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Predictive value of clinical toxicities of chemotherapy with fluoropyrimidines and oxaliplatin in colorectal cancer by *DPYD* and *GSTP1* gene polymorphisms

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Abstract

Background: Fluoropyrimidines and platinum are still widely used for colorectal cancer (CRC) management. Several studies have reported that mutations of dihydropyrimidine dehydrogenase (*DPYD*) and glutathione S-transferase pi-1 (*GSTP1*) polymorphisms are related to chemotherapy-related adverse events. In the present study, we purposed to assess the impact of *DPYD* and *GSTP1* variants on the toxicity of adjuvant chemotherapy risk among the Hakka population, minimize adverse events, and to maximize therapy outcome for individualized treatment.

Methods: Genotyping was examined in 104 patients diagnosed with CRC cases and receiving fluoropyrimidine and platinum drug-based chemotherapy regimen by direct sequencing of *DPYD* and *GSTP1* polymorphisms. Three *DPYD* variants including *2A, *5A, *9A, and *GSTP1* c.313A>G were analyzed and clinical outcomes were assessed.

Results: The data suggest that the incidence of *DPYD**5A, *DPYD**9A, and *GSTP1* c.313A>G variants were 38.4%, 24%, and 32.7%, respectively. *DPYD**2A variant was not found. A total of 23 patients (22.1%) suffered severe vomiting and 19 patients (18.3%) suffered severe anemia. *DPYD**5A polymorphism was found significantly associated with grade 3/4 ulceration ($p = 0.001$). *GSTP1* was determined to be an independent risk factor for severe vomiting and skin ulceration ($p = 0.042$ and $p = 0.018$, respectively). Patients with *GSTP1* c. 313A>G mutant type contributed to a higher risk for grade severe toxicity compared with wild genotype ($p = 0.027$). Nevertheless, no significant difference was found between patients with *DPYD**2A, *5A, and *9A for chemotherapeutic toxicity.

Conclusions: The results demonstrated that *GSTP1* polymorphisms were useful predictors of severe events. Screening of single-nucleotide polymorphisms of *GSTP1* in colorectal cancer patients before chemotherapy may help to realize personalized therapy.

Keywords: *DPYD*, *GSTP1*, Gene polymorphism, Chemotherapy toxicity, Hakka population

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Background

To date, the malignant tumor has become responsible for the majority of global deaths, and its incidence has been increasing over recent decades [1]. For both sexes combined, CRC is the third most prevalent diagnosed gastrointestinal malignancies globally and remains the fifth common cause of mortality by cancer in China, which is responsible for more than 191,000 deaths annually [1, 2].

5-FU and capecitabine, an oral prodrug of 5-FU, are the backbone of the therapeutic scheme for CRC and many other solid tumors including monotherapy and combined with other chemotherapy drugs. Common combination regimens include FOLFOX [3]: (oxaliplatin combined with bolus/infusional 5-FU and leucovorin) and XELOX [4]: (capecitabine plus oxaliplatin). Unfortunately, approximately 10 to 30% of patients display varying degrees of adverse effects [5], most frequent manifestations include anemia, neutropenia, nausea, vomiting, diarrhea, and neurological toxicities. Various clinical studies revealed that dihydropyrimidine dehydrogenase (DPD), encoded by the *DPYD* gene, is the limiting velocity enzyme in the catabolism of fluoropyrimidines. DPD is responsible for catabolism, more than 80% of given fluoropyrimidines. Deficiency of the *DPYD* gene results in excess fluoropyrimidine accumulation in the blood and increases the risk of toxicity. DPD enzyme activity detection currently commonly used methods are western blotting and HPLC [6, 7]. Western blotting requires preliminary experiments to determine the best conditions, and there are many interference factors. HPLC is expensive and not widely available in hospitals. Numerous studies have revealed that the polymorphism of the *DPYD* gene mutation results in a significantly decreased enzyme activity, such as well-known IVS14+1G>A (*DPYD**2A). The *DPYD* IVS14+1G>A alternate splicing in intron 14 resulting in creating a fragmentary protein and leading to the lack of normal DPD enzyme function. *DPYD* c. 1627A>G (*DPYD**5A) and *DPYD* c. 85T>C (*DPYD**9A) are linked with decreased enzyme activity. In codon 29 of *DPYD* at the 85 position, the point mutation of T to C gives rise to an amino acid alteration from Cys to Arg, and *DPYD* 1627A>G substitution results in the *DPYD**5A allele due to the change in I543V. In different ethnic groups, *DPYD* genetic polymorphisms vary widely [8].

Glutathione-S-transferase P1 (*GSTP1*) is a rate-limiting enzyme that catalyzes the detoxification pathway of platinum drugs, for cell protection. The *GSTP1* Ile105Val (rs1695, A>G) is a missense mutation and affects the activity of the *GSTP1* enzyme. Oxaliplatin-related cumulative peripheral neuropathy was reported to be more frequent and severe in patients with heterozygous (AG) genotype when compared to patients with

wild-type alleles (AA) [9]. Wiese et al. [10] conclude that *GSTP1* c.313A>G polymorphism was associated with the overall survival of patients treated with oxaliplatin-based therapy. From the above, we hypothesize that *DPYD* and *GSTP1* polymorphisms could be linked to acute toxicity in patients undergoing 5-FU and oxaliplatin-based chemotherapy.

Our study aims to board the possible relationship between *DPYD* and *GSTP1* genomic variations and adverse reactions with 5-FU and oxaliplatin-based treatment in the Meizhou Hakka population, southern China, a unique Han Chinese ethnic group having its civilization [11]. We performed a systematic retrospective pharmacokinetics study in a cohort of 104 Meizhou Hakka CRC patients for the first time to explore the associations.

Patients and methods

Population study and data collection

One hundred and four registered patients with histologically confirmed CRC who received adjuvant chemotherapy regimen containing 5-FU, capecitabine, or oxaliplatin between September 2016 and December 2017 at the Meizhou People's Hospital were investigated in this retrospective study. Patients had to follow the lists for inclusion criteria such as (1) histopathologically proven colorectal cancers in any stage; (2) aged over 18 years; (3) patients received adjuvant chemotherapy containing 5-FU, capecitabine, or oxaliplatin in any line of treatment and received at least 2 courses for treatment; (4) any previous chemotherapy completed half a year ago, without any other adjuvant chemotherapy or radiotherapy; (5) life expectancy > 4 months; (6) adequate hematological, liver, or kidney functions. Patients were excluded from enrollment as follows: (1) patients with concomitant neoplastic disease other than colorectal cancer; (2) any non-compliance of the inclusion criteria of above; (3) severe renal or hepatic disorder, severe hematological disease before treatment; (4) less than 6 months of previous treatment unless appear greater than grade 3 adverse events. Patients who disagree to sign an informed consent form were excluded from the research. Written informed consent was provided for each patient before any procedure of this study. All participants were then followed up 18 months to obtain their detailed clinical response information and recorded in a medical history sheet. The study protocol was approved by the institution's review and Human Ethics Committees of the Meizhou Peoples' Hospital (No. MPH-HEC 2016-A-40). Clinical characteristics distribution of 55 men and 49 women newly diagnosed with CRC from 2016 to 2017 were enrolled in the final study. The demographic and clinical-pathological features of our study cohort were evaluated as summarized in Table 1. The median age at diagnosis was 56 (range 25–78). All adverse drug

Table 1 Demographic and clinical characteristics of treated patients

Characteristics	Overall patients, n (%)	Adverse events	
		G1-2, n	G3-4, n
Age (years)			
Range	25-78	45-74	25-78
Median	56	61.5	53
≤ 56	50(48.1)	9	44
> 56	54(51.9)	17	29
Gender			
Male	55(52.8)	19	34
Female	49(47.1)	7	39
Smoking status			
Smoker	8(7.6)	2	5
Non-smoker	96(92.3)	24	68
Alcohol intake			
Yes	4(3.8)	2	2
No	100(96.1)	24	71
Anatomic site			
Rectum	39(37.5)	11	27
Colon	65(62.5)	15	46
Histopathological type			
Adenocarcinoma	104(100.0)	26	73
Squamous cell carcinoma	0(0)	0	0
Adenocarcinoma histology			
Poorly differentiated	1(1)	0	1
Moderately differentiated	57(54.8)	13	42
Mucinous adenocarcinoma	3(2.9)	0	3
Undefined	43(41.3)	13	27
Grade of tumor			
I	0(0)	0	0
II	30(28.8)	6	22
III	46(44.2)	15	31
IV	28(27)	5	20
Clinical T-stage			
1	2(1.9)	0	2
2	3(2.9)	1	2
3	65(62.5)	19	44
4	34(32.7)	6	25
Clinical N-stage			
0	33(31.7)	7	24
1	42(40.4)	12	30
2	28(26.9)	7	18
3	1(1)	0	1
Clinical M-stage			
0	76(73)	21	53
1	28(27)	5	20

reactions and toxicity underwent in the front 6 cycles of chemotherapy in each patient were monitored and graded in line with the National Cancer Institute Common Terminology Criteria for Adverse Events v3.0 (CTCAE) [12].

Genotyping

All collected blood samples were anticoagulants with EDTA. Genomic DNA from each enrolled patient was extracted using the DNA Isolation Kit for Blood/Bone Marrow/Tissue (Tiangen Biotech (Beijing) Co., Ltd.). DNA concentration and purity were evaluated using a NanoDrop2000 (Thermo Scientific). The primer sequences and the restriction enzymes used were provided by SINOMD Gene (Beijing) Co., Ltd. Genomic DNA was used for the cyclic condition consisted of one pre-denaturation step at 95 °C for 3 min initial cycle, then 45 cycles of 94 °C for 15 s, 63 °C for 1 min and extension at 72 °C for 1 min, and a final elongation step of 10 min at 72 °C. PCR products were purified with ExoSap-It (ABI PCR Product Cleanup Reagent). Single-nucleotide polymorphisms were analyzed using ABI 3500 Dx Genetic Analyzer (ABI Terminator v3.1 Cycle Sequencing kit) and Sequencing Analysis v5.4 (Life Technologies, CA, USA). We looked for the following SNPs: *DPYD**2A, *DPYD**5A, *DPYD**9A, and *GSTP1*.

Toxicity grading and statistical analysis

All quantitative variables such as age, gender, smoking, alcohol intake, tumor location, neoplasms histologic type, grade, and stage of the tumor were analyzed. Toxicity including hematopoietic toxicity (anemia, leucopenia, neutropenia, and thrombocytopenia), gastrointestinal toxicity (mucositis, vomiting, and diarrhea), and dermatologic toxicity (ulceration and hand-foot syndrome) were grouped by four classes. Association between *DPYD* and *GSTP1* variant status with a response and different toxicity was evaluated by Fisher's exact test. The Hardy-Weinberg equilibrium (HWE) of *DPYD* and *GSTP1* genotypes were assessed using the χ^2 test. A *p* value of less than 0.05 was deemed to be statistically significant. All tests were carried out by the SPSS software version 21.0 (SPSS Inc., Chicago, USA)

Results

Histopathological type and clinical stage

As shown in Table 1, colon cancer was more predominant than rectum cancer (62.54 and 37.5%, respectively) about tumor location. All histopathological types were adenocarcinoma. Seventy-four (46.9%) patients were affected by stage III–IV tumors. Following the American Joint Committee on Cancer's (AJCC) Cancer Staging 6th edition 2002 TNM grading systems, there were 28.8% of

Table 2 Toxicity of chemotherapy in the study participants

Toxicities	Grade 1–2 n (%)	Grade 3–4 n (%)
Hematological toxicity		
Anemia	46(44.2)	19(18.3)
Leucopenia	48(46.2)	10(9.6)
Neutropenia	39(37.5)	16(15.4)
Thrombocytopenia	39(37.5)	12(11.5)
Gastrointestinal toxicity		
Mucositis	4(3.8)	9(8.7)
Vomiting	27(25.9)	23(22.1)
Diarrhea	12(11.0)	9(8.7)
Dermatology toxicity		
Skin ulceration	5(4.8)	4(3.8)
Hand-foot skin reaction	11(10.6)	5(4.8)

stage II, 44.2% of stage III, and 27% of stage IV about the grade of the tumor, but none of stage I.

Chemotherapy toxicity

The toxicity of chemotherapy in the study participants is shown in Table 2. Treatment of fluoropyrimidine and platinum combination chemotherapy was made at the time of the first diagnosis of CRC. All patients received chemotherapy regimens for at least 2 cycles and were evaluated for adverse event outcomes. Of the total 104 cases, 72 patients (69.2%) develop severe chemotherapy-related toxicities, including 18.3% suffered from anemia, 9.6% for leukopenia, 15.4% for neutropenia, 11.5% for thrombocytopenia, 8.7% for mucositis, 8.7% for diarrhea, 22.1% for vomiting, and 8.6% for dermatological toxicities.

Dihydropyrimidine dehydrogenase and glutathione S-transferase pi-1 genotypes

In our cohort, all patients were genotyped for *DPYD**2A, *DPYD**5A, *DPYD**9A, and *GSTP1* variants. The *DPYD* [IVS] 14+1G>A mutation was not identified in any of this study patients. In the case of *DPYD**5A polymorphism, *DPYD* c. 1627A>G was present in 33 (31.7%) patients with heterozygous and 7 (6.7%) in homozygous, and there were 76% wild types and 24% heterozygote mutants to *DPYD* c. 85T>C. The results also show that the distribution of *GSTP1* A/A, *GSTP1* A/G, and *GSTP1* G/G was 67.3%, 29.8%, and 2.9%, respectively (Table 3). Determined SNPs were found to be in Hardy-Weinberg Equilibrium.

Adverse events

The distribution of toxicity classification of patients with *DPYD* and *GSTP1* wild type and mutant was shown in Tables 4 and 5, respectively. Among patients screened for *DPYD* mutant (*n* = 56), 40 patients had *DPYD* c.

Table 3 DPYD and GSTP1 genes frequency in the study patients

Gene	Genotypes	No. of patients	Percentage (%)
DPYD*2A	WT(GG)	104	100.0
	HET(GA)	0	0.0
	HOM(AA)	0	0.0
DPYD*5A	WT(AA)	64	61.5
	HET(AG)	33	31.7
	HOM(GG)	7	6.7
DPYD*9A	WT(TT)	79	76.0
	HET(TC)	25	24.0
	HOM(CC)	0	0.0
GSTP1	WT(AA)	70	67.3
	HET(AG)	31	29.8
	HOM(GG)	3	2.9

WT = wild type, HET = heterozygote, HOM = homozygote

1627A>G (DPYD*5A) variant, while DPYD c. 85T>C (DPYD*9A) variant exists in 25 patients. Out of them in 9 patients with both DPYD*5A and DPYD*9A heterozygous. The most commonly experienced grade 3 or grade 4 toxicity in both DPYD wild-type and mutant patients was anemia in hematological and vomiting in gastrointestinal toxicity (Table 4). The same results were found in patients with GSTP1 wild type and mutants (Table 5).

Association between SNPs and toxicities

Association between polymorphisms of DPYD and GSTP1 genes and toxicities of chemotherapy outline is shown in Tables 6, 7, and 8. Adverse events were

experienced in most patients who received chemotherapy after the first or second cycle. We further examined the association between these SNPs and adverse events in all individuals. The existence of the GSTP1 c. 313A>G polymorphism was associated with platinum related to several toxicities such as vomiting and skin ulceration ($p = 0.042$ and $p = 0.018$, respectively). We also found that patients with the AA genotype for GSTP1 presented lower rates of serious vomiting (35.3%) than patients with the AG and GG genotype (66.7% and 100%, respectively, $p = 0.027$). Hand-foot syndrome and hematopoietic toxicity could not be demonstrated a statistically significant association with GSTP1 genotype in our study. However, 5-FU and capecitabine-related toxicities were not correlated to DPYD polymorphism, although there was a statistical trend for more diarrhea in the patients when carrying the mutant DPYD*5A A/G genotype.

Discussion

Fluorouracil (FU) is the most common component of chemotherapy agents for malignant tumors like leukemia, lung, breast, gastric, and colorectal cancers. Falzacappa et al. demonstrated combined fluorouracil, and rucaparib was able to enhance the efficacy and specificity of acute myeloid leukemia [13]. Adrian Bogdan-Tigu et al. highlighted that the combination of allicin with 5-FU has an obvious against lung and colorectal cancer effect while reducing the dosage of fluorouracil [14]. For breast cancer, fluorouracil (5-FU) is widely used and a growing number of physicians are using capecitabine as first-line or second-line chemotherapy [15].

Table 4 The frequency of all grade toxicities in patients with DPYD wild type and mutants

Toxicities	DPYD wild type (n = 48)		DPYD mutant (n = 56)			
	G 1-2	G 3-4	DPYD*5A (n = 40*)	DPYD*9A (n = 25*)	G 1-2	G 3-4
Hematological toxicity						
Anemia	22(21.2)	9(8.7)	17(16.3)	8(7.7)	12(11.5)	4(3.8)
Leucopenia	21(20.2)	4(3.8)	20(19.2)	4(3.8)	11(10.6)	3(2.9)
Neutropenia	17(16.3)	5(4.8)	17(16.3))	8(7.7)	8(7.7)	6(5.8)
Thrombocytopenia	16(15.4)	4(3.8)	16(15.4)	6(5.8)	12(11.5)	4(3.8)
Gastrointestinal toxicity						
Mucositis	2(1.9)	7(6.7)	2(1.9)	2(1.9)	0(0)	0(0)
Vomiting	12(11.5)	13(12.5)	13(12.5)	7(6.7)	5(4.8)	4(3.8)
Diarrhea	7(6.7)	7(6.7)	5(4.8)	2(1.9)	1 (0.9)	1(0.9)
Dermatology toxicity						
Skin ulceration	1(0.9)	1(0.9)	4(3.8)	3(2.9)	0(0)	0(0)
Hand-foot skin reaction	4(3.8)	1(0.9)	6(5.8)	4(3.8)	1(0.9)	0(0)

Data are presented as n (%)

*9 patients had double heterozygous status for DPYD*5A and DPYD*9A variants

Table 5 The frequency of all grade toxicities in patients with *GSTP1* wild type and mutants

Toxicities	<i>GSTP1</i> wild type (n = 70)		<i>GSTP1</i> mutant (n = 34)	
	G 1–2	G 3–4	G 1–2	G 3–4
Hematological toxicity				
Anemia	31(29.8)	14(13.5)	15(14.4)	5(4.8)
Leucopenia	35(33.7)	7(6.7)	13(12.5)	3(2.9)
Neutropenia	30(28.8)	13(12.5)	9(8.7)	3(2.9)
Thrombocytopenia	26(25.0)	8(7.7)	13(12.5)	4(3.8)
Gastrointestinal toxicity				
Mucositis	2(1.9)	7(6.7)	2(1.9)	2(1.9)
Vomiting	22(21.2)	12(11.5)	5(4.8)	11(10.6)
Diarrhea	8(7.7)	7(6.7)	4(3.8)	2(1.9)
Dermatology toxicity				
Skin ulceration	5(4.8)	1(0.9)	0(0)	3(2.9)
Hand-foot skin reaction	8(7.7)	2(1.9)	3(2.9)	3(2.9)

Data are presented as n (%)

Adjuvant chemoradiotherapy based on 5-fluorouracil is the first choice after gastric cancer surgery. Li et al. suggested that accurate classification of GC patients who may benefit from 5-FU adjuvant chemoradiotherapy will help clinicians formulate more effective GC treatment regimens [16].

Fluorouracil-based treatment is generally well tolerated, except about 5 to 10% patient who suffers grade > 3 toxicity, which even impacts on the quality of life [17]. Intolerance of 5-FU is usually because of decreased activity of the fluoropyrimidine detoxifying enzyme dihydropyrimidine dehydrogenase (DPD) [18]. DPD is known to be the initial and rate-limiting enzyme catalyze

degradation of more than 85% of the given 5-FU. Activity levels vary caused by variable phenotypes and genetic alterations [19]. Single-nucleotide polymorphisms (SNPs) in its encoding gene *DPYD* is the most common cause of deficiency in DPD enzyme activity [20]. Analyzing the impact of *DPYD* genotype in serum levels of 5-FU and correlative toxicity is vital to achieving good curative effect and prevent adverse events. In this research, we looked for adverse events related to fluoropyrimidines and platinum drugs-based treatment and their association with *DPYD* and *GSTP1* gene polymorphisms. Vomiting and skin ulceration was the most chemotherapy-related toxicity observed in our study.

Table 6 Association between polymorphisms of *DPYD* and *GSTP1* genes and hematological toxicity of chemotherapy

Genotype		Anemia			Neutropenia			Thrombocytopenia			Leucopenia		
		Grade 1–2	Grade 3–4	P	Grade 1–2	Grade 3–4	P	Grade 1–2	Grade 3–4	P	Grade 1–2	Grade 3–4	P
DPYD*5A (c.1627A > G)	WT	29	11	Ref.	22	8	Ref.	23	6	Ref.	28	6	Ref.
	HET	16	7	0.804	14	7	0.607	14	5	0.650	17	4	0.896
	HOM	1	1	0.492	3	1	0.943	2	1	0.614	3	0	0.427
	HET + HOM	17	8	0.698	17	8	0.665	16	6	0.583	20	4	0.922
DPYD*9A (c.85T>C)	WT	34	15	Ref.	31	10	Ref.	27	8	Ref.	37	7	Ref.
	HET	12	4	0.668	8	6	0.189	12	4	0.867	11	3	0.794
	HOM	0	0	–	0	0	–	0	0	–	0	0	–
	HET + HOM	12	4	0.668	8	6	0.189	12	4	0.867	11	3	0.794
<i>GSTP1</i> (c.313A>G)	WT	31	14	Ref.	30	13	Ref.	26	8	Ref.	35	7	Ref.
	HET	14	4	0.480	9	3	0.724	11	3	0.875	11	2	0.913
	HOM	1	1	0.575	0	0	–	1	1	0.401	2	1	0.466
	HET + HOM	15	5	0.617	9	3	0.724	12	4	0.910	13	3	0.851

Table 7 Association between polymorphisms of *DPYD* and *GSTP1* genes and gastrointestinal toxicity of chemotherapy

Genotype		Mucositis			Vomiting			Diarrhea		
		Grade 1–2	Grade 3–4	P	Grade 1–2	Grade 3–4	P	Grade 1–2	Grade 3–4	P
DPYD*5A (c.1627A>G)	WT	2	7	Ref.	14	16	Ref.	7	7	Ref.
	HET	1	2	0.700	12	5	0.113	4	2	0.052
	HOM	1	0	0.107	2	2	0.900	1	0	0.533
	HET + HOM	2	2	0.317	14	7	0.158	5	2	0.107
DPYD*9A (c.85T>C)	WT	4	9	Ref.	23	19	Ref.	11	8	Ref.
	HET	0	0	–	5	4	0.965	1	1	0.830
	HOM	0	0	–	0	0	–	0	0	–
	HET + HOM	0	0	–	5	4	0.965	1	1	0.830
GSTP1 (c.313A>G)	WT	2	7	Ref.	22	12	Ref.	8	7	Ref.
	HET	2	2	0.317	5	10	0.042	4	2	0.577
	HOM	0	1	0.598	0	1	0.187	0	0	–
	HET + HOM	2	3	0.480	5	11	0.027	4	2	0.577

The allelic variant (A>G) at position 1627 amino acid in exon 13 of the *DPYD* gene results in an exchange of Isoleucine to Valine at codon 543 of *DPYD*5A*. There is a wide interethnic and inter geographical difference about gene polymorphism and frequency of *DPYD*5A*. *DPYD*5A* G/G + G/A genotype was identified in 38.4% participants, obviously more than that in African-Americans (22.7%) [21] and Caucasian subjects (28%) [22], and similar to that in Japanese (35%) [23]. *DPYD*9A* polymorphism is regarded as one prevalent *DPYD* variants. The TT and TC genotype proportion of *DPYD*9A* in our enrolled patients was 76% and 24%, respectively, whereas the homozygous *DPYD*9A* genotype was not found. The effect of *DPYD*9A* polymorphism on fluoropyrimidine treatment-related toxicity has been reported in previous researches. Khushman et al. [24]

showed that *DPYD* c. T85C mutant variant was associated with diarrhea ($p = 0.0055$). Joerger [25] reported that the subjects carrying the *DPYD*9A* C allele significantly increase the risk for severe diarrhea compared to patients with the T/T genotype ($p = 0.033$). Nevertheless, several other studies suggest different points of view. Maarten [26], Amirfallah [27], and McLeod et al. [28] revealed that *DPYD* heterozygous for 85T>C was not associated with a decreased risk for grade 3 to 4 toxicity. In our study cohort, we haven't found a statistically significant relationship between *DPYD*2A*, **5A*, **9A* polymorphism and severe chemotherapy-related toxicity, owing to the limited size of the cohort in our study, and different allele frequency of these gene variants among different populations in different regions.

Table 8 Association between polymorphisms of *DPYD* and *GSTP1* genes and dermatology toxicity of chemotherapy

Genotype		Skin ulceration			Hand-foot skin reaction		
		Grade 1–2	Grade 3–4	P	Grade 1–2	Grade 3–4	P
DPYD*5A (c.1627A>G)	WT	2	1	Ref.	5	1	Ref.
	HET	2	1	1.000	4	3	0.308
	HOM	2	2	0.659	2	1	0.571
	HET + HOM	4	3	0.778	6	4	0.330
DPYD*9A (c.85T>C)	WT	6	4	Ref.	10	5	Ref.
	HET	0	0	–	1	0	0.486
	HOM	0	0	–	0	0	–
	HET + HOM	0	0	–	1	0	0.486
GSTP1 (c.313A>G)	WT	5	1	Ref.	8	2	Ref.
	HET	0	3	0.018	3	3	0.210
	HOM	0	0	–	0	0	–
	HET + HOM	0	3	0.018	3	3	0.210

As a part of the phase II detoxification system, glutathione S-transferase (GST) superfamily members detoxifies cisplatin by the formation of glutathione conjugates, conversion to less toxic and increases its excretion from the body, resulting in the detoxification [29]. It is important to note that polymorphisms in GSTs are linked to the occurrence and development of various tumors by way of altering biological pathways and affecting protein expression [30]. Genes encoding GSTs, including *GSTP1*, different genotypes have different effects on the efficacy to detoxify exogenous and endogenous toxic species. Extensive research has been carried out to reveal the 313A>G (*GSTP1*) mutation is an A to G replacement at codon 105 leading to a transition in the amino acid from Isoleucine to Valine, which has been found to decrease enzymatic activity, and highly linked for causing platinum treatment-related toxicity in different cancers.

In previous studies, Santric [31] reported a significant association between *GSTP1* Ile105Val polymorphism and oxaliplatin-related toxicity. Kudhair [32] analyzed Arab populations and revealed that carrying *GSTP1* Ile105Val substitution is positively correlated with a higher risk for lung cancer in WP tobacco smokers. Conversely, Sophonnithiprasert [33] reported an insignificant difference between *GSTP1* Ile105Val polymorphism and oxaliplatin-induced toxicity. Our research showed that *GSTP1* c. 313A>G polymorphism was linked to acute toxicity in colorectal cancer patients undergoing skin ulceration, which was conflicting with research from Terrazzino [34] and Osti [35]. We also found that those participants having heterozygotes and homozygotes genotype showed a higher risk of high-grade severe toxicity compared to the wild type of *GSTP1*, in line with the reported study of Mir [36]. According to the research by Watson, it was suggested that GST enzyme activities were significantly reduced among individuals with Val-containing [37]. Besides, there was lower activity toward chemotherapy drug tolerance [38]. As mentioned above, the *GSTP1* c.313 A > G mutation leads to a decrease in enzyme activity, thereby reducing the ability to detoxify platinum and other substances. Skin ulceration may be a direct result of the transport of the residual oxaliplatin active substance to the skin surface via sweat. Oxaliplatin results in the formation of intra and inter strands crosslinks, causing potential errors in DNA repair and damaged DNA. Finally, it diminishes numbers of endothelial cells and dermal matrix regeneration, leading to the occurrence and development of skin ulceration.

In Table 7, we found an interesting association between *GSTP1* A/G genotype and severe vomiting in patients ($p = 0.042$), which consistent with Carron et al. [39]. As the detoxification ability of *GSTP1* A/G genotype decreases, platinum accumulates in the body causes

vomiting by inducing DNA damage in epithelial enterochromaffin cells of the intestine, thereby leading to serotonin release and stimulation of the vomiting center.

There are some limitations to our study. First, the number of subjects enrolling in the research is relatively small. Second, we did not investigate other SNPs of GST associated with oxaliplatin-related toxicities. The third limitation of this study is that we focus on only three out of more than 30,000 variants reported *DPYD* variants. Moreover, there is a difference in chemotherapy regimens. Future analysis will enroll a larger cohort and reveal the potential associations between more mutation combinations of *DPYD* and GST variants and severe toxicities.

Conclusions

In brief, skin ulceration and vomiting were the major dose-limiting toxicity of fluoropyrimidine and platinum-based regimens in this cohort. A significant correlation was confirmed between *GSTP1* polymorphism and toxicity like vomiting and skin ulceration strengthening *GSTP1* polymorphism as a predictor of severe events. Screening of genetic polymorphism of *GSTP1* in colorectal cancer patients before chemotherapy could contribute to effectively decrease the occurrence of serious adverse toxicity, improve the predictive specificity for a severe event. We indicate that the detection of *GSTP1* phenotype may become a routine laboratory test item. This may enable clinicians to implement the optimal therapeutic schedule to achieve satisfactory efficacy and minimize toxic and side effects, and then, lead to personalized therapy. Further studies should focus on validating results in a larger sample size, ascertain possible interactions between other *DPYD* and GST polymorphisms, and clinical outcomes.

Abbreviations

CRC: Colorectal cancer; *DPYD*: Dihydropyrimidine dehydrogenase; *GSTP1*: Glutathione S-transferase pi-1; SNP: Single-nucleotide polymorphisms

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Authors' contributions

Xunwei Deng performed data collection, analyzed the data, interpretation of the results, and wrote this paper. All of the authors designed this study, read, and gave final approval of the submitted version.

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Ethics approval and consent to participate

The study protocol was approved by the institution's review and Human Ethics Committees of the Meizhou Peoples' Hospital (No. MPH-HEC 2016-A-40).

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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References

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2008: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68:394–424.
- Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. *CA Cancer J Clin*. 2016;66:115–32.
- Pintova S, Dharmapuri S, Moshier E, Zubizarreta N, Ang C, Holcombe RF. Genistein combined with FOLFOX or FOLFOX-Bevacizumab for the treatment of metastatic colorectal cancer: phase I/II pilot study. *Cancer Chemotherapy Pharmacol*. 2019;84:591.
- Borner M, Mingrone W, Koeberle D, Von Moos R, Rauch D, Saletti P, Herrmann R, Dietrich D, Lanz D, Roth A. The impact of cetuximab on the capecitabine plus oxaliplatin (XELOX) combination in first-line treatment of metastatic colorectal cancer (MCC): A randomized phase II trial of the Swiss Group for Clinical Cancer Research (SAKK). *Annals Oncol*. 2006;19:1288–92.
- Twelves C, Wong A, Nowacki MP, Abt M, Burris H, Carrato A, Cassidy J, Cervantes A, Fagerberg J, Georgoulis V. Capecitabine as Adjuvant Treatment for Stage III Colon Cancer. *New England J Med*. 352:2696–704.
- Hishinuma E, Narita Y, Saito S, Maekawa M, Akai F, Nakanishi Y, Yasuda J, Nagasaki M, Yamamoto M, Yamaguchi H, et al. Functional characterization of 21 allelic variants of dihydropyrimidine dehydrogenase identified in 1070 Japanese Individuals. *Drug Metab Dispos*. 2018;46:1083–90.
- Hahn RZ, Galarza AF, Schneider A, Antunes MV, Schwartzmann G, Linden R. Improved determination of uracil and dihydrouracil in plasma after a loading oral dose of uracil using high-performance liquid chromatography with photodiode array detection and porous graphitic carbon stationary phase. *Clin Biochem*. 2015;48:915–8.
- Etienne MC, Lagrange JL, Dassonville O, Fleming R, Thyss A, Renee N, Schneider M, Demard F, Milano G. Population study of dihydropyrimidine dehydrogenase in cancer-patients. *Bull Cancer*. 1995;12:2248.
- Katayanagi S, Katsumata K, Mori Y, Narahara K, Shigoka M, Matsudo T, et al. GSTP1 as a potential predictive factor for adverse events associated with platinum-based antitumor agent-induced peripheral neuropathy. *Oncology Letters*. 2019;17:2897–904.
- Wiese MD, Sorich MJ, Kichenadasse G, Ridha Z. Pharmacogenetic and ethnicity influence on oxaliplatin therapy for colorectal cancer: a meta-analysis. *Pharmacogenomics*. 2016;17(15):1725–32.
- Pan S, Yang X, Yang L, Wei Q, Yang Y, Xu G. Human GSTs polymorphisms in the Hakka population of south China and their associations with family history of several chronic diseases. *Biomed Environmental Sci*. 2011;24:491–8.
- Program CTE: Common Terminology Criteria for Adverse Events v3.0 (CTCAE). 2003. <https://ctep.cancer.gov/reporting/ctcnewhtml>. Accessed 3 May 2019.
- Falzaccappa MV, Ronchini C, Faretta M, Iacobucci I, Di Rorà AG, Martinelli G, Meyer LH, Debatin KM, Orecchioni S, Bertolini F, Pelicci PG. The combination of the PARP inhibitor rucaparib and 5FU is an effective strategy for treating acute leukemias. *Mol Cancer Ther*. 2015;14:889–98.
- Tjigu AB, Toma VA, Moț AC, Jurj A, Moldovan CS, Fischer-Fodor E, Berindan-Neagoe I, Părvu M. The synergistic antitumor effect of 5-fluorouracil combined with alicin against lung and colorectal carcinoma cells. *Molecules*. 2020;25:1947.
- Claessens AKM, Ibragimova KIE, Geurts SME, Bos M, Erdkamp FLG, Tjan-Heijnen VCG. The role of chemotherapy in treatment of advanced breast cancer: an overview for clinical practice. *Crit Rev Oncol Hematol*. 2020;153:102988.
- Li M, Chen H, He J, Xie J, Xia J, Liu H, Shi Y, Guo Z, Yan H. A qualitative classification signature for post-surgery 5-fluorouracil-based adjuvant chemoradiotherapy in gastric cancer. *Radiother Oncol*. 2020;S0167-8140(20):30847–1.
- Koopman M, Antonini NF, Douma J, Wals J, Honkoop AH, Erdkamp FL, Jong RSD, Rodenburg CJ, Vreugdenhil G, Loosveldt OJ: Sequential versus combination chemotherapy with capecitabine, irinotecan, and oxaliplatin in advanced colorectal cancer (CAIRO): a phase III randomised controlled trial. *Lancet*. 370:0-142.
- Tuchman M. Familial pyrimidinemia and pyrimidinuria associated with severe fluorouracil toxicity. *N Engl J Med*. 1985;313.
- Amstutz U, Froehlich TK, Largiadèr CR. Dihydropyrimidine dehydrogenase gene as a major predictor of severe 5-fluorouracil toxicity. *Pharmacogenomics*. 2011;12:1321–36.
- Lunenburg CATC, Henricks LM, Guchelaar HJ, Swen JJ, Deenen MJ, Schellens JHM. Prospective DPYD genotyping to reduce the risk of fluoropyrimidine-induced severe toxicity: Ready for prime time. *Eur J Cancer (Oxford, England : 1990)*. 2016;54:40–8.
- Ridge SA, Sludden J, Brown O, Robertson L, Wei X, Sapone A, et al. Dihydropyrimidine dehydrogenase pharmacogenetics in Caucasian subjects. *British J Clin Pharmacol*. 1998;46:151–6.
- McLeod HL, Collie-Duguid ESR, Vreken P, Johnson MR, Wei X, Sapone A, Diasio RB, Fernandez-Salguero P, Van Kuilenberg ABP, Van Gennip AH. Nomenclature for human DPYD alleles. *Pharmacogenetics*. 1998;8:455–60.
- Kubota T. 5-Fluorouracil and dihydropyrimidine dehydrogenase. *Int J Clin Oncol*. 2003;8:127–31.
- Khushman MD, Patel GK, Hosein PJ, Laurini JA, Cameron D, Clarkson DR, Butler TW, Norden CW, Baliem W, Jones V. Germline pharmacogenomics of DPYD*9A (c.85T>C) variant in patients with gastrointestinal malignancies treated with fluoropyrimidines. *J Gastrointestinal Oncol*. 2018;9:416–24.
- Joerger M, Huitema ADR, Boot H, Cats A, Doodeman VD, Smits PHM, Vainchtein L, Rosing H, Meijerman I, Zueger M. Germline TYMS genotype is highly predictive in patients with metastatic gastrointestinal malignancies receiving capecitabine-based chemotherapy. *Cancer Chemotherapy Pharmacol*. 2015;75:763–72.
- Deenen MJ, Tol J, Burylo AM, Doodeman VD, De Boer A, Vincent A, Guchelaar HJ, Smits PHM, Beijnen JH, Punt CJA. Relationship between single nucleotide polymorphisms and haplotypes in DPYD and toxicity and efficacy of capecitabine in advanced colorectal cancer. *Clin Cancer Res Official J Am Assoc Cancer Res*. 2011;17:3455–68.
- Amirfallah A, Kocal GC, Unal OU, Ellidokuz H. DPYD, TYMS and MTHFR genes polymorphism frequencies in a series of Turkish colorectal cancer patients. *J Pers Med*. 2018;8(4):45.
- McLeod HL, Sargent DJ, Marsh S, Green EM, Goldberg RM. Pharmacogenetic predictors of adverse events and response to chemotherapy in metastatic colorectal cancer: results from North American Gastrointestinal Intergroup Trial N9741. *J Clin Oncol*. 2010;28:3227–33.
- Hayes JD, Strange RC. Glutathione S-transferase polymorphisms and their biological consequences. *Pharmacology*. 2000;61:154–66.
- Huang GZ, Shan W, Zeng L, Huang LG. The GSTP1 A1578G polymorphism and the risk of childhood acute lymphoblastic leukemia: results from an updated meta-analysis. *Genetics Molecular Res*. 2013;12:2481–91.
- Santric V, Djokic M, Suvakov S, Pljesa-Ercegovac M, Nikitovic M, Radic T, et al. GSTP1 rs1138272 polymorphism affects prostate cancer risk. *Medicina (Kaunas, Lithuania)*. 2020;56(3):128.
- Kudhair BK, Alabid NN, Asghar T-K, Lafta IJ. Correlation of GSTP1 gene variants of male Iraqi waterpipe (Hookah) tobacco smokers and the risk of lung cancer. *Molecular Biology Reports*. 2020;47(4):2677–84.
- Sophonithprasert T, Saelee P, Pongtheerat T. Glutathione S-transferase P1 polymorphism on Exon 6 and risk of hepatocellular carcinoma in Thai male patients. *Oncology*. 2020;98(4):243–7.
- Terrazzino S, La Mattina P, Masini L, Caltavuturo T, Gambaro G, Canonico PL, Genazzani AA, Krenghli M. Common variants of eNOS and XRCC1 genes may predict acute skin toxicity in breast cancer patients receiving radiotherapy after breast conserving surgery. *Radiother Oncol*. 2012;103:199–205.
- Osti MF, Nicosia L, Agolli L, Gentile G, Falco T, Bracci S, Di Nardo F, Minniti G, De Sanctis V, Valeriani M, et al. Potential role of single nucleotide polymorphisms of XRCC1, XRCC3, and RAD51 in predicting acute toxicity in rectal cancer patients treated with preoperative radiochemotherapy. *Am J Clin Oncol*. 2017;40:535–42.
- Mir O, Alexandre J, Tran A, Durand JP, Goldwasser F. Relationship between GSTP1 Ile 105Val polymorphism and docetaxel-induced peripheral

neuropathy: Clinical evidence of a role of oxidative stress in taxane toxicity. *Ann Oncol.* 2009;20:736–40.

37. Watson MA, Stewart RK, Smith GB, Massey TE, Bell DA. Human glutathione S-transferase P1 polymorphisms: relationship to lung tissue enzyme activity and population frequency distribution. *Carcinogenesis.* 1998;2:275–80.
38. Sweeney C, McClure GY, Fares MY, Stone A, Ambrosone CB. Association between survival after treatment for breast cancer and glutathione S-transferase P1 Ile105Val polymorphism. *Cancer Research.* 2000;60:5621–4.
39. Carron J, Lopes-Aguiar L, Costa EFD, Nogueira GAS, Lima TRP, Pincinato EC, Visacri MB, Quintanilha JCF, Moriel P, Lourenço GJ, Lima CSP. GSTP1 c.313A>G, XPD c.934G>A, XPF c.2505T>C and CASP9 c.-1339A>G Polymorphisms and severity of vomiting in head and neck cancer patients treated with cisplatin chemoradiation. *Basic Clin Pharmacol Toxicol.* 2017; 121:520–5.

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