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Original Paper

Association of Antioxidative Enzymes Polymorphisms with Efficacy of Platin and Fluorouracil-Based Adjuvant Therapy in Gastric Cancer

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Key Words
Gastric cancer • Adjuvant chemotherapy • Genetic polymorphism • Biomarker

Abstract
Background/Aims: Imbalance of oxidative/antioxidative enzymes in cells is associated with carcinogenesis and cancer cell chemoresistance. The aim of this study was to examine the clinical significance of potentially functional single nucleotides polymorphisms (SNPs) in antioxidative enzymes, *GPxs* and *CAT*, in stages II and III gastric cancer patients. ***Methods:*** A total of 591 gastric cancer patients who had radical gastrectomy were recruited. 207 patients received platinum and fluorouracil-based (PF-based) adjuvant chemotherapy and 384 patients were untreated. *GPx1* rs1050450, *GPx2* rs4902346, *GPx3* rs736775, rs3828599 and *CAT* rs769218 were genotyped in the DNA samples extracted from paraffin-embedded tumor tissue. ***Results:*** *CAT* rs769218 was significantly correlated with the overall survival (OS) in the dominant model (P = 0.014). Multivariate analysis revealed that *CAT* rs769218 GA/AA (HR, 0.715; 95%CI, 0.562-0.910, P = 0.006) was an independent prognostic marker indicating improved survival. After adjustments, *GPx3* rs736775 TC/CC was significantly associated with improved OS (HR, 0.621;

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95%CI, 0.399-0.965; *P*=0.034) in patients treated with PF-based adjuvant chemotherapy, and *CAT* rs769218 GA/AA was significantly associated with improved OS (HR, 0.646; 95% CI, 0.482-0.864; *P* = 0.003) in the untreated patients. PF-based chemotherapy significantly decreased risk of death for patients carrying *GPx3* rs736775 TC/CC and age ≤ 60 years or with diffused type adenocarcinoma compared to surgery alone. ***Conclusion:*** our findings suggested *CAT* rs769218 and *GPx3* rs736775 may be considered as prognostic markers in gastric cancer. Patient stratification by *GPx3* rs736775 and conventional pathological parameters may provide additional predictive information in treatment decision-making.

Introduction

Gastric cancer (GC) is the fourth most commonly diagnosed cancer worldwide and the second leading cause of cancer deaths in China [1-3]. Surgical resection is still the only curative treatment option for gastric cancer patients. Despite the implement of perioperative chemotherapy and chemoradiation therapy, the 5-year survival rate was only improved by 5-15% [4-7]._ENREF_1_ENREF_1 Currently, platin and fluorouracil-based (PF-based) adjuvant chemotherapy is recommended by the main clinical practice guidelines as the standard of care for post-operative gastric cancer patients with stage II and III disease. Conventional clinicopathological parameters or comprehensive molecular classification using The Cancer Genome Atlas (TCGA) datasets only provide prognostic and tumor biological information [8], but not able to predict patients who would be benefit from adjuvant chemotherapy. Therefore, it is necessary to identify biomarkers that can be used to guide personalized therapy, exempting those patients from unnecessary potential exposure to toxicity and the financial burden of chemotherapy treatments.

The intrinsic regulation of reactive oxygen species (ROS) in cancer cells is one of the various molecular mechanisms involved in multidrug resistance and maintenance of cancer stemness [9, 10], which is considered as major causes of poor survival after multi-modalities anti-cancer treatment. Increasing level of ROS has been observed in many cancer types and associated with activation of cancer initiation and progression related signaling, such as mitogen-activated protein (MAP) kinase/Erk [11], phosphoinositide-3-kinase (PI3K)/Akt [12] as well as the IκB kinase (IKK)/nuclear factor κ-B (NF-κB) pathways [13]. Chemotherapeutics such as platinum, taxane and fluorouracil may induce cancer cells death through inducing oxidative stress to highly toxic level. In the process of protecting cancer cells from oxidative damage, superoxide dismutases (SODs) convert superoxide radical into hydrogen peroxide, which is further detoxified into water by glutathione peroxidases (GPxs) and catalase (CAT). Cancer stem cells could survive through chemo drug-induced cells death and initiate recurrence by utilizing redox-regulatory mechanisms [14]. In response to chemotherapeutics and cytotoxic by-products, redox signaling pathways are frequently activated in cancer cells to acquire drug resistance [15, 16]. In pancreatic cancer stem cells, chemoresistance to 5-fluorouracil and gemcitabine was related with suppressed ROS levels [17]. Active GPx1 was found in glioma stem cell lines, which mediated their resistant to oxidative stress induced cell death [18].

Genetic variations of antioxidant genes may alter their activity or function, contributing to the imbalance of ROS production and scavenging. *GPx1* rs1050450 polymorphism causes a proline-leucine substitution at codon 198, which correlated with lipid peroxidation, GPx1 activity and breast cancer risk [19]. C-262T polymorphisms at *CAT* affected its expression in cells and serum level [20, 21]. Previously, we reported *SOD2* rs4880, which may regulate SOD2 catalyzation activity, could predict gastric patients' survival after receiving adjuvant chemotherapy [22]. However, the clinical significance of the genetic variations of the antioxidative enzymes regulating hydrogen peroxide scavenging has not been examined. Herein, we investigated the association of potentially functional single nucleotides polymorphisms (SNPs) in *CAT*, *GPx1*, *GPx2* and *GPx3* with adjuvant chemotherapy outcome in gastric cancer patients.

Materials and Methods

Patients

All patients received curative surgery and diagnosed at stage II-III according to disease histological examinations and imaging studies by board-certified pathologists at the Yixing People's Hospital (Yixing, Jiangsu Province, China) between 1999 and 2006 were recruited for retrospective analysis [23]. None of them had perioperative chemoradiation or neoadjuvant chemotherapy. 207 patients had PF-based adjuvant chemotherapy within one month after surgery, and 384 patients received no adjuvant therapy for various reasons. Overall survival (OS) was determined from the date of surgery to the date of death or last follow-up (March 31, 2009, ranging from 3-118 months). The demographic features and clinicopathologic data are summarized in Table 1. Surgical specimens were processed immediately after the operation by fixing in buffered paraformaldehyde before embedding in paraffin. The samples used for genotyping were reviewed and classified by 2 independent pathologists. The study protocol was approved by the Institutional Review Board of Nanjing Medical University (Nanjing, China). All patients have given written informed consents on the use of clinical specimens for medical research.

Table 1. Characteristics of the two cohorts of the gastric cancer patients. [a] Tumor size was measured by the length of the tumor. [b] Partial data were not available and statistics were based on available data. [c] Data were defined according to the TNM classification (AJCC 7th, seven edition of the American Joint Commission on Cancer Staging Manual) for gastric cancer. [d] Classification is based on the predominant pattern of tumor as tubular adenocarcinoma (well to moderately differentiated), poorly differentiated adenocarcinoma (poorly differentiated). Mucinous carcinoma and Signet-ring cell carcinoma are included as poorly differentiated

Clinicopathologic features	treated (n=207)	untreated (n=384)	P
Age (years)			
≤ 60	113	157	0.002
> 60	94	227	
Sex			
male	166	290	0.218
female	41	94	
Tumor size [a]			
≤ 5cm	118	205	0.436
> 5cm	89	179	
Tumor location [b]			
antrum	42	73	0.244
fundus or cardia	78	141	
body	51	120	
multiple locations	7	26	
Tumor stage [c]			
II	61	124	0.516
III	146	260	
Tumor differentiation [b, d]			
well to moderately	61	113	1.000
poorly	145	270	
Lauren classification [b]			
intestinal type	64	116	0.851
diffuse type	142	268	

Treatment plan

The adjuvant chemotherapy consisted of at least 4 cycles of PF-based regimens, including combinations of cisplatin and 5-fluorouracil, oxaliplatin and 5-fluorouracil. Chemotherapy was given only if the patient had neutrophil count of ≥ 1.5×10^9/L, platelet count of ≥ 100×10^9/L, hemoglobin level of ≥ 8 g/dl and no sign of organ toxicity. Antiemetics and mannitol diuresis were given according to institutional protocols.

SNP selection

All common (minor allele frequency, MAF > 0.05 in the Han Chinese) polymorphisms in hydrogen peroxide scavenging related genes, *GPx* and *CAT*, with potentially functional significance, that is, located at 5'-flanking regions (5'-FRs), 5'-untranslated regions (5'-UTRs), coding regions, or 3'-UTRs according to NCBI dbSNPs were identified. SNPs that were demonstrated to be of biological significance or associated with gene expression and/or cancer risk/survival according to the literature review were also included. If SNPs are in high linkage disequilibrium (LD) ($r^2 > 0.8$), only one SNP were genotyped. As a result, *GPx1* rs1050450, *GPx2* rs4902346, *GPx3* rs736775, rs3828599 and *CAT* rs769218 were selected for genotyping and analysis (Table 2).

Genotyping

Genomic DNA was extracted from tumor specimens by proteinase K digestion, isopropanol extraction, and ethanol precipitation [24]. The SNPs were examined by multiplex TaqMan technology using ABI fluorescence-based allelic discrimination method (Applied Biosystems, Foster City, CA) as described previously [25]. The SNPs were analyzed using ABI 7900, and the genotypes were determined by using

GeneMapper 4.0 software (Applied Biosystems). Genotyping was validated in randomly selected 10% of samples by Sanger sequencing, and the results were 100% concordant.

Statistical analysis

The SPSS Statistical Package for Windows (version 16; SPSS Inc. Chicago, IL) was used for data analysis. All statistical tests were two-sided, and an association was considered statistically significant with a P value of < 0.05. Kaplan-Meier survival curves and the log-rank test were used for survival analysis. Chi-squared test was used to assess differences in the frequencies of characteristics between patient sub-groups. Cox regression was used in the univariate survival analysis to determine the association of individual clinicopathologic variables with overall survival. All variables with $P < 0.05$ in addition to age, sex and treatment arms were subsequently subjected to the multivariate Cox regression analysis in the corresponding cohort to determine the hazards ratios (HRs) and the independence of effects. Because of the exploratory nature of the study, all P values were not adjusted for multiple comparisons [26].

Results

Patient Characteristics and Associations with the 5 SNPs

Among the 591 patients, *GPx1* rs1050450, *GPx2* rs4902346, *GPx3* rs736775, rs3828599 and *CAT* rs769218 were successfully determined in more than 96% of the samples (Table 2). The associations between their genotypes and patient characteristics were examined. *CAT* rs769218 GA/GG were correlated with tumor at antrum ($P = 0.003$). *GPX3* rs736775 TC/CC were associated with intestinal type of gastric adenocarcinoma ($P = 0.036$). In this population, 207 patients received PF-based adjuvant chemotherapy and 384 patients were untreated. As shown in the Table 1, patients over 60-year old tended to not have adjuvant chemotherapy ($P = 0.002$). The median age of patients treated with PF-based adjuvant chemotherapy was 59 years and that of untreated patients was 63 years. None of the rest clinicopathological parameters were found significantly different between the two cohorts. Excepted for *GPx1* rs1050450 ($P = 0.029$), the allele distributions of the rest SNPs were consistent between different treatment groups.

GPx3 rs736775 and CAT rs769218 as Prognostic Markers

Univariate Cox regression was used to examine the associations between the 5 SNPs and OS in the total patients by using different genetic models (Table 2). P-values of Hardy-Weinberg equilibrium (HWE) were all greater than 0.05. None of *GPx*s related SNPs were found to have significant association with the OS. *CAT* rs769218 was significantly correlated with the OS in the dominant model ($P = 0.014$). Comparing to the patients carrying rs769218 GG, those with GA/AA had a longer mean overall survival time (MST) of 69 months (Log-rank = 6.211, $P = 0.013$). As for the conventional clinicopathological variables, lymph node metastasis ($P = 0.011$) and tumor stage ($P < 0.001$) were significantly correlated with postoperative OS in the total patients. Multivariate analysis revealed that *CAT* rs769218 GA/AA (HR, 0.715; 95%CI, 0.562-0.910, $P = 0.006$) was an independent prognostic marker indicating improved survival beside advanced tumor stage (HR, 1.868; 95%CI, 1.145-3.047; $P = 0.012$, Table 3).

The overall survival time did not show significant difference between patients treated with PF-based adjuvant chemotherapy and those untreated. Poor tumor

Table 2. Genotyping results with patients' survival (n=591). SNP, single nucleotide polymorphism; MAF, minor allele frequency; NA, not available. [a] major > minor allele. [b] MAF in Patients. [c] Calculated in univariate Cox regression. [d] only TT and CT genotypes were identified

SNP	Base change [a]	Gene	Location	Genotyping Rate	MAF [b]	P [c]	
						Dominant model	Recessive model
rs1050450	T>C	GPx1	3p21.31	98.1%	0.057	0.149 [d]	NA
rs4902346	C>T	GPx2	14q23.3	98.5%	0.348	0.137	0.425
rs736775	T>C	GPx3	5q33.1	96.8%	0.341	0.608	0.742
rs3828599	T>C	GPx3	5q33.1	96.4%	0.480	0.944	0.177
rs769218	G>A	CAT	11p13	99.0%	0.445	0.014	0.244

differentiation (P = 0.003), positive lymph node metastasis (P = 0.033), diffused type of tumor (P = 0.004) and advanced tumor stage (P < 0.001) were associated with shorter overall survival time in untreated cohort. In contrast, none of these clinicopathological parameters was found to significantly associate with OS in the treated patients. We further analyzed the 5 SNPs with overall survival in the two clinical settings (Table 4). When adjusted by age and sex, *GPx3* rs736775 TC/CC was associated with improved OS (HR, 0.621; 95%CI, 0.399-0.965; P=0.034) in patients treated with PF-based adjuvant chemotherapy. The MST was 48 months for patients carrying *GPx3* rs736775 TT, compared to the MST of 69 months for those carrying TC/CC (Log-rank = 4.641, P = 0.031, Fig. 1A). No such association was observed for the patients without adjuvant chemotherapy (Fig. 1B). The OS was not statistically different in treated patients with *CAT* rs769218 GG or GA/AA genotypes (Fig. 2A). However, in the untreated arm, *CAT* rs769218 GG was associated with shorter MST of 55 months than those carrying GA/AA (MST, 69

Table 3. Multivariable Cox regression analysis on patients' overall survival (n=591). HR, hazard ratio; CI: confident interval. Values in bold denote a significant P value (P< 0.05). [a] Data were defined according to the TNM classification (AJCC 7th, seven edition of the American Joint Commission on Cancer Staging Manual) for gastric cancer

Variables	HR (95% CI)	P
Age (years)		0.141
<=60	1	
>60	1.196(0.942-1.518)	
Sex		0.218
Male	1	
Female	1.189(0.903-1.565)	
Lymph node metastasis [a]		0.562
N0	1	
N1/N2/N3	0.851(0.494-1.467)	
Tumor stage [a]		0.012
II	1	
III	1.868(1.145-3.047)	
CAT rs769218		0.006
GG	1	
GA/AA	0.715(0.562-0.910)	

Table 4. Association of 5 SNPs with gastric cancer patients' survival. HR, hazard ratio; CI, confidence intervals; Median, median overall survival time; Mean, mean overall survival time; NA, not available. [a] Calculated in the multivariate Cox regression. [b] Mean overall survival time and median overall survival time were calculated in Kaplan-Meier analysis

		Treated (n=207)				Untreated (n=384)			
Genetic models	Genotypes	Mean (months)	Median (months)	P [a]	HR (95%CI) [a]	Mean (months)	Median (months)	P [a]	HR (95%CI) [a]
GPX1 rs1050450 T>C									
	TT	63	NA	0.112	1	64	62	0.259	1
	CT	42	33		1.557(0.902-	55	38		1.302(0.824-2.059)
GPX2 rs4902346 C>T									
Codominant model	CC	53	62	0.380	1	60	43	0.147	1
	CT	64	NA		0.838(0.528-	62	63		0.803(0.594-1.086)
	TT	47	30		1.342(0.673-	73	88		0.639(0.379-1.077)
Dominant model	CC	53	62	0.687	1	63	54	0.074	1
	CT/TT	62	NA		0.914(0.590-	65	65		0.769(0.577-1.026)
Recessive model	CT/CC	62	NA	0.237	1	54	54	0.186	1
	TT	47	30		1.478(0.774-	73	88		0.713(0.431-1.177)
GPX3 rs736775 T>C									
Codominant model	TT	48	42	0.091	1	68	63	0.441	1
	TC	70	NA		0.757(0.370-	58	58		1.295(0.833-2.014)
	CC	46	NA		0.583(0.359-	60	50		1.162(0.851-1.587)
Dominant model	TT	48	42	0.034	1	68	63	0.239	1
	TC/CC	69	NA		0.621(0.399-	60	54		0.839(0.626-1.124)
Recessive model	TC/TT	61	NA	0.875	1	65	62	0.382	1
	CC	46	NA		0.945(0.471-	60	50		0.832(0.552-1.256)
GPX3 rs3828599 T>C									
Codominant model	TT	56	45	0.323	1	65	58	0.625	1
	TC	64	NA		0.952(0.524-	60	63		1.204(0.819-1.769)
	CC	50	62		0.699(0.414-	60	48		1.059(0.751-1.493)
Dominant model	TT	57	45	0.303	1	62	56	0.518	1
	TC/CC	61	NA		0.773(0.474-	65	58		1.110(0.809-1.523)
Recessive model	TC/TT	63	NA	0.457	1	60	48	0.361	1
	CC	50	62		1.208	63	63		0.859(0.620-1.190)
CAT rs769218 G>A									
Codominant model	GG	50	51	0.339	1	55	37	0.006	1
	GA	66	NA		0.705(0.436-	74	94		0.586(0.422-0.816)
	AA	44	NA		0.926(0.516-	58	47		0.754(0.523-1.087)
Dominant model	GG	51	51	0.234	1	55	37	0.003	1
	GA/AA	64	NA		0.767(0.495-	69	77		0.646(0.482-0.864)
Recessive model	GA/GG	62	NA	0.681	1	66	62	0.980	1
	AA	44	NA		1.118(0.656-	58	47		1.004(0.721-1.398)
Combined effects of GPX3 rs736775-C and CAT rs769218-A									
	0	43	31	0.076	1	56	35	0.548	1
	1-2	64	NA		0.590(0.357-	65	63		0.840(0.526-1.339)
	3-4	44	NA		0.503(0.231-	64	62		0.746(0.423-1.316)

months; Log-rank = 5.355, *P* = 0.021, Fig. 2B). The significant difference remained (*CAT* rs769218 GA/AA, HR, 0.646; 95% CI, 0.482-0.864; *P* = 0.003) after adjustment for age, sex, tumor differentiation, lymph node metastasis, Lauren classification and tumor stage (stage III, HR, 2.100; 95% CI, 1.172-3.764; *P* = 0.013). Then, we evaluated the combined effects on overall survival in the two clinical settings by adding the number of variant alleles of the two significant SNPs (GPX3 rs736775-C and CAT rs769218-A). The "0" allele means subjects with wide-type homozygotes of the two SNPs; "1-4" alleles means carrying one to four variant alleles of the two SNPs. When compared with the subjects with wide-type homozygotes of the two SNPs, subjects carrying "1-2" variant alleles had an improved survival (HR, 0.590; 95%CI, 0.357-0.973; *P* = 0.039).

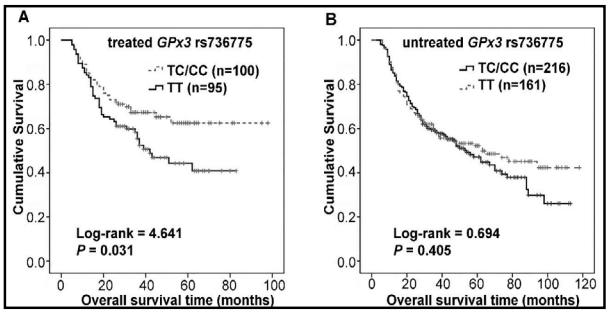

Fig. 1. Kaplan-Meier analyses of OS of treated/untreated patients by GPx3 rs736775 genotypes. (A) OS of treated patients by GPx3 rs736775 genotypes. (B) OS of untreated patients by GPx3 rs736775 genotypes.

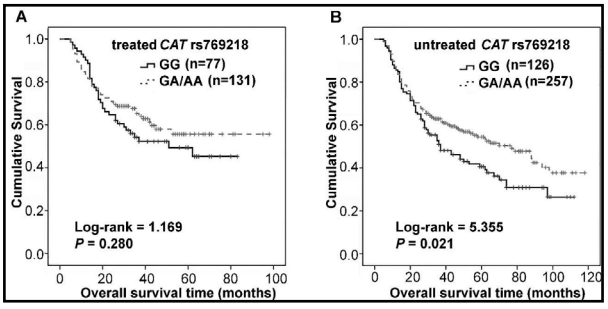

Fig. 2. Kaplan-Meier analyses of OS of treated/untreated patients by CAT rs769218 genotypes. (A) OS of treated patients by CAT rs769218 genotypes. (B) OS of untreated patients by CAT rs769218 genotypes.

GPx3 rs736775 Polymorphisms as a Predictive Marker

When the patients were divided by the genetic models, none of *GPx1* rs1050450, *GPx2* rs4902346, *GPX3* rs3828599 or *CAT* rs769218 genotypes associated with survival benefit from adjuvant chemotherapy (all *P* >0.05). Initial analysis showed the treatment benefit was present in patients with *GPx3* rs736775 TC/CC but not in those with TT genotype (Fig. 3A and 3B). The MST was 69 months for the patients treated with PF-based adjuvant chemotherapy, which is significantly longer than the MST (60 months) of untreated patients (Log-rank = 3.953, *P* = 0.047). For the patients carrying *GPx3* rs736775 TC/CC, lymph node metastasis (*P* = 0.008) and tumor stage (*P* = 0.001) were significantly associated with OS in univariate analysis. When adjusted by age, sex and these cofounders, adjuvant chemotherapy showed a trend towards lower risk of death than untreated patients with *GPx3* rs736775 TC/CC (HR, 0.698; 95% CI, 0.471-1.034; *P* = 0.073). We further performed stratification analysis. No treatment benefit was observed in patients with *GPx3* rs736775 TC/CC of stage II (HR, 0. 724; 95% CI, 0.310-1.694; *P* = 0.457) or stage III disease (HR, 0.708; 95% CI, 0.453-1.108; *P* = 0.131). PF-based chemotherapy significantly decreased risk of death for patients carrying *GPx3* rs736775 TC/CC and age ≤ 60 years (HR, 0.542; 95% CI, 0.303-0.970; *P* = 0.039; Fig. 4A) or with diffused type adenocarcinoma (HR,

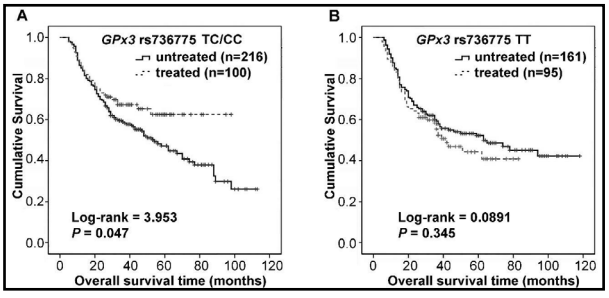

Fig. 3. Kaplan-Meier analyses of OS of patients carrying GPX3 rs736775 TC/CC or TT by treatment status. (A) OS of patients with GPx3 rs736775 TC/CC by treatment status. (B) OS of patients with GPx3 rs736775 TT by treatment status.

0.506; 95% CI, 0.308 to 0.831; P = 0.007; Fig. 4C) compared to surgery alone. As for patients carrying *GPx3* rs736775 TT, no protective effect from adjuvant chemotherapy was observed in those at younger age or with the diffused type of tumor (Fig. 4B and 4D).

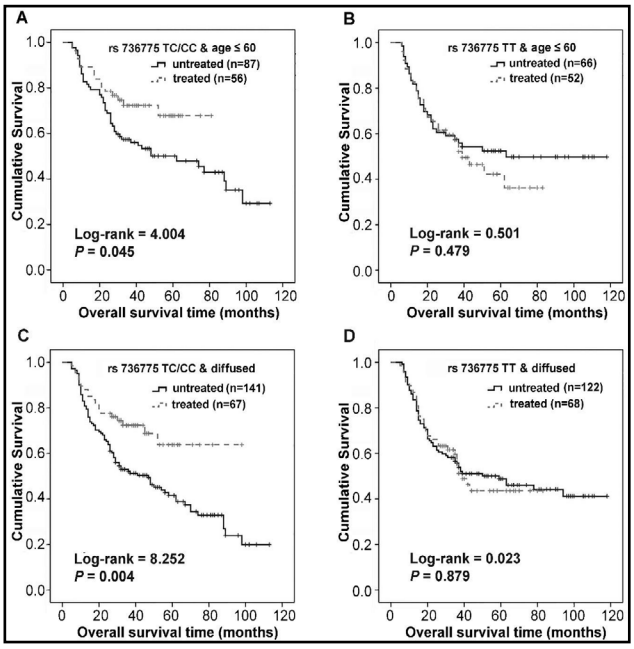

Fig. 4. (A) OS in patients ≤ 60 years and GPx3 rs736775 TC/CC by treatment status. (B) OS in patients with ≤ 60 years and GPx3 rs736775 TT by treatment status. (C) OS in patients with diffused type adenocarcinoma and GPx3 rs736775 TC/CC by treatment status. (D) OS in patients diffused type adenocarcinoma and GPx3 rs736775 TT by treatment status.

Discussion

Adjuvant PF-based therapy in patients with locally advanced (stage II or III) gastric adenocarcinoma is well accepted worldwide. Both CLASSIC (Capecitabine and Oxaliplatin Adjuvant Study in Stomach Cancer) and ACTS-GC (Adjuvant Chemotherapy Trial of TS-1 for Gastric Cancer) studies demonstrated a significant increase of 5-year overall survival rate by only 10-15% given the adjuvant chemotherapy [27, 28]. The modest clinical benefit of PF-based adjuvant chemotherapy in gastric cancer patients underlines the need for prognostic and predictive markers to optimize the surveillance and treatment strategy.

In the present study, the clinical associations of 5 SNPs of 4 redox related genes, *CAT* and *GPx*1-3, were explored in stage II-III postoperative gastric cancer patients. *CAT* rs769218 was a significant prognostic marker independent of age, sex, lymph node involvement, tumor differentiation, Lauren classification and tumor stage in postoperative patients. Further stratification analysis revealed the protective effect of rs769218 GA/GG on overall survival was observed in patients without adjuvant chemotherapy. On the other hand, *GPx3* rs736775 TC/CC was associated with improved overall survival in patients received PF-based adjuvant chemotherapy. Both of their prognostic effects maintained in multivariate models.

Regarding as predictive markers, none of the 5 SNPs was demonstrated of significant association with treatment benefit in multivariate models. Although PF-based adjuvant chemotherapy showed a trend towards lower risk of death for patients with *GPx3* rs736775 TC/CC (adjusted HR, 0.68; P = 0.053), our data do not support *GPx3* rs736775 TC/CC as a sole biomarker to recommend PF-based adjuvant treatment for patients with either stage II or III disease. For patients with *GPx3* rs736775 TC /CC, age (≤ 60 years) and tumor differentiation (diffused type) should be taken into consideration to recommend this regimen.

Patients after radical surgery and adjuvant therapy, cancer progression often occur and be subject to local recurrence and distant metastasis, which are partially related with chemoresistance and cancer stem cell persistence. Fluorouracil and platin are commonly used in adjuvant chemotherapy, which may be able to eliminate micrometastasis lesion through increasing intracellular ROS level and subsequently inducing cell death [17, 29]. Increased ROS have shown both pros and cons in cancer initiation and progression [30]. High level of ROS was found in tumor cells than adjacent nontumor counterparts, which cause damage to nucleic acids and lead to mutation, genomic instability and carcinogenesis. However, reducing ROS level through antioxidants accelerated tumor development and metastatic potential in lung [31], melanoma [32, 33] and breast cancer cells [34]. Activation

of redox signaling or its related elements have been found to associate with maintaining low intracellular ROS level and contribute to cancer stem cell survive and chemoresistance [35, 36].

CAT and GPxs are major cellular antioxidants reducing oxygen free radicals like H_2O_2. CAT expression was reported to increase [37] or decrease [38] in gastric carcinoma compared to non-tumor tissue. Under-expression of CAT in cancer cells related with chemoresistance to doxorubicin [39]. Retaining CAT activity showed enhancing cisplatin toxicity in breast cancer mouse models [40]. Genetic variation in *CAT* have been found to associate with impaired CAT activity and certain diseases [41]. Two common *CAT* SNPs, rs1001179 and rs794316, were found to be associated with prostate cancer risk and survival [42, 43]. So far, there is no risk-related *CAT* polymorphisms identified in gastric cancer [44, 45]. Rs769218, an intronic SNP in *CAT*, was found associated with breast overall survival in patients who did not received radiotherapy [46]. In our study, *CAT* rs769218 GG was associated with poor overall survival compared to GA/AA genotypes in patients without PF-based adjuvant chemotherapy. These results suggest *CAT* rs769218 may have potential effect modified by treatment and further functional studies are needed.

GPx3 was found frequently down-regulated in cancer tissues including breast, lung and gastric cancer [47], which may due to its promoter hypermethylation [48-50]. Silencing or decreased activity of GPX3 is correlated with increased ROS level and contributes to cancer initiation [51] and metastasis [52, 53]. Reduced expression of GPx3 could enhance platin sensitivity in colorectal cancer [54], and increased expression was related with low intracellular ROS level and maintaining cancer stem cell phenotype in leukemia stem cells [36]. Two intronic polymorphisms of *GPx3*, rs3805435 and rs3828599, were shown to influence gene expression and correlate with gastric cancer risk [55]. *GPx3* rs736775 C allele was found indicating better survival in colorectal cancer [56]. In our study, the protective effect of *GPx3* rs736775 C allele was demonstrated in patients treated with PF-based adjuvant therapy. Whether this polymorphism has an effect on its expression needs further investigation.

Although our study showed the presence of *CAT* rs769218 GA/AA or *GPx3* rs736775 TC/CC were important prognostic and/or predictive markers for certain gastric cancer patients, there are several cautions should be noted. Patients in this analysis were from a Chinese retrospective cohort and allele's distribution could be different worldwide. The clinical significance of *CAT* rs769218 and *GPx3* rs736775 in gastric cancer should be verified in other populations and patients from randomized clinical trials. Second, there is no consensus on adjuvant chemo regimen for gastric cancer. Taxane, platin and fluorouracil-based drugs such as capecitabine and S-1, were often used in different combinations. Our data only showed patients with *GPx3* rs736775 TC/CC could be benefit from PF-based chemotherapy, thus, it may not be appropriate for guiding adjuvant chemotherapy if other regimens were employed. Third, the biological functions of these genetic variations were not investigated or reported in the literature. Future studies that examining those variants related gene transcription and function would be useful for improve adjuvant chemotherapy efficacy.

In conclusion, *CAT* rs769218 GA/AA and *GPx3* rs736775 TC/CC could serve as prognostic markers for postoperative gastric cancer patients. Moreover, *GPx3* rs736775 genetic models showed specific influences on PF-based adjuvant chemotherapy outcome in some subtypes of gastric cancer. These results suggested that combined with pathological parameters and *GPx3* rs736775 genotypes could help clinical decision-making. Future prospective clinical trials to validate these findings and design for effective regimens for personalized therapy are needed.

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Disclosure Statement

There are no relevant conflicts of interest to disclose.

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