15 patients, and both the ABCC2 c.1249G>A and GSTP1 c.313A>G variants were prevalent in 15 and all but one patients, respectively.

Furthermore, the TP53 gene displayed the c.215C>G variant in 14 patients, XRCC1 exhibited the c.1196A>G variant in 16 patients, and the ERCC2 gene demonstrated the c.2251A>C variant in 14 patients.

Table 2. Non-Small Cell Lung Cancer Patients: Chemotherapeutics and Adverse Drug Reactions (ADRs) Graded from 1st to 5th Degree According to CTCAE

Patient			Treatment	ADRs from 1st to 5th degree					
	Age	Sex		anaemia	leukopenia	thrombocytopenia	hepatotoxicity	nephrotoxicity	rash
1	56	male	Pembrolizumab	**1**					
2	53	female	Paclitaxel, Carboplatin, Bevacizumab, Erlotinib, Atezolizumab	**1**					
3	64	male	Etoposid, Carboplatin, Atezolizumab	**1**			**1**		
4	54	male	Etoposid, Cisplatin, Docetaxel, Ramucirumab, Nivolumab	**1**			**3**	**1**	
5	59	female	Paclitaxel, Carboplatin, Pembrolizumab	**1**			**1**		**2**
6	72	male	Etoposid, Carboplatin, Atezolizumab	**2**	**2**	**1**	**4**		
7	74	male	Gemcitabine, Carboplatin, Atezolizumab, Docetaxel	**2**	**4**	**3**		**2**	**2**
8	64	male	Gemcitabine, Cisplatin, Docetaxel, Ramucirumab, Nivolumab, Erlotinib	**1**					
9	63	male	Gemcitabine, Cisplatin, Pembrolizumab	**2**			**1**		
10	53	female	Alectinib	**2**					
11	52	male	Paclitaxel, Carboplatin, Pembrolizumab	**1**	**2**				
12	65	male	Cisplatin, Vinorelbin, Erlotinib, Docetaxel, Atezolizumab	**1**				**2**	

Table 3. Breast Cancer Patients: Chemotherapeutics and Adverse Drug Reactions (ADRs) Graded from 1st to 5th Degree According to CTCAE

Patient			Treatment	ADRs from 1st to 5th degree											
	Age	Sex		**A***	**B**	**C**	**D***	**E**	**F**	**G**	**H**	**J**	**K**	**L**	**M**
1	34	female	Farmorubicin, Cyclophosphamide, Docetaxel	**1**							**2**		**2**		
2	51	female	Paclitaxel, Carboplatin, Docetaxel	**2**			**1**						**1**		
3	53	female	Farmorubicin, Cyclophosphamide, 5-Fluoro uracil, Paclitaxel							**2**			**2**	**2**	**2**
4	63	female	Pertuzumab, Trastuzumab, Docetaxel				**2**						**1**		
5	31	female	Farmorubicin. Docetaxel, Cyclophosphamide	**1**			**1**						**2**		
6	58	female	Farmorubicin, Docetaxel, Cyclophosphamide	**2**	**2**	**1**							**2**		
7	87	female	Arimidex	**1**											
8	37	female	Farmorubicin, Cyclophosphamide, Trastuzuma, Docetaxel, Tamoxifen, Zolendronic acid, Zoladex, Letrozole, Xgeva, Exemestane, Lapatinib	**1**	**1**	**1**	**2**		**2**	**2**	**2**	**2**	**2**		
9	67	female	Farmorubicin, Docetaxel, Cyclophosphamide				**2**			**2**	**2**	**2**	**2**		

***A** anaemia, **B** leukopenia, **C** thrombocytopenia, **D** hepatotoxicity, **E** nephrotoxicity, **F** neurotoxicity, **G** cardiotoxicity, **H** GIT toxicity, **J** rash, **K** alopecia, **L** lymphangitis, **M** bone/joint pain.

Exclusive identification of a singular variant within the EGFR gene was discerned within the patient cohort subjected to EGFR inhibitors, denoted as c.1562G>A (Table 6). Remarkably, the entire patient ensemble unequivocally exhibited carrier status for this specific genetic variant, as delineated in Table 6.

Within the cohort of patients subjected to taxane-based treatments, comprising a total of 15 individuals, our comprehensive genetic analysis unveiled the presence of six distinct variants distributed across five genes, each exhibiting significant associations with drug toxicity (Table 7). Specifically, the c.47T>C variant of the SOD2 gene was identified in

Table 4. Patients Treated with Platinum-Based Chemotherapeutics and Genes Associated with the Metabolism of These Agents (HMZ – homozygote; HTZ – heterozygote)

Patient			Disease	Treatment	Genes, associated with the metabolism of platinum-based chemotherapeutics										
	Age	Sex			MTHFR	MTHFR	XPC	XPC	SLC22A2	ABCC2	GSTP1	SLCO183	SLCO183	ERCC2	ERCC2
					c.1286A>C	c.665C>T	c.2815C>A	c.1496>T	c.808T>G	c.1249G>A	c.313A>G	c334T>G	c.699G>A	c.2251A>A	c.934G>A
					rs1801131	rs1801133	rs2228001	rs2228000	rs316019	rs2273697	rsl695	rs4149117	rs7311358	rs13181	rs1799793
1	48	male	CRC	Oxaliplatin			HTZ(CA)	HMZ(CC)		HTZ(AG)	HTZ(AG)	HTZ(GT)	HTZ(AG)		HMZ(GG)
2	68	male	CRC	Oxaliplatin			HTZ(CA)	HMZ(CC)		HMZ(GG)	HMZ(AA)		HMZ(AA)	HTZ(CA)	
3	70	male	CRC	Oxaliplatin		HTZA(TC)				HMZ(GG)	HTZ(AG)		HMZ(AA)		HMZ(GG)
4	65	male	CRC	Oxaliplatin		HTZA(TC)	HMZ(CC)	HMZ(CC)			HMZ(AA)	HMZ(GG)	HMZ(AA)	HTZ(CA)	HTZ(GA)
5	62	female	CRC	Oxaliplatin	HTZ(CA)		HTZ(CA)	HTZ(CT)	HTZ(TG)	HMZ(GG)	HMZ(AA)		HMZ(AA)	HTZ(CA)	
6	67	male	CRC	Oxaliplatin		HTZA(TC)		HMZ(CC)	HTZ(TG)	HMZ(GG)	HMZ(AA)	HMZ(GG)	HMZ(AA)		HTZ(GA)
7	75	male	CRC	Oxaliplatin		HMZ(TT)		HTZ(CT)		HMZ(GG)	HMZ(AA)	HMZ(GG)	HMZ(AA)	HTZ(CA)	HTZ(GA)
8	73	female	CRC	Oxaliplatin	HTZ(CA)	HTZA(TC)	HTZ(CA)	HMZ(CC)	HTZ(TG)		HTZ(AG)	HMZ(GG)	HMZ(AA)	HTZ(CA)	HMZ(GG)
9	75	male	CRC	Oxaliplatin		HTZA(TC)		HTZ(CT)		HMZ(GG)	HMZ(AA)	HMZ(GG)	HMZ(AA)	HTZ(CA)	HTZ(GA)
10	64	female	CRC	Oxaliplatin			HTZ(CA)	HTZ(CT)		HMZ(GG)	HMZ(AA)	HMZ(GG)	HMZ(AA)	HMZ(CC)	
11	70	male	CRC	Oxaliplatin	HTZ(CA)		HTZ(CA)	HTZ(CT)	HTZ(TG)	HMZ(GG)		HMZ(GG)	HMZ(AA)	HTZ(CA)	HTZ(GA)
12	63	male	CRC	Oxaliplatin		HTZA(TC)		HMZ(CC)		HMZ(GG)	HTZ(AG)	HMZ(GG)	HMZ(AA)	HTZ(CA)	HTZ(GA)’
13	74	male	CRC	Oxaliplatin	HTZ(CA)		HTZ(CA)	HMZ(CC)		HMZ(GG)	HMZ(AA)	HMZ(GG)		HTZ(CA)	HTZ(GA)
14	63	male	CRC	Oxaliplatin	HTZ(CA)			HTZ(CT)		HTZ(AG)	HTZ(AG)	HMZ(GG)	HMZ(AA)	HTZ(CA)	HMZ(GG)
15	46	female	CRC	Oxaliplatin	HTZ(CA)	HTZA(TC)	HTZ(CA)	HTZ(CT)	HTZ(TG)	HMZ(GG)	HMZ(AA)	HMZ(GG)	HMZ(AA)	HTZ(CA)	HTZ(GA)
16	61	female	CRC	Oxaliplatin	HTZ(CA)		HTZ(CA)	HMZ(CC)	HTZ(TG)	HMZ(GG)	HTZ(AG)	HMZ(GG)	HMZ(AA)	HTZ(CA)	HTZ(GA)
17	53	female	NSCLC	Carboplatin	HTZ(CA)		HTZ(CA)	HMZ(CC)	HTZ(TG)	HMZ(GG)	HTZ(AG)	HTZ(GT)	HTZ(AG)	HMZ(CC)	
18	64	male	NSCLC	Carboplatin		HTZA(TC)	HMZ(CC)	HMZ(CC)		HTZ(AG)	HTZ(AG)	HMZ(GG)	HMZ(AA)		HMZ(GG)
19	54	male	NSCLC	cisPlatin		HTZA(TC)		HTZ(CT)		HMZ(GG)	HMZ(AA)	HMZ(GG)	HMZ(AA)	HTZ(CA)	HTZ(GA)
20	59	female	NSCLC	Carboplatin		HMZ(TT)		HTZ(CT)		HTZ(AG)	HMZ(AA)	HMZ(GG)	HMZ(AA)		
21	72	male	NSCLC	Carboplatin			HMZ(CC)	HMZ(CC)	HTZ(TG)	HMZ(GG)	HTZ(AG)		HMZ(AA)	HMZ(CC)	HMZ(GG)
22	74	male	NSCLC	Carboplatin	HTZ(CA)					HMZ(GG)	HTZ(AG)	HMZ(GG)	HMZ(AA)	HTZ(CA)	HMZ(GG)
23	64	male	NSCLC	cisPlatin	HTZ(CA)	HTZA(TC)	HMZ(CC)	HMZ(CC)		HTZ(AG)	HMZ(AA)	HTZ(GT)	HTZ(AG)	HTZ(CA)	HTZ(GA)
24	63	male	NSCLC	cisPlatin			HTZ(CA)	HTZ(CT)		HTZ(AG)	HMZ(AA)	HMZ(GG)	HMZ(AA)	HTZ(CA)	HTZ(GA)
25	52	male	NSCLC	Carboplatin		HMZ(TT)		HMZ(CC)		HMZ(GG)	HTZ(AG)	HMZ(GG)	HMZ(AA)		HMZ(GG)
26	65	male	NSCLC	cisPlatin	HTZ(CA)	HTZA(TC)		HTZ(CT)		HMZ(GG)	HMZ(AA)	HTZ(GT)	HTZ(AG)		HTZ(GA)
27	51	female	BC	Carboplatin			HTZ(CA)	HMZ(CC)		HTZ(AG)	HTZ(AG)	HTZ(GT)	HTZ(AG)		HTZ(GA)

Table 5. Patients Treated with Pyrimidine Analogues and Genes Associated with the Metabolism of These Agents (HMZ – homozygote; HTZ – heterozygote)

	Patient		Disease	Treatment	Genes associated with the metabolism of 5-FU/ Capecitabine												
	Age	Sex			MTHFR	MTHFR	DPYD	DPYD	DPYD	DPYD	CYP1B1	ABCC2	ABCC2	GSTP1	TPS3	XRCC1	ERCC2
					c.1286A>C	c.665C>T	c.2194G>A	C.1627A>G	c.496A>G	c.85C>T	c.1294G>C	c.421C>A	c.1249G>A	c.313A>G	c.215C>G	c.1196A>G	c.2251A>C
					rsl801131	rsl801133	rsl801160	rsl801159	rs2297595	rsl801265	rsl056836	rs2231142	rs273697	rs1695	rs10425222	rs25487	rs13181
1	48	male	CRC	5-Fluoro uracil					HTZ(GA)	HTZ(TC)	HTZ(CG)		HTZ(AG)	HTZ(AG)	HMZ(GG)	HTZ(AG)	
2	68	male	CRC	Capecitabine						HMZ(TT)	HTZ(CG)		HMZ(GG)	HMZ(AA)	HTZ(GC)	HTZ(AG)	HTZ(CA)
3	70	male	CRC	5-Fluoro uracil		HTZ(TC)				HTZ(TC)			HMZ(GG)	HTZ(AG)	HMZ(GG)	HMZ(AA)	
4	65	male	CRC	5-Fluoro uracil		HTZ(TC)	HTZ(AG)	HTZ(GA)		HMZ(TT)	HTZ(CG)			HMZ(AA)	HMZ(GG)	HTZ(AG)	HTZ(CA)
5	62	female	CRC	5-Fluoro uracil	HTZ(CA)		HTZ(AG)			HMZ(TT)			HMZ(GG)	HMZ(AA)		HTZ(AG)	HTZ(CA)
6	67	male	CRC	5-Fluoro uracil		HTZ(TC)				HTZ(TC)	HTZ(CG)		HMZ(GG)	HMZ(AA)	HTZ(GC)	HTZ(AG)	
7	75	male	CRC	5-Fluoro uracil		HMZ(TT)	HMZ(AA)						HMZ(GG)	HMZ(AA)	HMZ(GG)	HMZ(AA)	HTZ(CA)
8	73	female	CRC	5-Fluoro uracil	HTZ(CA)	HTZ(TC)	HTZ(AG)			HTZ(TC)	HMZ(CC)			HTZ(AG)	HTZ(GC)	HMZ(AA)	HTZ(CA)
9	75	male	CRC	5-Fluoro uracil		HTZ(TC)	HTZ(AG)	HTZ(GA)		HTZ(TC)			HMZ(GG)	HMZ(AA)	HTZ(GC)	HMZ(AA)	HTZ(CA)
10	64	female	CRC	5-Fluoro uracil			HMZ(AA)			HMZ(TT)	HMZ(CC)		HMZ(GG)	HMZ(AA)	HTZ(GC)	HTZ(AG)	HMZ(CC)
11	70	male	CRC	5-Fluorouracil, Capecitabine	HTZ(CA)					HMZ(TT)	HTZ(CG)	HTZ(AC)	HMZ(GG		HTZ(GC)	HMZ(AA)	HTZ(CA)
12	63	male	CRC	Capecitabine					HMZ(GG)		HMZ(CC)		HMZ(GG	HTZ(AG)	HTZ(GC)	HTZ(AG)	HTZ(CA)
13	74	male	CRC	5-Fluoro uracil	HTZ(CA)		HTZ(AG)	HMZ(GG)		HMZ(TT)			HMZ(GG	HMZ(AA)		HMZ(AA)	HTZ(CA)
14	63	male	CRC	5-Fluoro uracil	HTZ(CA)					HTZ(TC)	HTZ(CG)		HTZ(AG)	HTZ(AG)		HMZ(AA)	HTZ(CA)
15	46	female	CRC	5-Fluoro uracil	HTZ(CA)	HTZ(TC)		HTZ(GA)		HTZ(TC)	HTZ(CG)	HTZ(AC)	HMZ(GG	HMZ(AA)	HMZ(GG)		HTZ(CA)
16	61	female	CRC	5-Fluorouracil, Capecitabine	HTZ(CA)				HTZ(GA)	HTZ(TC)		HTZ(AC)	HMZ(GG	HTZ(AG)	HMZ(GG)	HMZ(AA)	HTZ(CA)
17	53	female	BC	5-Fluoro uracil	HTZ(CA)					HTZ(TC)	HTZ(CG)		HMZ(GG	HTZ(AG)	HMZ(GG)	HTZ(AG)	HTZ(CA)

Table 6. Patients Treated with EGFR Inhibitors and Genes Associated with the Metabolism of These Agents (HMZ – homozygote; HTZ – heterozygote)

Patient			Disease	Treatment	Genes, associated with the metabolism of EGFR Inhibitors
	age	sex			
					EGFR, c.1562G>A, rs 2227983
1	70	male	CRC	Panitumumab	HMZ(GG)
2	67	male	CRC	Panitumumab	HMZ(GG)
3	75	male	CRC	Panitumumab	HMZ(GG)
4	75	male	CRC	Panitumumab	HMZ(GG)
5	70	male	CRC	Panitumumab, Cetuximab	HMZ(GG)
6	63	male	CRC	Panitumumab	HMZ(GG)
7	61	female	CRC	Panitumumab	HMZ(GG)
8	53	female	NSCLC	Erlotinib	HMZ(GG)
9	64	male	NSCLC	Erlotinib	HTZ(GA)
10	65	male	NSCLC	Erlotinib	HTZ(GA)

Table 7. Patients Treated with Taxanes and Genes Associated with the Metabolism of These Agents (HMZ – homozygote; HTZ – heterozygote)

Patient			Disease	Treatment	Genes associated with the metabolism of 5-FU/ Capecitabine					
	Age	Sex			SOD2	ABCB1	ABCC2	SLCO1B3	SLCO1B3	ERCC2
					c.47T>C	c.22677T>G	c.1249G>A	c.334T>G	c.699G>A	c.2251A>C
					rs4880	rs2032582	rs2273697	rs4149117	ra7311358	rs131814
1	34	female	BC	Docetaxel	HTZ(AC)	HTZ(TG)	HMZ(GG)	HTZ(GT)	HTZ(AG)	HTZ(AC)
2	51	female	BC	Paclitaxel, Docetaxel	HTZ(AC)	HTZ(TG)	HTZ(GA)	HTZ(GT)	HTZ(AG)	HMZ(AA)
3	53	female	BC	Paclitaxel	HMZ(AA)	HTZ(TG)	HMZ(GG)	HMZ(GG)	HMZ(AA)	HTZ(AC)
4	63	female	BC	Docetaxel	HTZ(AC)	HMZ(TT)	HMZ(GG)	HMZ(GG)	HMZ(AA)	
5	31	female	BC	Docetaxel		HTZ(TG)	HMZ(GG)	HMZ(GG)	HMZ(AA)	HMZ(AA)
6	58	female	BC	Docetaxel	HMZ(AA)	HTZ(TG)	HMZ(GG)	HMZ(GG)	HMZ(AA)	HTZ(AC)
7	37	female	BC	Docetaxel	HTZ(AC)	HTZ(TG)	HTZ(GA)	HTZ(GT)	HTZ(AG)	HTZ(AC)
8	67	female	BRCA	Docetaxel			HMZ(GG)	HTZ(GT)	HTZ(AG)	HTZ(AC)
9	53	female	NSCLC	Paclitaxel			HMZ(GG)	HTZ(GT)	HTZ(AG)	
10	54	male	NSCLC	Docetaxel	HTZ(AC)			HMZ(GG)	HMZ(AA)	HTZ(AC)
11	59	female	NSCLC	Paclitaxel		HMZ(TT)	HTZ(GA)	HMZ(GG)	HMZ(AA)	HMZ(AA)
12	74	male	NSCLC	Docetaxel	HTZ(AC)		HMZ(GG)	HMZ(GG)	HMZ(AA)	HTZ(AC)
13	64	male	NSCLC	Docetaxel	HTZ(AC)		HTZ(GA)	HTZ(GT)	HTZ(AG)	HTZ(AC)
14	52	male	NSCLC	Paclitaxel		HTZ(TG)	HMZ(GG)	HMZ(GG)	HMZ(AA)	HMZ(AA)
15	65	male	NSCLC	Docetaxel		HTZ(TG)	HMZ(GG)	HTZ(GT)	HTZ(AG)	HMZ(AA)

10 patients, underscoring its substantial representation. Furthermore, the ABCB1 c.2677T>G variant and the ABCC2 c.1249G>A variant were each identified in 10 and all but one patients, respectively.

The intricate genetic landscape also revealed the presence of two variants within the SLCO1B3 gene—c.334T>G and c.699G>A—both universally present across the entire patient cohort. Lastly, the ERCC2 c.2251A>C variant was identified in 13 patients.

Both patient cohorts, namely those treated with Cyclophosphamide and Farmorubicin, exhibit an

identical composition of six individuals (Table 8). Our meticulous genetic investigation identified six genes harboring variants intricately associated with the metabolism and manifestation of adverse drug reactions within this shared patient subset. Notably, the CYP1B1 c.1294G>C variant was prevalent in 5 patients, while the ABCG2 c.421C>A and ATM c.5557G>A variants were identified in 2 patients each. Additionally, the ABCC2 c.1249G>A and GSTP1 c.313A>G variants were uniformly present across the entirety of both patient groups. Moreover, the TP53 c.215C>G and XRCC1 c.1196A>G variants were identified in all but one patient.

DISCUSSION

The study aimed to identify germline pharmacogenetic variants associated with increased chemotherapy-related toxicity risk. The MTHFR gene, encoding methylenetetrahydrofolate reductase involved in DNA and RNA synthesis, features the c.1286A>C variant linked to reduced enzyme activity. Kristensen et al. associated the CC genotype with heightened toxicity risk in colorectal neoplasm patients treated with Capecitabine, Fluorouracil, Leucovorin, and Oxaliplatin (2). In our cohort, c.1286A>C was detected in 11 of 27 platinum-treated patients (allele frequency 0.2037) and 8 of 17 pyrimidine analogue-treated patients (allele frequency 0.2353). Another MTHFR variant, c.665C>T, correlated with increased adverse effects in colorectal cancer patients (3). In our study, c.665C>T occurred in 14 platinum-treated patients (allele frequency 0.3148) and 8 pyrimidine analogue-treated patients (allele frequency 0.2647).

The DPYD gene, pivotal in pyrimidine metabolism, encodes dihydropyrimidine dehydrogenase, crucial for processing chemotherapy drugs like 5-Fluorouracil and Capecitabine. DPYD variants can diminish DPD activity, heightening toxic effects, particularly with 5-Fluorouracil (linked to DPD deficiency) (4). Four DPYD variants identified in our pyrimidine analogue-treated patients—c.2194G>A (7/17, allele frequency 0.2647), c.1627A>G (4/17, allele frequency 0.1471), c.496A>G (3/17, allele frequency 0.0294), and c.85C>T (all but two, allele frequency 0.6176) – may elevate the risk of adverse reactions to chemotherapy, aligning with previous research (5).

The CYP1B1 gene encodes cytochrome P450 1B1, pivotal in metabolizing various substances, including drugs and toxins. The c.1294G>C variant has been studied for its association with chemotherapeutic toxicity, notably Cyclophosphamide, Doxorubicin, and 5-Fluorouracil (6). In the pyrimidine analogue-treated group (n=17), 11 carriers were identified (allele frequency 0.4118). In the anthracyclines and Cyclophosphamide-treated group (n=6), carriers, all but one, exhibited the variant (allele frequency 0.5833). This highlights the potential role of the CYP1B1 variant in chemotherapy response across distinct patient groups.

Table 8. Patients Treated with Farmorubicin and Cyclophosphamide, and Genes Associated with the Metabolism of These Agents (HMZ – homozygote; HTZ – heterozygote)

Patient			Disease	Treatment	Genes associated with the metabolism of Farmorubicin						
	Age	Sex			CYP11B1	ABCG2	ABCC2	GSTP1	ATM	TP53	XRCC1
					c.1294G>C	c.421C>A	c.1246G>A	c.313A>G	c.5557G>A	c.215C>G	c.1196A>G
					rs1056836	rs2231142	rs2273697	rs1695	rs1801516	rs1042522	rs25487
1	34	female	BC	Farmorubicin, Cyclophosphamide	HMZ(CC)		HMZ(GG)	HTZ(AG)	HTZ(AG)	HTZ(GC)	HTZ(AG)
2	53	female	BC	Farmorubicin, Cyclophosphamide	HTZ(GG)		HMZ(GG)	HTZ(AG		HMZ(GG)	HTZ(AG)
3	31	female	BC	Farmorubicin, Cyclophosphamide	HTZ(GG)	HTZ(AC)	HMZ(GG)	HMZ(AA)		HTZ(GC)	HMZ(AA) GSTP1
4	58	female	BC	Farmorubicin, Cyclophosphamide	HTZ(GG)		HMZ(GG)	HMZ(AA)		HMZ(GG)	HTZ(AG)
5	37	female	BC	Farmorubicin, Cyclophosphamide	HMZ(CC)		HTZ(AG)	HMZ(AA)		HMZ(GG)	HTZ(AG)
6	67	female	BC	Farmorubicin, Cyclophosphamide		HTZ(AC)	HMZ(GG)	HTZ(AG)	HTZ(AG)		

DNA damage, implicated in cell death, aging, and cancer, is recognized and addressed by XPC, a crucial gene in damage removal. Commonly observed XPC gene variants among our patients are c.2815C>A and c.1496C>T. Studies suggest increased adverse reactions risk in individuals with AA and AC genotypes of c.2815C>A and CC and CT genotypes of c.1496C>T when treated with platinum-based chemotherapeutics (7). In our Cisplatin/Oxaliplatin-treated patients (n=27), c.2815C>A was found in 17 (allele frequency 0.4074), and c.1496C>T in all but two patients (allele frequency 0.7222), emphasizing potential implications for platinum-based chemotherapy responses.

The ABCG2 gene, linked to drug resistance in cancer cells, features the c.421C>A variant affecting the pharmacokinetics and toxicity of chemotherapy drugs. Research suggests increased toxicity risk, especially with Cyclophosphamide, Doxorubicin, and Fluorouracil (6). In the pyrimidine analogue-treated group (n=17), 3 carriers were identified (allele frequency 0.0882). In the Cyclophosphamide and Anthracyclines-treated group (n=6), carriers were 2 out of 6 (allele frequency 0.1667), highlighting the potential impact of the ABCG2 variant on chemotherapy responses.

The overexpression of the SLC22A2 gene, observed in various cancers, is linked to multidrug resistance, reducing chemotherapy efficacy (8). The SLC22A2 c.808T>G variant, associated with adverse chemotherapeutic effects, is implicated in increased toxicity in non-small cell lung and colorectal cancer patients receiving platinum-based compounds (9). In our patient group, 8 carriers were identified (allele frequency 0.1667), emphasizing the potential impact of the SLC22A2 variant on platinum-based chemotherapy responses.

In cancer research, the SOD2 gene has been scrutinized for its involvement in tumorigenesis and malignancy progression. The c.47T>C variant is linked to heightened sensitivity to chemotherapy-induced adverse reactions, particularly with taxanes, where T allele homozygotes and heterozygotes face increased risks compared to CC homozygotes (10). In our Docetaxel/Paclitaxel-treated patient group (n=15), 10 carriers were identified (allele frequency 0.4333), highlighting the potential impact of the SOD2 variant on taxane chemotherapy responses.

EGFR signaling inhibitors, including tyrosine kinase inhibitors (TKIs) and monoclonal antibodies, are pivotal targeted therapies for cancer. Recent studies reveal an association between the EGFR c.1562G>A variant and cytotoxicity with EGFR inhibitors (11). In our EGFR inhibitor-treated patient cohort, all individuals were carriers of the variant, yielding an allele frequency of 0.9091, underscoring the potential impact of the EGFR variant on treatment outcomes.

ABCB1 overexpression in tumors is linked to multidrug resistance in cancer chemotherapy (12). Gonzalez-Haba et al. found increased adverse drug reactions in homozygous mutant or heterozygous carriers of the c.2677T>G polymorphism during 5-FU/Capecitabine therapies (13). In our pyrimidine analogue-treated group, all but two were carriers (allele frequency 0.5588). Taxane-treated patients with the wild-type allele, in homozygous or heterozygous state, are at heightened risk of adverse reactions (14). In our taxane-treated group (n=15), carriers were 10 patients (allele frequency 0.4). This underscores the potential impact of ABCB1 polymorphisms on chemotherapy outcomes.

In cancer research, ABCC2 is extensively examined for its role in drug resistance, where overexpression can actively expel drugs from cancer cells, reducing intracellular concentrations. The c.1249G>A variant is associated with increased susceptibility to adverse reactions induced by chemotherapeutics like 5-Fluorouracil, Cyclophosphamide, and Farmorubicin (6). In our pyrimidine analogue-treated group (n=17), all but two were carriers (allele frequency 0.8235). In the Cyclophosphamide and Farmorubicin-treated groups (n=6), all but one were carriers (allele frequency 0.8), highlighting the potential impact of ABCC2 variant on chemotherapy responses.

GSTP1 has been implicated in cancer, potentially contributing to chemotherapy and radiotherapy resistance or influencing carcinogenesis by regulating compound metabolism (15). Literature data suggest a higher risk of toxicity in cancer patients with the c.313A>G polymorphism (genotype AA/AG) receiving 5-Fluorouracil and platinum compounds (16). In our platinum-based chemotherapeutics group (n=27), carriers of the variant constituted the majority, with an allele frequency of 0.7407. Similarly, in the 5-Fluorouracil/Capecitabine group (n=17), carriers predominated, with an allele frequency of 0.7353. This underscores the potential role of the GSTP1 variant in influencing chemotherapy responses.

The ATM gene, vital for maintaining genome stability and DNA repair, plays a pivotal role in safeguarding against chemotherapy-induced DNA damage. Variants in the ATM gene, notably c.5557G>A, have been scrutinized for their asso-

ciation with chemotherapeutic toxicity. Individuals with AA and AG genotypes face a higher risk than those with the GG genotype (6). In our sample, the incidence of the c.5557G>A variant in patients treated with Cyclophosphamide and Farmorubicin was low—2 out of 6 with an allele frequency of 0.1667. This underscores the potential impact of the ATM variant on chemotherapy responses in this specific treatment context.

SLCO1B3 overexpression is noted in cancers like non-small cell lung carcinoma, breast cancer, and colorectal cancer, associated with poor prognosis and chemotherapy resistance, especially to taxanes and platinum-containing medications. The c.334T>G and c.699G>A variants of SLCO1B3 are linked to chemotherapeutic toxicity and drug resistance (17). In our taxane-treated patients (n=27), carriers of c.334T>G were 24 (allele frequency 0.7963), and for c.699G>A, all but one were carriers (allele frequency 0.8704). In the taxane-treated group, all 15 patients were carriers of both variants (allele frequency for both variants 0.7667). This emphasizes the potential impact of SLCO1B3 variants on chemotherapy responses.

The TP53 gene, pivotal in cell growth regulation and tumor suppression, is often disrupted in cancers due to its crucial role in maintaining genomic integrity. Studies on the TP53 c.215C>G variant indicate heightened susceptibility to chemotherapy-induced adverse reactions, including hematological, neurotoxicity, or gastrointestinal toxicity (6). In our pyrimidine analogue-treated group (n=17), carriers were 14 patients with an allele frequency of 0.6471, while in the Farmorubicin and Cyclophosphamide-treated group (n=6), carriers were 5 individuals with an allele frequency of 0.6667. This underscores the potential impact of the TP53 variant on chemotherapy responses in specific treatment contexts.

The XRCC1 gene, crucial in DNA repair, features the c.1196A>G variant associated with chemotherapeutic toxicity, particularly haematological or gastrointestinal toxicity, when treated with 5-Fluorouracil/Capecitabine, Farmorubicin, or Cyclophosphamide (6). In our pyrimidine analogue-treated group, the allele frequency is 0.7059, with carriers constituting all but one individual. Similarly, in the Farmorubicin and Cyclophosphamide-treated group, the allele frequency is 0.5, with all but one individual being carriers. This emphasizes the possible influence of the XRCC1 variant on how chemotherapy is responded to in these particular treatment scenarios.

The ERCC2 gene, alias XPF, encodes the ERCC2 protein, a vital element in the nucleotide excision repair (NER) pathway, countering DNA damage from UV radiation. Studies link the c.2251A>C variant to elevated drug toxicity risk in colorectal neoplasm patients treated with 5-Fluorouracil and Leucovorin or 5-Fluorouracil, Leucovorin, and Oxaliplatin (18). In our pyrimidine analogue-treated group, carriers of the variant were all but three (allele frequency 0.4412), while in the platinum-based chemotherapeutics group, 19 individuals were carriers (allele frequency 0.4074). Another ERCC2 variant, c.934G>A, linked to XPD protein function in DNA repair, may increase drug toxicity risk with platinum-containing chemotherapeutics (19). In our Carboplatin/Oxaliplatin-treated group, carriers numbered 22 individuals, with an allele frequency of 0.5556.

CONCLUSIONS

In conclusion, our study delves into the realm of pharmacogenetics, investigating how genetic variations impact drug metabolism in patients with oncological diseases. The identified 23 germline pharmacogenetic variants within 16 genes present potential associations with the metabolism of chemotherapy drugs, revealing a complex interplay between genetic factors and adverse drug reactions in our cohort of colorectal cancer, non-small cell lung cancer, and breast cancer patients. These findings underscore the importance of personalized medicine in optimizing therapeutic outcomes and minimizing adverse effects. The incorporation of these pharmacogenetic markers into preemptive testing protocols offers a promising avenue for tailoring treatment regimens, ensuring the selection of the most effective medication at precise dosages, while minimizing the risk of toxicity. Embracing pharmacogenetic markers represents a modern and vital approach to individualizing therapy in oncology, paving the way for more effective and safer treatment strategies.

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