

Influence of *GSTP1* I105V polymorphism on cumulative neuropathy and outcome of FOLFOX-4 treatment in Asian patients with colorectal carcinoma

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Glutathione S-transferase P1 (*GSTP1*) participates in detoxification of potentially genotoxic compounds that may alter the efficacy and toxicity of platinum-based chemotherapy. We analyzed the influence of I105V polymorphism of *GSTP1* on clinico-pathological features and outcomes in 166 Chinese patients with metastatic colorectal carcinoma who had been treated with first-line FOLFOX-4. Combined analysis of *GSTP1* I105V, *ERCC1*-118, and *XPB*-751 polymorphisms was also conducted. The results showed that, in comparison with Caucasian populations, a remarkably lower prevalence of Val105 allele variants was noted (24.7%). Patients with Val105 allele variants had a higher response to FOLFOX-4 (56.1% vs 37.6%, $P = 0.04$), and a longer progression-free ($P < 0.01$) as well as overall ($P < 0.01$) survival. By adjusted analysis, this polymorphism was identified as an independent prognostic factor ($P = 0.01$). In combined analysis, patients without any risk genotype, including *GSTP1*-105 Ile/Ile, *ERCC1*-118 C/T or T/T, and *XPB*-751 Lys/Gln, had significantly longer progression-free and overall survivals ($P < 0.01$). In addition, patients with Val105 allele variants had a higher incidence of grade 3/4 cumulative neuropathy after different cycles of treatment. These data suggest that Asian populations have a lower prevalence of I105V polymorphism in *GSTP1*. I105V polymorphism in *GSTP1*, by reducing its enzymatic activity and consequential detoxification to oxaliplatin, could be a key determinant for a better outcome, but more neurotoxicity, to FOLFOX-4 treatment. (*Cancer Sci* 2010; 101: 530–535)

Colorectal carcinoma (CRC) is one of the leading causes of cancer-related mortality in Taiwan and its incidence has increased steadily over the past few decades. Oxaliplatin is very effective in treating metastatic CRC patients,^(1,2) and improves the disease-free survival of patients with stage II/III CRC.⁽³⁾ Neuropathy is the major toxicity of oxaliplatin, and the incidence of oxaliplatin-induced severe neuropathy has varied from 12% to 18%.^(3–5) Oxaliplatin-induced neuropathy includes an acute, transient peripheral nerve hyperexcitability,⁽⁶⁾ and a chronic, dose-related, peripheral sensory neuropathy with symptoms similar to those caused by cisplatin.⁽⁷⁾ Development of chronic neuropathy results in severe disturbance of neurologic functions. Therefore, identification of factors predictive of neurotoxicity to oxaliplatin treatment, including genes involved in the nucleotide excision repair (NER)^(8,9) and detoxification pathways,⁽¹⁰⁾ is of extreme interest.

Glutathione S-transferases (GST) are a multigene family of enzymes which catalyze the conjugation of glutathione to electrophilic xenobiotics to inactivate them, and thus prevent DNA damage and adduct formation.⁽¹¹⁾ At least five major classes of the GST superfamily have been identified,⁽¹⁰⁾ among them, the *GSTP1*, *GSTT1*, and *GSTM1* genotypes have been studied

extensively for their influences in the inter-individual variations of outcome to chemotherapeutic agents.^(12,13) The isoenzyme *GSTP1* is highly expressed in human CRC tissues,⁽¹⁴⁾ and participates in the detoxification of platinum drugs that may mediate the resistance to platinum-based chemotherapy. A single nucleotide polymorphism (A313G) in exon 5 of the *GSTP1* gene causing isoleucine to valine substitution in the 105th amino acid (I105V) significantly decreases *GSTP1* activity,⁽¹¹⁾ and has a profound impact on chemotherapy for CRC patients.^(12,13) *GSTP1* I105V polymorphism also predicts cumulative neuropathy in patients treated with docetaxel- as well as oxaliplatin-based chemotherapy,^(15,16) but controversies exist. In addition, this polymorphism is associated with the susceptibility to several cancers.⁽¹⁷⁾

In addition to detoxification pathways, genetic polymorphisms in genes involved in DNA repair also attribute to resistance to oxaliplatin.^(8,9) From a genetic viewpoint, in a multi-factorial disease, it is sometimes difficult with single polymorphisms in single genes to alter the extent of a physiologic or pathologic phenotype. Therefore, combined analysis of genes involved in the NER pathways, including *ERCC1*, *XPB*, and detoxification pathways, may more actually identify patients with maximal benefit, or toxicity, from oxaliplatin-based chemotherapy.

Ethnic differences have a profound influence on the response and toxicity to chemotherapeutic agents. Due to a higher prevalence of epidermal growth factor receptor mutations, gefitinib becomes very effective in Asian patients with non-small-cell lung cancer.⁽¹⁸⁾ The *UGT1A1**28 polymorphism is rare in Asian populations,⁽¹⁹⁾ leading to decreased risk of severe neutropenia after being treated with irinotecan. Furthermore, Asian populations have a remarkably lower prevalence of *ERCC1* codon 118 C → T and *XPB* K751Q polymorphisms, which may affect platinum-based chemotherapy.^(8,9) However, little is known about ethnic difference in *GSTP1* I105V polymorphism and its impact on Asian CRC patients treated with oxaliplatin.

Based on these earlier findings, we propose that the *GSTP1* I105V polymorphism may account for altered susceptibility and neuropathy to oxaliplatin-based chemotherapy in CRC patients. Ethnic difference of this polymorphism in Asian populations was analyzed, and combined analysis of the *GSTP1* I105V, *ERCC1*-118, and *XPB*-751 polymorphisms was conducted.

Materials and Methods

Patient characteristics. To understand the impact of the *GSTP1* I105V polymorphism on response and neurotoxicity to

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FOLFOX-4 treatment, we examined 217 Chinese patients with unresectable metastatic CRC, who had received FOLFOX-4 as a first-line treatment, from June 2003 to December 2007. Among them, 166 patients were enrolled and analyzed (patients' characteristics are shown in Table 1). The remaining patients were excluded. They either lacked measurable lesions ($n = 9$), or did not have primary tumor removed to know the accurate T and N stages ($n = 7$), or died before blood sampling ($n = 6$), or were unwilling to participate ($n = 4$), or were lost in follow-up ($n = 3$). Patients with diabetes mellitus ($n = 10$), central nervous system metastasis ($n = 7$), pre-existing neuropathy ($n = 3$), and alcoholic disease ($n = 2$), were also excluded from this study. The FOLFOX-4 regimen consisted of oxaliplatin (Sanofi-Aventis, Paris, France) 85 mg/m² (1-h infusion, day 1) and folinic acid (FA) (200 mg/m², 2-h infusion, days 1 and 2) before bolus 5-fluorouracil (5-FU) (400 mg/m², days 1 and 2) and infusional 5-FU (600 mg/m², 22-h infusion immediately after bolus 5-FU, days 1 and 2) was administered every 2 weeks. Patients with or without the *GSTP1* I105V polymorphism were followed up at a similar intensity with a median duration of 18.0 months. The responses of treatment were evaluated on the basis of standard RECIST criteria. Patients with complete response (CR), partial response (PR), or stable disease remained in the protocol until progressive disease or unacceptable toxicity was documented. Common toxicities were assessed according to the National Cancer Institute Common Toxicity Criteria (NCI-CTC).

In this study, we primarily focused on oxaliplatin-induced 'chronic cumulative neuropathy' because this neuropathy may result in a severe disturbance of neurologic function, and have a significant impact on oxaliplatin treatment. A detailed neurological history was obtained including possible risk factors for the development of peripheral neuropathy (e.g. diabetes mellitus, alcohol abuse, central nervous system diseases, or prior history of neurotoxic chemotherapy or neuropathy). Symptoms (paresthesias, dysesthesias, numbness, etc.) as well as whether symptoms interfered with function were assessed separately and were graded according to the NCI-CTC criteria. Complete neurological examinations were performed at baseline and after four, eight, and 12 cycles of treatment. Treatment was delayed until recovery if grade 3–4 toxicity occurred, and the doses of oxaliplatin and 5-FU were reduced by 20% in subsequent cycles. In the case of intolerable toxicity or failure on front-line FOLFOX-4, treatment was discontinued, and irinotecan-based or fluoropyrimidine-only regimens were subsequently administered according to physicians' decision. During treatments, chest X-ray, ultrasonography of the abdomen, or computed tomography scan was conducted every 2 months. An institutional review board approved this study and an informed consent was given by all patients before blood testing for genotyping.

Examination of the *GSTP1* I105V polymorphism. Genomic DNA, extracted from patients' leukocytes obtained via 0.5-mL whole blood using standard phenol-chloroform procedures, was subjected to *GSTP1* codon 105 testing. The *GSTP1* I105V polymorphism was examined by the PCR–restriction fragment length polymorphism (PCR-RFLP) method as previously described.⁽¹⁷⁾ 0.1 µg of genomic DNA, forward primer 5'-ACC CCA GGG CTC TAT GGG AA-3' and reverse primer 5'-TGA GGG CAC AAG AAG CCC CT-3', were used for PCR amplification. Initial denaturation was carried out at 95°C for 5 min. Cycling conditions were: primer annealing at 55°C for 30 s, polymerization at 72°C for 30 s, and strand separation at 94°C for 30 s. Thirty cycles were carried out. A final polymerization step of 72°C for 5 min was carried out to complete the elongation processes. PCR products, after being digested by *Bsm*AI restriction enzyme (New England Biolabs, Beverly, MA, USA) at 37°C for 2 h, were separated on 2% Nusieve ethidium bromide-stained agarose gels to visualize the bands.

Table 1. Clinico-pathological features of metastatic colorectal cancer patients with or without glutathione S-transferase P1 (*GSTP1*) codon 105 Ile → Val polymorphism ($n = 166$)

Characteristics	Ile/Ile (wild-type) (%)	Ile/Val or Val/Val (%)	P-values
All patients	125 (100)	41 (100)	
Age (years)			
<50	43 (34.4)	13 (31.7)	0.75
≥50	82 (65.6)	28 (68.3)	
Gender			
Male	77 (61.6)	27 (68.9)	0.63
Female	48 (38.4)	14 (34.1)	
Performance status			
0	94 (75.2)	29 (70.7)	0.57
1, 2	31 (24.8)	12 (29.3)	
Primary tumor			
Colon	85 (68.0)	27 (65.9)	0.80
Rectum	40 (32.0)	14 (34.1)	
Histological differentiation			
Well/moderate	111 (88.8)	36 (87.8)	0.86
Poorly/unknown	14 (11.2)	5 (12.2)	
Extent of invasion			
T1-2	44 (35.2)	16 (39.0)	0.66
T3-4	81 (64.8)	25 (61.0)	
Lymph node involvement			
N0	26 (20.8)	11 (26.8)	0.42
N1-3	99 (79.2)	30 (73.2)	
Serum CEA level (ng/mL)			
≤6	13 (10.4)	5 (29.3)	0.75
>6	112 (89.6)	36 (87.8)	
TSER 28-bp polymorphism			
2R/2R or 2R/3R	42 (33.6)	14 (34.1)	0.95
3R/3R	83 (66.4)	27 (65.9)	
<i>ERCC1</i> codon 118 genotype			
C/C (wild type)	59 (47.2)	19 (46.3)	0.92
C/T or T/T	66 (52.8)	22 (53.7)	
<i>XPD</i> codon 751 status			
Lys/Lys (wild type)	105 (84.0)	34 (82.9)	0.87
Lys/Gln	20 (16.0)	7 (17.1)	

CEA, carcinoembryonic antigen; TSER, 5'-enhancer region of the thymidylate synthase gene.

Examinations of genetic polymorphisms involved in the NER pathway and expression of the thymidylate synthase gene. An enhanced repair of DNA-platinum lesions through the NER pathway results in poor response to platinum-based chemotherapy. Because polymorphisms of genes involved in the NER pathway, including *ERCC1* and *XPD*, have contributed to resistance to FOLFOX-4 treatment in Asian populations,^(8,9) the influence of these polymorphisms between different *GSTP1* codon 105 genotypes warrants further analysis. *ERCC1* codon 118 C → T and *XPD* K751Q polymorphisms were examined by PCR-RFLP as previously described.^(8,9) Primers 5'-GCA GAG CTC ACC TGA GGA AC-3' (forward) and 5'-GAG GTG CAA GAA GAG GTG GA-3' (reverse) were used for PCR amplification for examining *ERCC1*-118 C → T, whereas 5'-CCT CTC CCT TTC CTC TGT TC-3' (forward) and 5'-CAG GTG AGG GGG ACA TCT-3' (reverse) were used for examining *XPD* K751Q. PCR products, after being digested with *Bsr*D1 (for *ERCC1*) or *Mbo*II (for *XPD*) restriction enzymes (New England Biolabs), were separated on Nusieve ethidium bromide-stained agarose gels.

Since 5-FU was used in combination with oxaliplatin for treating these patients, and germ-line polymorphisms of a 28-bp tandemly repeated sequence in the 5'-enhancer region of the thymidylate synthase gene (*TSER*) remarkably affect the

response and survival of CRC patients receiving 5-FU,⁽²⁰⁾ the influence of this polymorphism on patients with different *GSTP1* codon 105 genotypes was also analyzed. The primers used for TSER analysis were 5'-GTG GCT CCT GCG TTT CCC CC-3' (forward) and 5'-CCA AGC TTG GCT CCG AGC CGG CCA CAG GCA TGG CGC GG-3' (reverse).⁽²¹⁾ Then the amplified DNA fragments were analyzed by electrophoresis on a 4% agarose gel to determine the number of a 28-bp tandemly repeated sequence in TSER.

Statistical analysis and survival curve plotting. All statistical analyses were performed using the SPSS software system (SPSS for Windows, version 14.0; SPSS, Chicago, IL, USA). The progression-free and overall survival curves were plotted using the Kaplan–Meier product limit method, and the statistical differences in survival among subgroups were compared by log-rank test. The correlations between different *GSTP1*-105 genotypes and clinico-pathological characteristics, response, and neurotoxicity to FOLFOX-4 treatment were analyzed with the chi-squared test. To assess the independent prognostic values of different polymorphisms, we used Cox's proportional hazards regression analysis (multivariate) including *GSTP1*-105, *ERCC1*-118, and *XPB*-751 genotypes and other clinico-pathological parameters. Cox hazard ratio and its 95% confidence interval (CI) were analyzed accordingly. Two-sided *P*-values less than 0.05 were considered statistically significant.

Results

Significantly lower prevalence of *GSTP1* I105V polymorphism is identified in Asian populations, but no difference in this polymorphism between patients with or without CRC. Representative PCR-RFLP patterns of different *GSTP1*-105 genotypes are shown in Figure 1. The prevalence of Ile/Ile, Ile/Val, and Val/Val genotypes was 75.3% (*n* = 125), 22.9% (*n* = 38), and 1.8% (*n* = 3), respectively, in Chinese populations. It has been reported that in Caucasian populations, the prevalence of Ile/Ile, Ile/Val, and Val/Val genotypes was 45% (*n* = 34), 46% (*n* = 35), and 9% (*n* = 7), respectively.⁽¹²⁾ In another study, the prevalence of Ile/Ile, Ile/Val, and Val/Val genotypes was 61% (*n* = 39), 31% (*n* = 20), and 8% (*n* = 5), respectively.⁽¹⁶⁾ In comparison with Caucasian populations, a significantly lower prevalence of Val105 allele variants was clearly demonstrated in our cases (24.7%), indicating the existence of ethnic difference.

Regarding clinico-pathologic characteristics, there were no between-group differences in patients with or without *GSTP1* I105V polymorphism (Table 1). Because I105V polymorphism of *GSTP1* is associated with reduced enzymatic activity and detoxification efficiency,⁽¹¹⁾ and is associated with susceptibility to bladder and testicular cancers,⁽¹⁷⁾ we wondered whether a higher percentage of 105V allele variants may present, and thus contribute to the malignant progression, in CRC patients. A total of 63 patients free of malignant diseases were analyzed, including hypertension (*n* = 23), diabetes mellitus (*n* = 22), chronic obstructive pulmonary diseases (*n* = 10), upper respiratory tract infection (*n* = 6), and urinary tract infection (*n* = 2). The percentages of Ile/Ile, Ile/Val, and Val/Val genotypes were 74.6% (*n* = 47), 23.8% (*n* = 15), and 1.6% (*n* = 1), respectively (*P* = 0.88), indicating no between-group difference in patients with or without CRC.

***GSTP1* I105V polymorphism leads to a higher response to and favorable prognosis for FOLFOX-4 treatment.** In comparison with Ile/Ile (wild type), patients with Ile/Val or Val/Val genotypes in *GSTP1* have a significantly higher response to FOLFOX-4 treatment (56.1% vs 37.6%, *P* = 0.04) (Table 2). Accordingly, longer progression-free (12.0 months vs 6.0 months, *P* < 0.01) and overall (25.0 months vs 16.0 months, *P* < 0.01) survivals were observed in those with Val105 allele variants (Fig. 2), which is consistent with previous findings.^(12,13) By

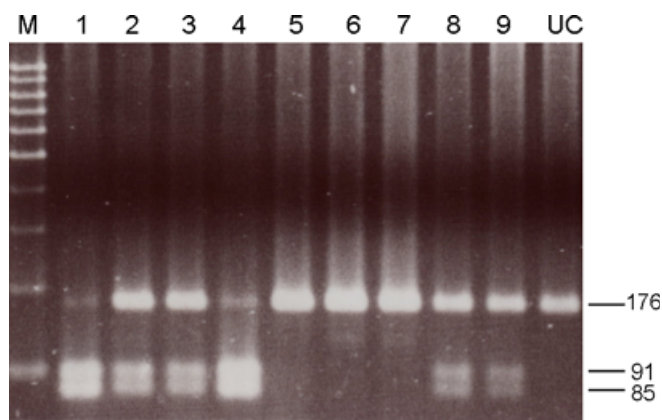


Fig. 1. Representative PCR-restriction fragment length polymorphism (RFLP) patterns of different glutathione *S*-transferase P1 (*GSTP1*) codon 105 genotypes examined by patients' blood samples. Genomic DNA obtained from patients' leukocytes was subjected to PCR amplification using 5'-ACC CCA GGG CTC TAT GGG AA-3' and 5'-TGA GGG CAC AAG AAG CCC CT-3' as forward and reverse primers, respectively. PCR products after being digested by *Bsm*AI were separated by agarose gel electrophoresis. Lanes 1 and 4 represent Val/Val; lanes 2, 3, 8, and 9 represent Ile/Val; lanes 5, 6, and 7 represent Ile/Ile. Lane UC indicates PCR product that has not been digested.

Table 2. Response to FOLFOX-4 treatment in patients with different genetic polymorphisms (*n* = 166)

Genotypes	Responders (%)	Non-responders (%)	<i>P</i> -values
Overall	70 (42.2)	96 (57.8)	
<i>GSTP1</i> -105 status			
Ile/Ile (<i>n</i> = 125)	47 (37.6)	78 (62.4)	0.04
Ile/Val or Val/Val (<i>n</i> = 41)	23 (56.1)	18 (43.9)	
<i>ERCC1</i> -118 genotype			
C/C (<i>n</i> = 78)	41 (52.6)	7 (47.4)	0.01
C/T or T/T (<i>n</i> = 88)	29 (33.0)	59 (67.0)	
<i>XPB</i> -751 status			
Lys/Lys (<i>n</i> = 139)	64 (46.0)	75 (54.0)	0.02
Lys/Gln (<i>n</i> = 27)	6 (22.2)	21 (77.8)	
TSER 28-bp polymorphism			
2R/2R or 2R/3R (<i>n</i> = 56)	26 (46.4)	30 (53.6)	0.43
3R/3R (<i>n</i> = 110)	44 (40.0)	66 (60.0)	
Any risk genotype			
Absence (<i>n</i> = 26)	18 (69.2)	8 (30.8)	<0.01
Presence (<i>n</i> = 140)	52 (37.1)	88 (62.9)	

Risk genotypes: *GSTP1*-105 Ile/Ile, *ERCC1*-118 C/T or T/T, and *XPB*-751 Lys/Gln. Non-responders, patients with stable or progressive diseases; responders, patients with complete or partial remission after treatment with FOLFOX-4.

adjusted analysis, this polymorphism was further identified as an independent prognostic factor (*P* = 0.01; Table 3).

With regard to genes involved in the NER pathway, patients with *ERCC1*-118 C/T or T/T genotypes had a lower response to FOLFOX-4 than those with C/C genotype (33.0% vs 52.6%, *P* = 0.01). And patients with *XPB*-751 Lys/Gln genotype also had a lower response rate (22.2% vs 46.0%, *P* = 0.02) (Table 2). In combined analysis with three risk genotypes, including *GSTP1*-105 Ile/Ile, *ERCC1*-118 C/T or T/T, and *XPB*-751 Lys/Gln, patients without the risk genotypes had a remarkably higher response rate than those with any one of the risk genotypes (69.2% vs 37.1%, *P* < 0.01) (Table 2). Accordingly, remarkably longer progression-free (17.0 months

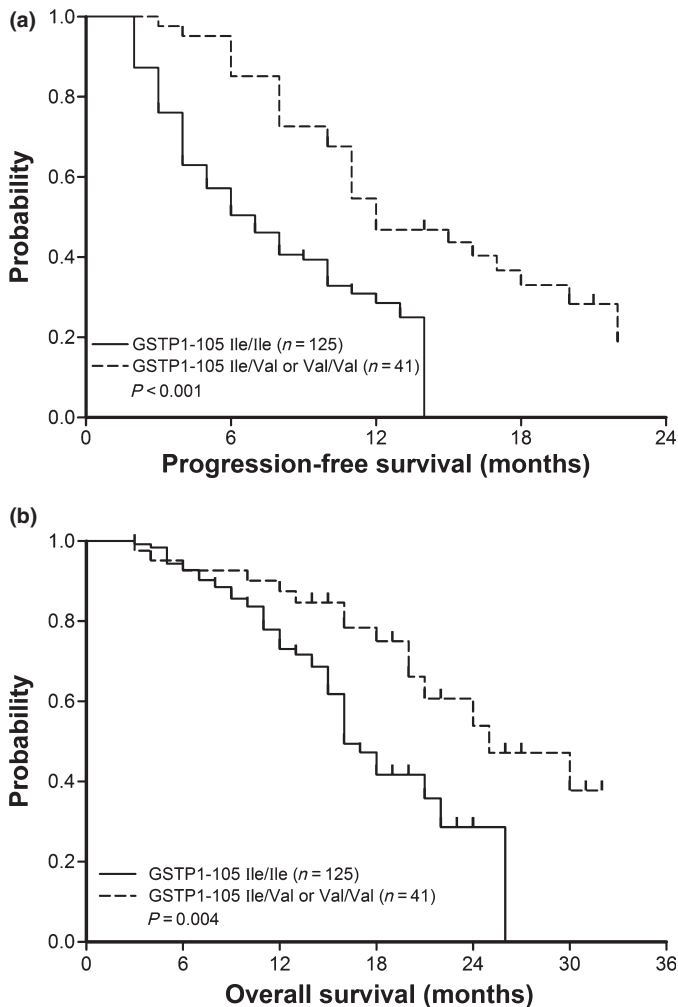


Fig. 2. Patients with glutathione *S*-transferase P1 (*GSTP1*) codon 105 Ile → Val polymorphism have significantly longer progression-free and overall survivals after being treated with FOLFOX-4. (a) Progression-free survival curves of 166 patients with *GSTP1*-105 Ile/Ile (wild type) or Ile/Val, Val/Val genotypes are plotted by Kaplan-Meier method ($P < 0.01$, log-rank test). (b) A similar method has been used to plot overall survival curves of patients with different *GSTP1* codon 105 genotypes ($P < 0.01$).

vs 6.0 months, $P < 0.01$) and overall (30.0 months vs 16.0 months; $P < 0.01$) survivals were observed in patients without risk genotypes (Fig. 3). Furthermore, a better effect in predicting survival was found in combined analysis with three risk genotypes (HR = 5.73, Table 3). Although polymorphisms in TSER affect the response to 5-FU in CRC patients;⁽²⁰⁾ in the current study, patients with different TSER genotypes had a similar response to FOLFOX-4 treatment (Table 2).

***GSTP1* I105V polymorphism is associated with higher incidence of severe oxaliplatin-related cumulative neuropathy.** Later, we proposed that the *GSTP1* I105V polymorphism, by reducing enzymatic activity of *GSTP1* and thus decreased metabolism of potentially cytotoxic compounds, might account for a higher incidence of severe neuropathy after treatment with oxaliplatin. As shown in Table 4, there were significantly more neurotoxicities in patients with Val105 allele variants than those without them. After eight cycles of treatment, the percentage of grade 3–4 peripheral sensory neuropathy was higher in patients with Val105 allele variants (19.5% vs 7.2%, $P = 0.02$). After 12 cycles, the percentage of grade 3–4 neuropathy remained higher in patients

Table 3. Analysis of factors that may affect the survival of patients with metastatic colorectal carcinoma receiving FOLFOX-4 ($n = 166$)

Characteristics	<i>P</i> -values (univariate)	<i>P</i> -values (multivariate)	HR	95% CI
TSER 28-bp polymorphism				
2R/3R + 3R/3R vs 3R/3R	0.06	NA	NA	NA
<i>GSTP1</i> -105 status				
Ile/Ile vs Ile/Val + Val/Val	0.04	0.01	2.45	1.30–4.62
<i>ERCC1</i> -118 genotype				
T/T+T/C vs C/C	0.03	<0.01	3.15	1.89–5.23
<i>XPB</i> -751 status				
Lys/Gln vs Lys/Lys	0.02	<0.01	4.41	2.51–7.75
Any risk genotype				
Presence vs absence	<0.01	<0.01	5.73	2.22–14.78

Risk genotypes: *GSTP1*-105 Ile/Ile, *ERCC1*-118 C/T or T/T, and *XPB*-751 Lys/Gln. 95% CI, 95% confidence interval for Cox hazard ratio; HR, Cox hazard ratio; NA, not analyzed.

with Val105 allele variants (36.6% vs 14.4%, $P < 0.01$). In the current study, patients with different *ERCC1*-118 and *XPB*-751 genotypes had a similar incidence of grade 3–4 neurotoxicity after treatment with FOLFOX-4 ($P = 0.56$ and 0.85, respectively).

Discussion

In comparison with Caucasian populations,^(12,16) a remarkably lower prevalence of the *GSTP1* I105V polymorphism was noted in our patients (24.7%). Ethnic differences have a profound influence on chemotherapy for CRC patients. For example, the prevalence of homozygous triple-repeat polymorphism in TSER was twice as common in Chinese as in Caucasian subjects, which accounts for an impaired response to fluoropyrimidine regimens.⁽²⁰⁾ With regard to genes involved in the NER pathway, a remarkably lower prevalence of *ERCC1* codon 118 C/T or T/T genotypes and *XPB* Gln751 allele variants have been found in Asian populations, which may account for an increased susceptibility to platinum-based chemotherapy.^(8,9) It has been shown that Asian populations seem to have a lower prevalence of *GSTP1* Val105 allele variants,⁽¹⁰⁾ but the sample size was quite small ($n = 11$). In our study, a larger sample size ($n = 166$) was analyzed, and a remarkably lower percentage of Val105 allele variants (24.7%) was identified, compared to that in Caucasian populations.^(12,16) These results implicated that Asian populations might have a better detoxification pathway. However, to date, no randomized study has been conducted to compare the outcome of FOLFOX treatment in different racial populations. Whether these polymorphisms may account for altered treatment outcome in Asian patients warrants further studies.

Altered expression of *GST* genes has been associated with different susceptibility to cancer.^(22–24) For example, methylation of the regulatory sequences near the *GSTP1*, resulting in its inactivation, has been identified in the early stages of prostatic carcinogenesis.⁽²²⁾ Polymorphisms in *GSTM1* and *GSTT1* are associated with the susceptibility to colon cancer and age at onset,⁽²³⁾ and I105V polymorphism of the *GSTP1* has been associated with susceptibility to testicular and bladder cancers.⁽¹⁷⁾ Because I105V polymorphism of the *GSTP1* is associated with reduced detoxification efficiency in comparison with the wild-type one,⁽¹¹⁾ a higher prevalence of the I105V allele variants might contribute to susceptibility in CRC. However, in the current study, there was no statistically difference between patients with CRC and control groups in different *GSTP1* codon 105 genotypes, which was similar to a previous report.⁽²⁴⁾

It has been reported that the *ERCC1* codon 118 C → T and *XPB* K751Q polymorphism results in a higher NER

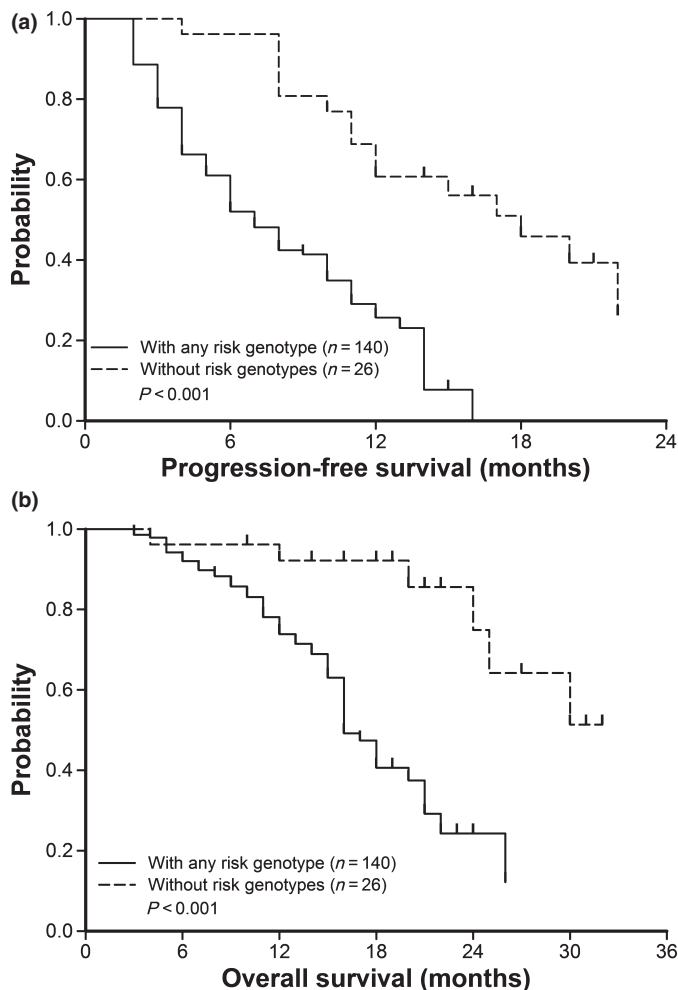


Fig. 3. Patients without risk genotypes (*GSTP1*-105 Ile/Ile, *ERCC1*-118 C/T or T/T, and *XPB*-751 Lys/Gln) have a significantly longer progression-free as well as overall survival time after treatment with FOLFOX-4. (a) Progression-free survival curves of 166 patients with or without any risk genotype plotted by Kaplan–Meier method ($P < 0.01$, log-rank test). (b) A similar method has been used to plot overall survival curves of patients with or without any risk genotype ($P < 0.01$).

proficiency;^(8,9) we proposed that these polymorphisms may have an influence in the development of neuropathy after treatment with oxaliplatin. But in the current study, patients with different *ERCC1* codon 118 and *XPB* codon 751 genotypes had a similar incidence of severe neurotoxicity after treatment with FOLFOX-4 ($P = 0.56$ and 0.85 , respectively). These results indicate that different NER proficiency and oxaliplatin metabolisms between nerve and tumor tissues might exist. Another explanation is that nerve cells are not in actively dividing status; therefore, oxaliplatin neurotoxicity may be mediated by a different mechanism than the platinum-DNA lesions. For example, oxalate, an oxaliplatin metabolite, may alter the properties of voltage-gated sodium channels or slow down the clearance of platinum compounds from the peripheral nervous system.⁽²⁵⁾

We found that patients with *GSTP1* Val105 allele variants had a significantly higher incidence of grade 3/4 cumulative neuropathy after treatment with FOLFOX-4 (Table 4). To date, in spite of the biochemical evidence that GSTs mediate the inactivation of platinum drugs, the association between polymorphisms in the *GST* genes and platinum-induced neurotoxicity remains controversial.^(16,26,27) It has been shown that the Val105 allele variants of the *GSTP1* were an unfavorable

Table 4. Incidence of oxaliplatin-induced neuropathy after different cycles of treatment of patients with different glutathione S-transferase P1 (*GSTP1*) codon 105 status ($n = 166$)

Neurotoxicity	Ile/Ile (wild type) (%)	Ile/Val or Val/Val (%)	<i>P</i> -values
All patients enrolled	125 (100)	41 (100)	
After 4 cycles			
Grade 0–2	124 (99.2)	40 (97.6)	0.40
Grade 3–4	1 (0.8)	1 (2.4)	
After 8 cycles			
Grade 0–2	116 (92.8)	33 (80.5)	0.02
Grade 3–4	9 (7.2)	8 (19.5)	
After 12 cycles			
Grade 0–2	107 (85.6)	26 (63.4)	<0.01
Grade 3–4	18 (14.4)	15 (36.6)	

Neurological toxicity defined by the National Cancer Institute Common Toxicity Criteria (NCI-CTC).

factor for developing neurotoxicity.⁽²⁶⁾ However, in another study, this polymorphism confers a decreased risk of developing severe cumulative neuropathy.⁽¹⁶⁾ In another study, this polymorphism was not identified as having any correlation with oxaliplatin neurotoxicity.⁽²⁷⁾ In some cases, these results should be interpreted with caution because some studies were analyzed on a retrospective basis with very limited sample size. Furthermore, concurrent medications and environmental factors may also play important roles in inter-individual variability. Prospective studies with larger sample sizes and a homogenous population of patients treated with the same regimens are warranted in order to clarify this issue.

In the current study, patients with *GSTP1* Val105 allele variants had a significantly higher response to FOLFOX-4 (56.1% vs 37.6%, $P = 0.04$) (Table 2) and longer progression-free and overall survivals ($P < 0.01$, Fig. 2), consistent with previous findings.^(12,13) *GSTP1* is directly involved in the detoxification of cisplatin by the formation of cisplatin-glutathione adducts,⁽²⁸⁾ indicating that *GSTP1* plays a role in the acquisition of resistance to this platinum compound.⁽²⁹⁾ Patients with head and neck carcinoma with a lower level of intra-tumor GST expression also have a better response and survival to platinum-based treatment.⁽³⁰⁾ However, discrepancies existed in the association between *GSTP1*-105 variants and survival of CRC patients treated with oxaliplatin.^(26,27) The authors explained that these controversies might result from the exertion of effects through a multistep cascade by most chemotherapeutic drugs. In addition, reduced enzymatic activity in Val105 allele variants leading to increased toxicities might have influenced patient survival.⁽²⁶⁾

Interestingly, a better effect in predicting treatment outcome was found in combined analysis with genes involved in detoxification and NER pathways (Tables 2,3). In combined analysis with three risk genotypes, including *GSTP1*-105 Ile/Ile, *ERCC1*-118 C/T or T/T, and *XPB*-751 Lys/Gln, patients without risk genotypes had a remarkably higher response rate than those who had any one of the genotypes ($P < 0.01$, Table 2). Accordingly, remarkably longer progression-free and overall survivals ($P < 0.01$) are observed in patients without risk genotypes (HR = 5.73, Fig. 3). Because polymorphisms in genes involved in DNA repair also attributed to resistance to oxaliplatin,^(8,9) combined analysis of several polymorphisms in genes involved in NER and detoxification pathways may be more predictive of treatment outcome of oxaliplatin-based chemotherapy.

Except for FOLFOX-4, the *GSTP1* I105V polymorphism also has effects on other types of chemotherapy. In non-small-cell lung cancer patients, *GSTP1* I105V polymorphism increases the response to platinum-based chemotherapy.⁽³¹⁾ In acute myeloid

leukemia patients, a significantly longer relapse-free, as well as overall survival, was found in patients with Val105 allele variants.⁽³²⁾ For gastric cancer patients treated with cisplatin plus 5-FU, carriage of the Val/Val genotype showed a significantly higher response rate and longer survival compared to those with at least one Ile allele.⁽³³⁾ However, for metastatic breast cancer patients treated with high-dose chemotherapy and autologous stem cell transplantation, the risks for disease progression and cancer specific death were higher in patients with AG or GG (Ile/Val or Val/Val) genotypes.⁽³⁴⁾ Whether this is due to pre-conditioning regimens that did not contain platinum drugs or simply due to different tumor type deserves further studies. With regard to irinotecan, this polymorphism was predictive of the response to irinotecan-based chemotherapy in metastatic CRC patients, with the Val105 allele variants being associated with a better outcome.⁽³⁵⁾

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