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RESEARCH ARTICLE

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Dihydropyrimidine dehydrogenase (DPYD) gene c.1627A>G A/G and G/G genotypes are risk factors for lymph node metastasis and distant metastasis of colorectal cancer

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Abstract

Background: Dihydropyrimidine dehydrogenase (DPD) acts as the key enzyme catabolizing pyrimidines, and may affect the tumor progression. *DPYD* gene mutations affect DPD activity. The relationship between *DPYD* IVS14+1G>A, c.1627A>G, c.85T>C and lymph node metastasis (LNM) and distant metastasis (DM) of colorectal cancer (CRC) was investigated.

Methods: A total of 537 CRC patients were enrolled in this study. *DPYD* polymorphisms were analyzed by polymerase chain reaction (PCR)-Sanger sequencing. The relationship between *DPYD* genotypes and clinical features of patients, metastasis of CRC was analyzed.

Results: About *DPYD* c.1627A>G, A/A (57.7%) was the most common genotype, followed by A/G (35.6%), G/G (6.7%) genotypes. In c.85T>C, T/T, T/C, and C/C genotypes are accounted for 83.6%, 16.0%, and 0.4%, respectively. Logistic regression analysis revealed that *DPYD* c.1627A>G A/G and G/G genotypes in the dominant model (A/G + G/G vs. A/A) were significant risk factors for the LNM ($p = 0.029$, OR 1.506, 95% CI = 1.048–2.165) and DM ($p = 0.039$, OR 1.588, 95% CI = 1.041–2.423) of CRC. In addition, *DPYD* c.1627A>G polymorphism was more common in patients with abnormal serum carcinoembryonic antigen (CEA) (>5 ng/ml) ($p = 0.003$) or carbohydrate antigen 24–2 (CA24-2) (>20 U/ml) level ($p = 0.015$).

Conclusions: The results suggested that *DPYD* c.1627A>G A/G, G/G genotypes are associated with increased risk of LNM and DM of CRC.

KEYWORDS

colorectal cancer, dihydropyrimidine dehydrogenase, distant metastasis, *DPYD*, lymph node metastasis

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1 | INTRODUCTION

With the burden of cancer morbidity and mortality rapidly growing worldwide, cancer is a major barrier to increasing life expectancy worldwide.[1] Colorectal cancer (CRC) is one of the most common gastrointestinal malignancies. According to the Global Cancer Statistics in 2020 by International Agency for Research on Cancer (IARC), CRC is the third most prevalent cancer and the second leading cause of cancer death in the world.[2] In clinical treatment, CRC can be treated with endoscopic treatment, surgical resection, chemotherapy drugs, targeted drugs, immunotherapy, and radiation.[3,4] The multiple disciplinary team (MDT) model also improved the treatment level of CRC.[5] However, the recurrence and metastasis of CRC are the major problems affecting the survival of the patients. Metastasis is the process by which cancer cells spread from the primary lesion to the distal organs and is the leading cause of cancer mortality.[6] Metastasis of CRC includes lymph nodes metastasis (LNM) and distant metastasis (DM).[7]

Capecitabine is an oral prodrug of 5-fluorouracil (5-FU) and has been approved for the treatment of various malignancies.[8] There has been reports that the curative effect and toxic effects of 5-FU exist noticeable individual differences.[9] After fluorouracil administration, 5-FU can be transformed into 5-fluoro-2'-deoxyuridine 5' monophosphate (FdUMP), 5-fluoro-2'-deoxyuridine 5'-triphosphate (FdUTP), and 5-fluorouridine 5'-triphosphate (FUTP) in cells, which are three cytotoxic metabolites.[10] FdUMP inhibits the thymine ceoxyribonucleotide synthetase, the enzyme is necessary for DNA replication and repair, while FdUTP and FUTP disrupt the processing and function of DNA and RNA.[11] Dihydropyrimidine dehydrogenase (DPD) is a rate-limiting enzyme in the catabolic pathway of fluorouracil. DPD can inactivate up to 85% of 5-Fu into 5, 6-dihydro-5-fluorouracil, and the intermediate is further metabolized to β-alanine or β-aminoisobutyric acid.[12] These processes will increase nucleotide synthesis, which is conducive to DNA synthesis and cell growth. While DPD enzyme activity is decreased, fluorouracil clearance rate in vivo is decreased, the half-life is prolonged and cytotoxicity is enhanced.[13] DPD enzyme activity is affected by *DPYD* gene polymorphisms.[14] In addition, DPD is associated with epithelial-to-mesenchymal transition (EMT). EMT has been implicated in carcinogenesis and tumor metastasis by enhancing mobility, invasion, and resistance to apoptotic stimuli.[15] *DPYD* gene polymorphisms may affect the process of EMT by changing the activity of DPD, thus participating in the metastasis of tumor cells.

The human *DPYD* gene is located on chromosome 1p21.3, it is 850 kb in length encompassing 23 exons. Genetic variations of *DPYD* lead to changes in DPD enzyme activity, which could result in some adverse side effects. The *DPYD* gene has more than 1700 different genetic variants, and more than 600 are missense variants impacting on the DPD protein sequence, according to the report in the GnomAD database (https://gnomad.broadinstitute.org/). So far, the variants or polymorphisms of *DPYD* gene attracted more attention including: *DPYD* IVS14+1 G>A (rs3918290, *DPYD* *2A), *DPYD* c. 1627 A>G (rs1801159, *DPYD* *5A), *DPYD* c. 85 T>C (rs1801265, *DPYD* *9A).[16,17]

Studies have shown that the clinical outcome, the survival of CRC is associated with gene polymorphisms and gene expression level.[18] One study showed that polymorphisms of *DPYD* have a significant effect on toxicity and clinical outcome in colorectal or gastroesophageal cancer patients receiving capecitabine-based chemotherapy.[19] Another study showed that the mRNA expression of *DPYD* is associated with clinicopathological characteristics and may be useful for predicting survival in CRC patients.[20] The relationship between *DPYD* gene polymorphisms and metastasis of CRC has not been studied. In the present study, the relationship between *DPYD* gene polymorphisms and the clinical features of CRC patients, metastasis of CRC (including LNM and DM) was analyzed. It is expected to provide a valuable marker for the prognosis of CRC and a valuable target for the clinical treatment of metastatic CRC. This study may provide a valuable reference for the relationship between gene polymorphism and pathological features and metastasis of CRC.

2 | MATERIALS AND METHODS

2.1 | Subjects

A total of 537 CRC patients were recruited from Meizhou People's Hospital, from January 2016 to May 2019. Inclusion criteria: (1) Imaging diagnosis and histologically confirmed diagnosis met the diagnostic criteria for CRC. (2) Patients without serious cardiovascular and cerebrovascular diseases and infectious diseases. Exclusion criteria: (1) Patients without colorectal cancer. (2) Patients with dysfunction of vital organs. (3) Patients who also have other tumors. This study was supported by the Ethics Committee of the Meizhou People's Hospital. The flow chart of the present study is shown in Figure 1.

2.2 | Genotyping of *DPYD* gene

Two milliliters of venous blood sample were obtained from each subject. Genomic DNA was extracted using a QIAamp DNA Kit (Qiagen GmbH). *DPYD* IVS14+1 G>A variant and polymorphisms of *DPYD* c. 1627 A>G and *DPYD* c. 85 T>C were analyzed. *DPYD* Genotyping Test Kit (SINOMD Gene Detection Technology Co. Ltd.) based on Sanger sequencing was used for testing. Polymerase chain reaction (PCR) was performed according to the following procedure: Initial denaturation at 95℃ for 3 min, followed by 45 cycles of denaturation at 94℃ for 15 s, annealing at 63℃ for 1 min, and extension at 72℃ for 1 min. PCR products were purified with ExoSap-It (ABI PCR Product Cleanup Reagent). DNA sequences determination was detected using ABI Terminator v3.1 Cycle Sequencing kit and performed on ABI 3500 Dx Genetic Analyzer, analyzed with Sequencing Analysis v5.4 (Life Technologies).

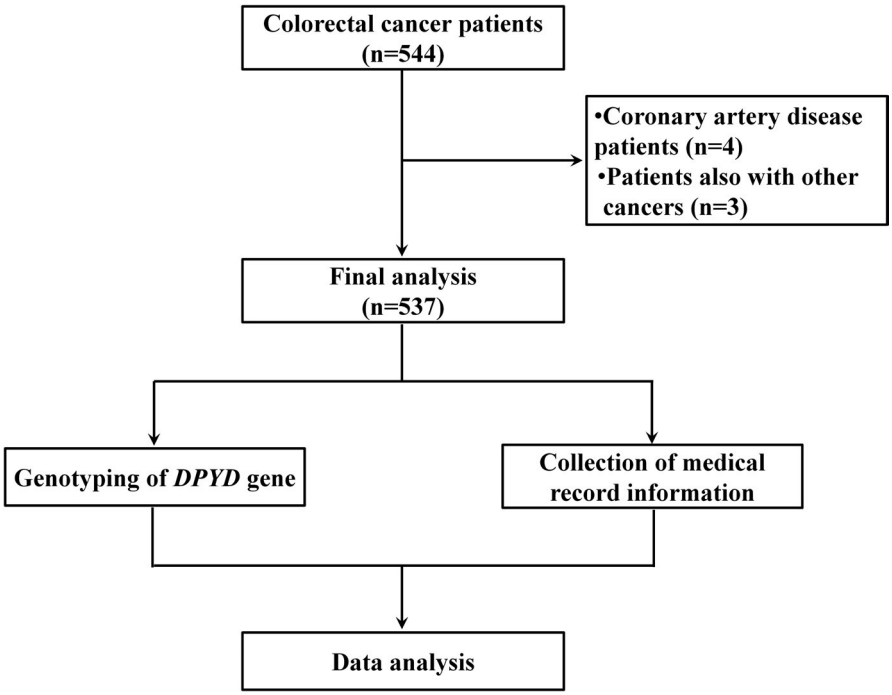

FIGURE 1 The flow chart of the present study

TABLE 1 Baseline characteristics of study objects

	Colorectal cancer patients (n = 537)
Gender	
Male, n (%)	349 (65.0)
Female, n (%)	188 (35.0)
Age, mean ± SD (years)	59.34 ± 10.14 (26–85)
≤60, n (%)	273 (50.8)
>60, n (%)	264 (49.2)
Differentiation	
Well, n (%)	8 (1.5)
Moderate, n (%)	497 (92.5)
Poor, n (%)	26 (5.0)
Unknown, n (%)	6 (1.0)
T stages	
pT1, n (%)	3 (0.6)
pT2, n (%)	27 (5.0)
pT3, n (%)	364 (67.8)
pT4, n (%)	142 (26.4)
Unknown, n (%)	1 (0.2)
N stages	
N0, n (%)	192 (35.8)
N1, n (%)	196 (36.5)
N2, n (%)	145 (27.0)
N3, n (%)	4 (0.7)
M stages	
M0, n (%)	428 (79.7)
M1, n (%)	109 (20.3)

2.3 | Data collection and statistical analysis

Relevant information and medical records of these participants were collected. Clinical information, including age, gender, histopathological type, degree of tumor differentiation, TNM stage, and tumor grade, was collected. SPSS statistical software version 21.0 (IBM Inc.) was used for the data analysis. The Hardy–Weinberg equilibrium (HWE) of *DPYD* genotypes was assessed using the χ^2 test. Association between *DPYD* variants status with the clinical features of patients and metastasis of CRC were evaluated by Fisher's exact test. A p value <0.05 was set as statistically significant.

3 | RESULTS

3.1 | Population characteristics

A total of 537 CRC patients were enrolled in this study, including 349 (65.0%) men and 188 (35.0%) women. The average age of the patients was 59.34 ± 10.14 years (26–85 years), 273 (50.8%) patients with ≤60 years old, and 264 (49.2%) patients with >60 years old. According to the pathological degree of tumor differentiation, 8 (1.5%) samples were well-differentiated tumors, 497 (92.5%) samples were moderately differentiated tumors, 26 (5.0%) samples were poorly differentiated tumors, and 6 samples were unknown. According to the tumor stage, 3 (0.6%), 27 (5.0%), 364 (67.8%), and 142 (26.4%) cases were pT1, pT2, pT3, and pT4 stage, respectively. The proportion of higher stage tumors (pT3+ pT4 categories) was 94.2%. According to the lymph nodes status, 192 (35.8%), 196 (36.5%), 145 (27.0%), and 4 (0.7%) cases were N0, N1, N2, and N3 stage, respectively. In addition, 428 (79.7%) and 109 (20.3%) cases were M0 and M1 stage, respectively (Table 1).

3.2 | The frequency of *DPYD* gene polymorphisms in the patients

In this study, the *DPYD* IVS14+1 G>A, *DPYD* c. 1627 A>G, *DPYD* c. 85 T>C genotypes in the patients were identified. About the *DPYD* IVS14+1G>A variant, there were 537 (100%) cases with G/G genotype (wild type), 0 (0%) cases with G/A heterozygous, and 0 (0%) cases with A/A homozygous. That is to say, no *DPYD* IVS14+1G>A mutation was found in the patients in this study. In the *DPYD* c.1627A>G, there were 310 (57.7%) cases with A/A genotype (wild type), 191 (35.6%) cases with A/G heterozygous, and 36 (6.7%) cases with G/G homozygous. Among *DPYD* c.85T>C, there were 449 (83.6%) cases with T/T genotype (wild type), 86 (16.0%) cases with T/C heterozygotes, and 2 (0.4%) cases with C/C homozygous. The genotype distributions of *DPYD* c.1627A>G, and *DPYD* c.85T>C in the CRC patients were consistent with Hardy–Weinberg equilibrium ($\chi^2 = 0.425$, $p = 0.802$ and $\chi^2 = 0.715$, $p = 0.750$, respectively).

3.3 | Association of *DPYD* polymorphisms with metastasis of CRC

Logistic regression analysis of the relationship between the genotype of *DPYD* polymorphisms and the LNM status of CRC was studied. The frequency of *DPYD* c.1627A>G A/G genotype (39.4%) in the LNM group was obviously higher than that (28.6%) in the non-LNM CRC patients. It was demonstrated that the A/G genotype of *DPYD* c.1627A>G might increase the risk of LNM in CRC patients ($p = 0.016$, OR = 1.626, 95% CI = 1.104–2.395). The variants were analyzed under different genetic models. It was showed that *DPYD* c.1627A>G A/G and G/G genotypes in the dominant model (*DPYD* c.1627A>G A/G + G/G vs. *DPYD* c.1627A>G A/A) were the significant risk factors ($p = 0.029$, OR 1.506, 95% CI = 1.048–2.165) for the LNM of CRC (Table 2).

Logistic regression analysis of the relationship between the genotype of *DPYD* polymorphisms and DM status of CRC was studied. The frequency of *DPYD* c.1627A>G A/G genotype (45.0%) in the DM group was obviously higher than that (33.2%) in the non-DM group. It was demonstrated that the A/G genotype of *DPYD* c.1627A>G might increase the risk of DM in CRC patients ($p = 0.023$, OR = 1.673, 95% CI = 1.079–2.596). In addition, *DPYD* c.1627A>G A/G and G/G genotypes in the dominant model (*DPYD* c.1627A>G A/G + G/G vs. *DPYD* c.1627A>G A/A) were the significant risk factors ($p = 0.039$, OR = 1.588, 95% CI = 1.041–2.423) for the DM of CRC (Table 2).

3.4 | Association of *DPYD* polymorphisms with clinicopathological parameters in the CRC patients

The association between *DPYD* c.1627A>G, c.85T>C polymorphisms, and clinicopathological features of CRC patients have been evaluated. The clinical features including gender, age, degree of differentiation of the tumor sample, serum tumor marker levels (carcinoembryonic antigen (CEA), carbohydrate antigen 24–2 (CA24-2), carbohydrate antigen 19–9 (CA19-9)), tumor stage, lymph nodes status, and distant metastasis status was collected. There was no relationship between the *DPYD* c.1627A>G, c.85T>C polymorphisms and gender, degree of differentiation of the tumor sample, serum CA19-9 level, and tumor stage (T stage) of CRC patients. However, the frequency of *DPYD* c.1627A>G A/G+G/G genotypes in older patients (>60 years old) was significantly higher than that in the younger patients (≤60 years old) ($p = 0.036$). The frequency of *DPYD* c.1627A>G A/G+G/G genotypes in patients with abnormal serum CEA level (>5 ng/ml) and abnormal serum CA24-2 level (>20 U/ml) was significantly higher than that in the patients with normal serum CEA level (≤5 ng/ml) ($p = 0.003$) and normal serum CA24-2 level (≤20 U/ml) ($p = 0.015$), respectively (Table 3).

4 | DISCUSSION

CRC is one of the common malignant tumors in human digestive tracts.[21,22] Metastasis is a biological phenotype of malignant tumors and an important factor affecting the prognosis of malignant tumors. Tumor metastasis is a dynamic process in which multiple factors are involved in multiple stages of development, including the biology of tumor cells and the interaction between tumor and microenvironment.[23,24] At present, the research on tumor metastasis mainly focuses on tumor metastasis genes and tumor metastasis suppressor genes, tumor angiogenesis, extracellular matrix, cell adhesion, tumor microenvironment, and so on.[25,26]

Studies have shown that some gene polymorphisms were associated with the metastasis of cancer. It is a lower risk of LNM in oral cancer patients carrying A/A genotype of the single nucleotide polymorphism (SNP) rs10399805 or rs6691378 in chitinase-3-like protein 1 (*CHI3L1*) gene.[27] Polymorphisms in the promoter regions of matrix metalloproteinase (*MMP*)1, 3, 7, and 9 genes are associated with metastasis of head/neck and breast cancer.[28] Luminal A and luminal B breast cancer patients with the A/G genotype of C-C motif chemokine ligand 4 (*CCL4*) gene SNP rs10491121 were less likely to develop LNM.[29] The SNPs rs1143630, rs1143633, and rs1143643 of interleukin-1 beta (*IL-1B*) gene showed a relationship with LNM of papillary thyroid carcinoma (PTC).[30] SNP rs1989839 C/T genotype of Ras-association domain family 1 isoform A (*RASSF1A*) gene increases the risk of lung metastasis of osteosarcoma.[31] Transforming growth factor-β1 (*TGFB1*) gene promoter −509C/T polymorphism affected the metastasis of CRC.[32] Granzyme B (*GZMB*) gene polymorphisms were not associated with the metastasis of CRC.[33] Studies have shown that *DPYD* gene polymorphisms were associated with the susceptibility to CRC[12] and the toxicity of chemotherapy drugs.[34] However, the relationship between *DPYD* gene polymorphisms and metastasis of CRC has not been studied.

DPYD IVS14+1G>A variant was not found in this study, and this result was similar to those reported in other populations, such as Caucasians, African-Americans, Egyptians, Turks, and

TABLE 2 Association of *DPYD* polymorphisms with metastasis of CRC patients

Genotype	LNM n (%)	Non-LNM n (%)	OR (95% CI)	*p* value	DM n (%)	Non-DM n (%)	OR (95% CI)	*p* value
DPYD c. 1627 A>G								
A/A	187 (54.2)	123 (64.1)	1.000 (reference)		53 (48.6)	257 (60.0)	1.000 (reference)	
A/G	136 (39.4)	55 (28.6)	1.626 (1.104–2.395)	**0.016**	49 (45.0)	142 (33.2)	1.673 (1.079–2.596)	**0.023**
G/G	22 (6.4)	14 (7.3)	1.034 (0.509–2.097)	1.000	7 (6.4)	29 (6.8)	1.170 (0.487–2.813)	0.816
Dominant model (A/G+G/G vs. A/A)			1.506 (1.048–2.165)	**0.029**			1.588 (1.041–2.423)	**0.039**
Recessive model (G/G vs. A/A+A/G)			0.866 (0.432–1.735)	0.720			0.944 (0.402–2.217)	1.000
Allele frequency			1.280 (0.952–1.722)	0.104			1.333 (0.956–1.860)	0.094
A allele	510 (73.9)	301 (78.4)			155 (71.1)	656 (76.6)		
G allele	180 (26.1)	83 (21.6)			63 (28.9)	200 (23.4)		
DPYD c. 85 T>C								
T/T	290 (84.1)	159 (82.8)	1.000 (reference)		90 (82.6)	359 (83.9)	1.000 (reference)	
T/C	54 (15.7)	32 (16.7)	0.925 (0.574–1.492)	0.806	19 (17.4)	67 (15.7)	1.131 (0.647–1.979)	0.770
C/C	1 (0.3)	1 (0.5)	0.548 (0.034–8.825)	1.000	0 (0)	2 (0.5)	—	1.000
Dominant model (T/C+C/C vs. T/T)			0.914 (0.569–1.466)	0.716			1.098 (0.629–1.919)	0.772
Recessive model (C/C vs. T/T+T/C)			0.555 (0.035–8.927)	1.000			—	1.000
Allele frequency			0.909 (0.582–1.420)	0.731			1.056 (0.622–1.793)	0.891
T allele	634 (91.9)	350 (91.1)			199 (91.3)	785 (91.7)		
C allele	56 (8.1)	34 (8.9)			19 (8.7)	71 (8.3)		

Abbreviations: CRC, colorectal cancer; DM, distant metastasis; LNM, lymph node metastasis.

Bold numbers indicate significant values ($p < 0.05$).

TABLE 3 Association of *DPYD* polymorphisms with clinicopathological parameters in the CRC patients

	DPYD c. 1627 A>G						*DPYD* c. 85 T>C					
	Dominant model			Recessive model			Dominant model			Recessive model		
Parameters	A/A	A/G+G/G	*p* value	A/A+A/G	G/G	*p* value	T/T	T/C+C/C	*p* value	T/T+T/C	C/C	*p* value
Gender												
Male	200	149	0.855	325	24	0.859	289	60	0.542	348	1	1.000
Female	110	78		176	12		160	28		187	1	
Age, years												
≤60	170	103	**0.036**	259	14	0.168	228	45	1.000	271	2	0.499
>60	140	124		242	22		221	43		264	0	
Differentiation												
Well	4	4	0.242	6	2	0.069	8	0	0.329	8	0	1.000
Moderate	291	206		463	34		417	80		495	2	
Poor	11	15		26	0		20	6		26	0	
Serum CEA												
≤5 ng/ml	244	152	**0.003**	370	26	0.845	332	64	0.895	394	2	1.000
>5 ng/ml	66	75		131	10		117	24		141	0	
Serum CA24-2												
≤20 U/ml	290	198	**0.015**	459	29	0.036	407	81	0.697	486	2	1.000
>20 U/ml	20	29		42	7		42	7		49	0	
Serum CA19-9												
≤37 U/ml	272	187	0.084	430	29	0.460	378	81	0.068	457	2	1.000
>37 U/ml	38	40		71	7		71	7		78	0	
T stages												
pT1-2	15	15	0.447	28	2	1.000	27	3	0.450	30	0	1.000
pT3-4	295	211		472	34		421	85		504	2	
N stages												
N0	123	69	**0.029**	178	14	0.720	159	33	0.716	191	1	1.000
N1-3	187	158		323	22		290	55		344	1	
M stages												
M0	257	171	**0.039**	399	29	1.000	359	69	0.772	426	2	1.000
M1	53	56		102	7		90	19		109	0	

Abbreviations: CA19-9, carbohydrate antigen 19–9; CA24-2, carbohydrate antigen 24–2; CEA, carcinoembryonic antigen.
Bold numbers indicate significant values ($p < 0.05$).

Taiwanese.[35] Many studies have reported that CRC patients with *DPYD* IVS14+1G>A variant might suffer from severe toxicity and even death after the 5-FU administration.[36,37] However, *DPYD* IVS14+1G>A variant is rare in most populations. In this study, *DPYD* c.1627A>G, A/A, A/G, and G/G genotypes accounted for 57.7%, 35.6%, and 6.7%, respectively. The result is in line with those of another Chinese population study.[17] *DPYD* c.85T>C T/T, T/C, and C/C genotypes accounted for 83.6%, 16.0%, and 0.4%, respectively. The result in this study was consistent with that in the previous study.[17] A study of a population of a mixed racial background showed that *DPYD* c.85T>C T/C and C/C genotypes were 41% and 10%, respectively.[38] The frequencies of *DPYD* c.85T>C variants in patients were higher than that in this study.

In this study, *DPYD* c.1627A>G A/G and G/G genotypes in the dominant model (A/G + G/G vs. A/A) were significant risk factors for the LNM and DM of CRC. DPD activity is in association with the epithelial-to-mesenchymal transition (EMT). EMT is a process during which the epithelial features of cancer cells are lost, the cytoskeletal architecture is re-organized, the cell shape is changed, and some genes are activated, which leads to increased cell motility and dissemination of tumor to distant metastatic sites.[39] EMT results in decreased adhesion and enhanced migration or invasion. Studies have shown that dihydrothymine and dihydrouracil, the metabolites catabolized by DPD, play an important role in tumor EMT.[40,41] DPD is necessary for cells to acquire mesenchymal characteristics in vitro and tumorigenic cells overflow. It is a metabolic

process essential associated with the acquisition of metastatic and aggressive cancer cell traits for the EMT.[40] Mechanistically, DPD may act as a regulator of EMT by targeting the p38/NF-κB/Snail1 pathway.[41]

In the present study, the frequency of *DPYD* c.1627A>G A/G+G/G genotypes in patients with abnormal serum CEA levels was significantly higher than that in patients with normal serum CEA levels. Serum CEA levels can be used as biomarkers for diagnosis, postoperative recurrence, or efficacy monitoring of colorectal cancer.[42] The CEA gene family belongs to the immunoglobulin (Ig) superfamily and codes for a vast number of glycoproteins that differ greatly both in amino acid composition and function. The CEA family is divided into two groups, the carcinoembryonic antigen-related cell adhesion molecules (CEA-CAMs) and the pregnancy-specific glycoproteins. CEA expression on epithelial cells may directly influence tumor development by CEA-CEA bridges between tumor cells or tumor-stromal cells.[43] That is to say, *DPYD* gene mutations may affect the process of EMT by changing the activity of DPD, thus participating in the metastasis of tumor cells. Elevated CEA expression level and *DPYD* gene mutations may be associated with CRC metastasis.

CA24-2 is a serum tumor marker, which is one of the indicators reflecting the number and activity of tumor cells.[44] A study has shown that the CA24-2 level was higher in gastric cancer patients with distant metastasis than in patients without distant metastasis.[45] Increased serum CA24-2 concentrations were significantly associated with the risk of invasiveness of intraductal papillary mucinous neoplasm (IPMN).[46] CEA, CA19-9, CA24-2, and CA72-4, examined postoperatively during follow-up, were useful to find early tumor recurrence and metastasis, and evaluate prognosis.[47] Tumorigenesis is dependent on the reprogramming of cellular metabolism. A common feature of metabolism in the cancer cells is the ability to acquire necessary nutrients from a frequently nutrient-poor environment and utilize these nutrients to both maintain viability and build new biomass.[48] Some studies have shown that Pantothenate and CoA biosynthesis signaling pathway was significantly altered in tumor cells.[49–51] DPD is a key enzyme in the Pantothenate and CoA biosynthesis signaling pathway (https://www.genome.jp/pathway/ko00770+K00207). So, *DPYD* c.1627A>G A/G+G/G genotypes may affect the activity of DPD, and regulate tumor cells tumorigenesis through signaling pathway regulation in the reprogramming of cellular metabolism, which is manifested as changes in serum tumor markers.

Tumor invasion and metastasis is a dynamic and complex process, including multiple simultaneous steps. The persistent emergence of populations of cells with different invasion and metastasis capabilities is a barrier to tumor therapy.[52] In order to prevent the invasion and metastasis of tumor, it is a hot spot of research to design modulatory blocking methods specifically aiming at some key links in tumor invasion and metastasis.[53] With the deepening understanding of the occurrence and mechanism of tumor invasion and metastasis, it can promote the design and search for effective anti-tumor drugs, provide new ideas for the treatment of tumors, and have a positive significance to reduce the mortality of tumor patients.

This is the first study about the relationship of *DPYD* gene variants/polymorphisms and lymph node metastasis, distant metastasis of CRC. There are some limitations to this study that should be noted. First of all, the number of cases included in this study is not large, which may lead to some deviations in the results. Second, the number of gene polymorphisms included in this study was relatively single. Tumor cell metastasis is affected by tumor metastasis-related genes and tumor metastasis-suppressor genes, tumor angiogenesis, extracellular matrix degradation, cell adhesion, tumor microenvironment, and other factors. It may be more meaningful to include some related genes for comprehensive analysis. In addition, a tumor is a kind of multifactorial disease caused by genetic and environmental factors. As a retrospective analysis, the limitations of the original data included in this study constrained assessment of potential gene-environment interactions.

5 | CONCLUSION

DPYD c.1627A>G A/G and G/G genotypes are associated with the increased risk of lymph node metastasis and distant metastasis of CRC. Future studies need to include more relevant genes for analysis and to assess potential gene-environment interactions. This study may provide a valuable reference for the relationship between gene polymorphism and pathological features and metastasis of CRC.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

Zhixiong Zhong, Heming Wu, and Juanzi Zeng designed the study. Juanzi Zeng, Qingyan Huang, and Zhikang Yu performed the experiments. Juanzi Zeng and Jiaquan Li collected the clinical data. Heming Wu and Juanzi Zeng analyzed the data. Heming Wu and Juanzi Zeng prepared the manuscript. All authors were responsible for critical revisions, and all authors read and approved the final version of this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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REFERENCES

1. Brokaar EJ, van den Bos F, Visser LE, Portielje JEA. Deprescribing in older adults with cancer and limited life expectancy: an integrative review. *Am J Hosp Palliat Care*. 2021;10499091211003078
2. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209-249.
3. Kuipers EJ, Grady WM, Lieberman D, et al. Colorectal cancer. *Nat Rev Dis Primers*. 2015;1:15065.
4. Johdi NA, Sukor NF. Colorectal cancer immunotherapy: options and strategies. *Front Immunol*. 2020;11:1624.
5. Hu L, Zhu JY, Fang L, Yu XC, Yan ZL. Isolated metachronous splenic multiple metastases after colon cancer surgery: a case report and literature review. *World J Clin Cases*. 2020;8(15):3320-3328.
6. Suhail Y, Cain MP, Vanaja K, et al. Systems biology of cancer metastasis. *Cell Syst*. 2019;9(2):109-127.
7. Nanduri LK, Hissa B, Weitz J, Schölch S, Bork U. The prognostic role of circulating tumor cells in colorectal cancer. *Expert Rev Anticancer Ther*. 2019;19(12):1077-1088.
8. Lam SW, Guchelaar HJ, Boven E. The role of pharmacogenetics in capecitabine efficacy and toxicity. *Cancer Treat Rev*. 2016;50:9-22.
9. Amstutz U, Henricks LM, Offer SM, et al. Clinical Pharmacogenetics Implementation Consortium (CPIC) guideline for dihydropyrimidine dehydrogenase genotype and fluoropyrimidine dosing: 2017 update. *Clin Pharmacol Ther*. 2018;103(2):210-216.
10. Derissen EJ, Jacobs BA, Huitema AD, Rosing H, Schellens JH, Beijnen JH. Exploring the intracellular pharmacokinetics of the 5-fluorouracil nucleotides during capecitabine treatment. *Br J Clin Pharmacol*. 2016;81(5):949-957.
11. Gmeiner WH. Chemistry of fluorinated pyrimidines in the era of personalized medicine. *Molecules*. 2020;25(15):3438.
12. Matáková T, Halašová E, Škovierová H, Dzian A, Dobrota D, Škereňová M. DPYD genotype and haplotype analysis and colorectal cancer susceptibility in a case-control study from Slovakia. *Gen Physiol Biophys*. 2017;36(5):557-563.
13. Del Re M, Michelucci A, Di Leo A, et al. Discovery of novel mutations in the dihydropyrimidine dehydrogenase gene associated with toxicity of fluoropyrimidines and viewpoint on preemptive pharmacogenetic screening in patients. *EPMA J*. 2015;6(1):17.
14. Edwards L, Gupta R, Filipp FV. Hypermutation of DPYD deregulates pyrimidine metabolism and promotes malignant progression. *Mol Cancer Res*. 2016;14(2):196-206.
15. Mittal V. Epithelial mesenchymal transition in tumor metastasis. *Annu Rev Pathol*. 2018;13:395-412.
16. Offer SM, Fossum CC, Wegner NJ, Stuflesser AJ, Butterfield GL, Diasio RB. Comparative functional analysis of DPYD variants of potential clinical relevance to dihydropyrimidine dehydrogenase activity. *Cancer Res*. 2014;74(9):2545-2554.
17. Li GY, Duan JF, Li WJ, Liu T. DPYD*2A/*5A/*9A and UGT1A1*6/*28 polymorphisms in Chinese colorectal cancer patients. *J Cancer Res Ther*. 2016;12(2):782-786.
18. Ried T, Meijer GA, Harrison DJ, et al. The landscape of genomic copy number alterations in colorectal cancer and their consequences on gene expression levels and disease outcome. *Mol Aspects Med*. 2019;69:48-61.
19. Joerger M, Huitema ADR, Boot H, et al. Germline TYMS genotype is highly predictive in patients with metastatic gastrointestinal malignancies receiving capecitabine-based chemotherapy. *Cancer Chemother Pharmacol*. 2015;75(4):763-772.
20. Goto T, Shinmura K, Yokomizo K, et al. Expression levels of thymidylate synthase, dihydropyrimidine dehydrogenase, and thymidine phosphorylase in patients with colorectal cancer. *Anticancer Res*. 2012;32(5):1757-1762.
21. Haraldsdottir S, Einarsdottir HM, Smaradottir A, Gunnlaugsson A, Halfdanarson TR. Colorectal cancer - review. *Laeknabladid*. 2014;100(2):75-82.
22. Wang J, Liang W, Wang X, et al. The value of biomarkers in colorectal cancer: protocol for an overview and a secondary analysis of systematic reviews of diagnostic test accuracy. *Medicine*. 2019;98(24):e16034.
23. Yeung KT, Yang J. Epithelial-mesenchymal transition in tumor metastasis. *Mol Oncol*. 2017;11(1):28-39.
24. Lin Y, Xu J, Lan H. Tumor-associated macrophages in tumor metastasis: biological roles and clinical therapeutic applications. *J Hematol Oncol*. 2019;12(1):76.
25. Zeeshan R, Mutahir Z. Cancer metastasis - tricks of the trade. *Bosn J Basic Med Sci*. 2017;17(3):172-182.
26. Ganesh K, Massagué J. Targeting metastatic cancer. *Nat Med*. 2021;27(1):34-44.
27. Su CW, Chen MK, Hung WC, Yang SF, Chuang CY, Lin CW. Functional variant of CHI3L1 gene is associated with neck metastasis in oral cancer. *Clin Oral Investig*. 2019;23(6):2685-2694.
28. Faraji F, Pang Y, Walker RC, Nieves Borges R, Yang L, Hunter KW. Cadm1 is a metastasis susceptibility gene that suppresses metastasis by modifying tumor interaction with the cell-mediated immunity. *PLoS Genet*. 2012;8(9):e1002926.
29. Hu GN, Tzeng HE, Chen PC, et al. Correlation between CCL4 gene polymorphisms and clinical aspects of breast cancer. *Int J Med Sci*. 2018;15(11):1179-1186.
30. Ban JY, Kim MK, Park SW, Kwon KH. Interleukin-1 beta polymorphisms are associated with lymph node metastasis in Korean patients with papillary thyroid carcinoma. *Immunol Invest*. 2012;41(8):888-905.
31. Xu H, Zhan W, Chen Z. Ras-association domain family 1 isoform A (RASSF1A) gene polymorphism rs1989839 is associated with risk and metastatic potential of osteosarcoma in young Chinese individuals: a multi-center, case-control study. *Med Sci Monit*. 2016;22:4529-4535.
32. Stanilova S, Stanilov N, Julianov A, Manolova I, Miteva L. Transforming growth factor-β1 gene promoter -509C/T polymorphism in association with expression affects colorectal cancer development and depends on gender. *PLoS One*. 2018;13(8):e0201775.
33. Mhaidat NM, Al-azzam SI, Alzoubi KH, Khabour OF, Gharaibeh BF. Granzyme B gene polymorphisms, colorectal cancer risk, and metastasis. *J Cancer Res Ther*. 2014;10(3):587-590.
34. Bruera G, Ricevuto E. Pharmacogenomic assessment of patients with colorectal cancer and potential treatments. *Pharmgenomics Pers Med*. 2020;13:601-617.
35. Sulzyc-Bielicka V, Bińczak-Kuleta A, Pioch W, et al. 5-Fluorouracil toxicity-attributable IVS14 + 1G > A mutation of the dihydropyrimidine dehydrogenase gene in Polish colorectal cancer patients. *Pharmacol Rep*. 2008;60(2):238-242.
36. De Falco V, Natalicchio MI, Napolitano S, et al. A case report of a severe fluoropyrimidine-related toxicity due to an uncommon DPYD variant. *Medicine*. 2019;98(21):e15759.
37. Terrazzino S, Cargnin S, Del Re M, Danesi R, Canonico PL, Genazzani AA. DPYD IVS14+1G>A and 2846A>T genotyping for the prediction of severe fluoropyrimidine-related toxicity: a meta-analysis. *Pharmacogenomics*. 2013;14(11):1255-1272.
38. Maharjan AS, McMillin GA, Patel GK, et al. The prevalence of DPYD*9A(c.85T>C) genotype and the genotype-phenotype correlation in patients with gastrointestinal malignancies treated with fluoropyrimidines: updated analysis. *Clin Colorectal Cancer*. 2019;18(3):e280-e286.

39. Nieszporek A, Skrzypek K, Adamek G, Majka M. Molecular mechanisms of epithelial to mesenchymal transition in tumor metastasis. *Acta Biochim Pol*. 2019;66(4):509-520.
40. Shaul Y, Freinkman E, Comb W, et al. Dihydropyrimidine accumulation is required for the epithelial-mesenchymal transition. *Cell*. 2014;158(5):1094-1109.
41. Zhu WP, Liu ZY, Zhao YM, et al. Dihydropyrimidine dehydrogenase predicts survival and response to interferon-α in hepatocellular carcinoma. *Cell Death Dis*. 2018;9(2):69.
42. Calinescu A, Turcu G, Nedelcu RI, et al. On the dual role of carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) in human malignancies. *J Immunol Res*. 2018;2018:7169081.
43. Beauchemin N, Arabzadeh A. Carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) in cancer progression and metastasis. *Cancer Metastasis Rev*. 2013;32(3–4):643-671.
44. Dou H, Sun G, Zhang L. CA242 as a biomarker for pancreatic cancer and other diseases. *Prog Mol Biol Transl Sci*. 2019;162:229-239.
45. Jiexian J, Xiaoqin XU, Lili DU, et al. Clinical assessment and prognostic evaluation of tumor markers in patients with gastric cancer. *Int J Biol Markers*. 2013;28(2):192-200.
46. You L, Ma L, Zhao WJ, Zhao YP, Dai MH. Emerging role of tumor markers and biochemistry in the preoperative invasive assessment of intraductal papillary mucinous neoplasm of the pancreas. *Clin Chim Acta*. 2016;454:89-93.
47. Jing JX, Wang Y, Xu X-Q, et al. Tumor markers for diagnosis, monitoring of recurrence and prognosis in patients with upper gastrointestinal tract cancer. *Asian Pac J Cancer Prev*. 2014;15(23):10267-10272.
48. Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. *Cell Metab*. 2016;23(1):27-47.
49. You R, Wang L, Liu LI, et al. Probing cell metabolism on insulin like growth factor(IGF)-1/tumor necrosis factor(TNF)-α and chargeable polymers co-immobilized conjugates. *J Tissue Eng Regen Med*. 2021;15(3):256-268.
50. Wang Z, Chen H, Xue LU, et al. High throughput proteomic and metabolic profiling identified target correction of metabolic abnormalities as a novel therapeutic approach in head and neck paraganglioma. *Transl Oncol*. 2021;14(8):101146.
51. Liu X, Cheng X, Liu X, et al. Investigation of the urinary metabolic variations and the application in bladder cancer biomarker discovery. *Int J Cancer*. 2018;143(2):408-418.
52. Beckham TH, Yang TJ, Gomez D, Tsai CJ. Metastasis-directed therapy for oligometastasis and beyond. *Br J Cancer*. 2021;124(1):136-141.
53. Lin XL, Li K, Yang Z, Chen B, Zhang T. Dulcitol suppresses proliferation and migration of hepatocellular carcinoma via regulating SIRT1/p53 pathway. *Phytomedicine*. 2020;66: 153112.

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