Original Paper

Chemotherapy Effectiveness and Prognosis
of Gastric Cancer Influenced by *PTPN11*
PolymorphismsChuanjun Zhuo^{a,b,c,d} Mingjing Shao^e Ce Chen^c Chongguang Lin^c Deguo Jiang^c
Guangdong Chen^c Hongjun Tian^a Lina Wang^a Jie Li^a Xiaodong Lin^c^aTianjin Anding Hospital, Tianjin, ^bTianjin Anning Hospital, Tianjin, ^cWenzhou Seventh People's Hospital, Wenzhou, Zhejiang, ^dInstitute of Mental Health, Jining Medical University, Jining, Shandong, ^eChina-Japan Friendship Hospital, Beijing, China

Key Words

Gastric cancer • *PTPN11* • SNP • Chemotherapy • Prognosis • Gene-environment interaction

Abstract

Objective: Since gastric cancer (GC) cells exhibited higher grades of SHP-2 encoded by *PTPN11* than normal cells, it would be intriguing to explore whether *PTPN11* single nucleotide polymorphisms (SNPs) would influence chemotherapy effectiveness and GC prognosis among a Chinese population. **Methods:** Altogether 430 late-stage GC patients and 960 healthy controls matched with age and sex were incorporated. Three *PTPN11* SNPs (i.e. rs7958372, rs12229892 and rs2301756) were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Chemotherapies of cisplatin and 5-fluorouracil were performed for 4 cycles. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using the logistic regression. Survival curves were plotted with Kaplan-Meier method and the COX proportional hazard model was used to analyze independent factors for GC prognosis. **Results:** For rs12229892, AA and GA genotypes would cause 1.60-fold increase of GC risk in comparison to homozygote GG (OR = 1.60; 95% CI = 1.23-2.07; $P < 0.001$). The A allele of rs2301756 was significantly associated with a decrease in the risk of GC when compared with G allele (OR = 0.81; 95% CI = 0.65-0.99; $P = 0.043$). Results from both 2-cycle and 4-cycle chemotherapy suggested that chemotherapy was significantly more effective for GA and AA genotypes of rs2301756 compared with homozygote GG ($P < 0.001$). Besides, the joint impact of rs12229892 (AA) and environmental factors (i.e. smoking, family history, intake of processed food and *H. pylori* infection) on GC risk was considered as positive interaction, while that of rs2301756 (AA) and the above parameters was deemed as negative interaction. Finally, differentiation degree, axillary lymph node metastasis, rs12229892 and rs2301756 appeared as independent risk factors for GC development (all $P < 0.05$). **Conclusion:** Since rs2301756 polymorphism of *PTPN11* was associated with reduced risk of GC and better effects of chemotherapy on GC, it can be considered as a predictor of GC prognosis and the treatment target for GC.

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C. Zhuo, M. Shao and C. Chen contributed equally to this work as co-first author.

Xiaodong Lin,

Wenzhou Seventh People's Hospital, No. 552 East Xishan Road, Ouhai District,
Wenzhou, Zhejiang, 325005, (China)
Tel. +86 0577-88209928. Fax +86 0577-88432238. E-Mail lidongww@yeah.net

Introduction

Gastric cancer (GC) is one common malignancy with high incidence and mortality in Eastern Asia, and the incidence of GC in males is nearly two times higher than that in females [1, 2]. To date, GC is divided into intestinal and diffuse types based on histopathological classification: the former type is usually environment-induced and featured by cells with irregular tubular structures, while the latter type is mainly triggered by genetic factors (e.g. *E-cadherin* mutations) and characterized by poorly-differentiated cells that produce non-adhesive and secreted mucus [3]. As was suggested by several researchers, *Helicobacter pylori* (*H. pylori*) has been identified as a chief risk factor for GC development as it induces gastric atrophy and develops precancerous lesions [4, 5]. Meanwhile, the degree of gastric damage induced by *H. pylori* infection seemed to vary among individuals, suggesting that the host genetic traits could play key roles in the development of gastritis and even GC [6-8].

One gene of interest appeared as tyrosine-protein phosphatase non-receptor 11 (*PTPN11*), which encodes Src homology 2 domain-containing protein tyrosine phosphatase (SHP-2) within gastric epithelial cells [9]. The binding of SHP-2 to cytotoxin-associated gene A (CagA) of *H. pylori* would facilitate formation of a scattering phenotype, the so-called hummingbird phenotype, through stimulating phosphatase activities of SHP-2 and thereby extending activation of Erk [10, 11]. The hummingbird shape has been commonly recognized as a cellular alteration that symbolizes gastritis, which was identified as the previous stage of gastric carcinogenesis [11, 12]. The key role of SHP-2 in GC development was also certified with evidence that functional polymorphisms of *PTPN11* could modulate several signaling pathways, including mitogenic activation, cell migration and metabolic control, in patients with *H. pylori* infection and that GC cells exhibited higher grades of SHP-2 than normal cells [10, 13].

Apart from GC development, GC prognosis was also noteworthy. The 5-year survival rate of GC remained depressingly low, since that GC usually could not be diagnosed until in its advanced stages [14], during which period GC progression can merely be controlled, yet not cured, by either adjuvant radiotherapy or chemotherapy [15, 16]. Notably, variations of genes (e.g. *GSTP1* and *VEGF*) seemed to confer GC patients distinct prognoses and resistances to fluoropyrimidine-/platinum- based chemotherapies [17]. For instance, for advanced GC patients, subjects carrying ≥ 1 ¹⁰⁵Val allele of *GSTP1* were predisposed to enjoy superior prognosis and effective responses to regimens based on oxaliplatin/5-FU [16]. Therefore, it was hypothesized that identification of *PTPN11* polymorphisms may help predict GC prognosis after chemotherapies.

To validate the above hypothesis, this study was specifically designated to assess whether single-nucleotide polymorphisms (SNPs) of *PTPN11* are associated with both GC development and chemotherapy effects on GC. Since rs2301756 which is located in intron3 was connected with elevated risk of gastric atrophy among a Japanese population with *H. pylori* infection, several rs2301756-centered tag SNPs were arranged for this study [18]. Moreover, environmental factors that might also account for GC development were also researched on, including smoking, family history, intake of processed food and *Helicobacter pylori* (*H. pylori*) infection.

Materials and Methods

Study subjects

In total, 1390 subjects were incorporated in this study, including 430 patients with GC (case group) and 960 healthy subjects (control group). The case group consisted of 314 males and 116 females who were diagnosed with late-stage GC between March 2007 and March 2011. The average age of the case group was 62.75 ± 11.40 years old. All GC patients in the case group were selected according to the following criteria: (1) late-stage GC confirmed by pathology or cytology; (2) patients had at least 1 lesion measurement confirmed by CT; (3) normal bone marrow function and key normal organ (e.g. liver, kidney, heart, lung) functions were

retained; (4) No chemotherapy program had been performed one-month prior to the inclusion of patients. All subjects were independent Han Chinese and there was no consanguinity identified among the subjects. Besides that, 960 cases of healthy individuals (609 males and 351 females) were incorporated in the control group and they were selected during the same period with an average age of 63.26 ± 10.50 years old. All subjects have signed the informed consent form and this research was approved by the Ethics Committee of Wenzhou Seventh People's Hospital.

Identification of *PTPN11* polymorphisms

Specimen collection and DNA extraction: we extracted 2ml peripheral venous blood from both fasting patients and healthy subjects into tubes with ethylene diamine tetraacetic acid (EDTA) anticoagulant, then they were stored at -20°C ; DNA extraction Kit (United States Promega Corporation) was applied to extract DNA according to the protocols and the extracted DNA were stored at -80°C .

Primer Design and synthesis: As suggested by the target gene sequence and the selection of polymorphic loci, the Sequenom Mass ARRAY @Assay Design 3.1 software (United States Sequenom Company) was used to design multiple PCR amplification primers and single base extension primers and the synthesis of these primers were performed by Shanghai Chun Biological Company (Invitrogen). In order to identify *PTPN11* gene polymorphism of rs7958372, rs12229892 and rs2301756, the method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was utilized in different actions with primers shown in Table 1. The PCR amplification system was set as follows: 100 ng genomic DNA, 1.5 μl of Taq polymerase, 25 mmol of dNTP and 2.5 pmol of PCR primers. PCR reaction conditions were set as follows: stage I, predegenerated PCR cycling at 95°C for 4 minutes, then 35 cycles of 45 seconds at 95°C , 30 seconds at 56°C and 30 seconds at 72°C , extension with 7 minutes at 72°C ; stage II, *PTPN11* (rs7958372, rs12229892, rs2301756) synthesis, PCR cycling at 94°C for 5 minutes, then 35 cycles of 45 seconds at 94°C , 45 seconds at 60°C and 30 seconds at 72°C , extension with 5 minutes at 72°C . After that, 5 μl of PCR products was taken and incubated with restriction endonucleases overnight and the temperature was set to 65 degrees centigrade. The amplified DNA were electrophoresed in the 3% agarose gel, stained with ethidium bromide, then scanned using ultraviolet light transilluminator and finally images were obtained and analyzed.

Chemotherapy

Chemotherapy of cisplatin (20mg/m², intravenous drip, from day 1 to day 4, then withdrawal, regained from day 25 to 28) and 5-fluorouracil (750mg/m², intravenous drip, from day 1 to day 4, then withdrawal, regained from day 25 to 28) were performed for 4 cycles. Then the chemotherapy effects on solid tumors were evaluated every two cycles based on the WHO clinical solid tumor evaluation standards.

Based on response evaluation criteria in solid tumors (RECIST), around 4 evaluation standards were identified in the present study, namely, complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). Their definitions were enlisted as follows: CR, all target lesions disappeared; PR, the sum of baseline long diameters shortened by $\geq 30\%$; SD, change of the sum of baseline long diameters was between PR and SD; PD, the sum of baseline long diameters lengthened by $\geq 20\%$ or new lesions appeared.

Follow-up period

We designed questionnaires and collected all relevant information in order to assess the survival status of 430 patients with GC. All relevant information was collected by mail and telephone up to January 2015. Overall survival was calculated from date of post-chemotherapy to the deadline of the follow-up period or date of death. The expiration date was defined as postmortem interval (PMI) because of GC or last follow-up day. And for GC patients who died of other causes, their survival time was recorded as censored data.

Table 1. Primer sequences of *PTPN11* polymorphisms

SNP	Sense	Anti-sense
rs12229892	5'-GAATGAATGGTGCTGCTAC-3'	5'-CTGGGATTACAGGCGTGAG-3'
rs7958372	5'-CCCCTATGTGCCCTATTGT-3'	5'-CTGGGATTACAGGCGTGAG-3'
rs2301756	5'-TTAGCTCTACTATGGGTTCA-3'	5'-TTCCAAAGTGTGGGATTA-3'

Table 2. Clinical characteristics of gastric cancer patients and healthy controls included in our study

Characteristics	Control (n = 960)	Case (n = 430)	P value
Age (yrs)	63.26 ± 10.50	62.75 ± 11.40	0.415
Sex			
Female	351 (36.56%)	156 (36.28%)	0.952
Male	609 (63.44%)	274 (63.72%)	
Smoking status			
Yes	425 (44.27%)	228 (53.02%)	0.003
No	535 (55.73%)	202 (46.98%)	
Family history			
Yes	143 (14.90%)	109 (25.35%)	<0.001
No	817 (85.10%)	321 (74.65%)	
Intake of processed food			
< 8 kilograms/year	458 (47.71%)	287 (66.74%)	<0.001
≥ 8 kilograms/year	502 (52.29%)	143 (33.26%)	
<i>H. pylori</i> infection			
Positive	472 (49.17%)	300 (69.77%)	<0.001
Negative	488 (50.83%)	130 (30.23%)	
Pathological subtype			
Adenocarcinoma		304 (70.70%)	
Mucoid carcinoma		37 (8.60%)	
Signet-ring cell carcinoma		49 (11.39%)	
Others		40 (9.31%)	
Differentiation degree			
Well differentiated		14 (3.26%)	
Moderately differentiated		167 (38.84%)	
Poorly differentiated		222 (51.63%)	
Others		27 (6.27%)	
Clinical stages			
IIIA		13 (3.02%)	
IIIB		51 (11.86%)	
IIIC		225 (52.33%)	
IV		141 (32.79%)	
Axillary lymph node metastasis			
N ₀₋₁		79 (18.37%)	
N ₂₋₃		351 (91.63%)	
CEA			
< 5 ng/mL		255 (58.30%)	
≥ 5 ng/mL		175 (41.70%)	

Statistical analysis

Age was expressed in the form of mean ± standard deviation and compared using the Student's t-test. Categorical data were analyzed using the Chi-square test. The Hardy-Weinberg equilibrium (HWE) test was used to assess whether the genotype frequency in the control group was complied with the expected frequency which were evaluated using the Hardy-Weinberg Equilibrium Theory (p = allele frequency, q = $1-p$, $p^2 + q^2 = 1$). Differences in genotype and allele frequency between the control and case group were evaluated using the χ^2 test. Odds ratios (ORs) and 95% confidence intervals (CI) adjusting for gender and age were calculated using the method of logistic regression. Type-II gene-environment interaction (GEI) model was applied to analyze interactive effects of genetic and relevant environmental factors on GC development. And we judged whether the interaction reactions existed and which type the gene-environment action

Table 3. Association of *PTPN11* SNPs (rs12229892, rs7958372 and rs2301756) with development of gastric cancer risk

Genotype	Case (n, %)	Control (n, %)	OR	95% CI	P value
rs12229892					
Genotype					
GG	104 (24.19%)	324 (33.75%)	Reference		
GA	255 (59.30%)	455 (47.40%)	1.75	1.33-2.29	<0.001
AA	71 (16.51%)	181 (18.85%)	1.22	0.86-1.74	0.264
GA + AA	326 (75.81%)	636 (66.25%)	1.60	1.23-2.07	<0.001
Allele					
G	463 (53.84%)	1103 (57.45%)	Reference		
A	397 (46.16%)	817 (42.55%)	1.16	0.98-1.36	0.076
rs7958372					
Genotype					
TT	317 (73.72%)	712 (74.17%)	Reference		
TC	106 (24.65%)	231 (24.06%)	1.03	0.79-1.34	0.824
CC	7 (1.63%)	16 (1.67%)	0.98	0.40-2.41	0.969
TC + CC	113 (26.28%)	247 (25.73%)	1.02	0.79-1.33	0.837
Allele					
T	740 (86.05%)	1655 (86.20%)	Reference		
C	120 (13.95%)	263 (13.80%)	1.02	0.81-1.29	0.865
rs2301756					
Genotype					
GG	295 (68.60%)	634 (66.04%)	Reference		
GA	123 (28.60%)	261 (27.19%)	1.01	0.78-1.31	0.922
AA	12 (2.80%)	65 (6.77%)	0.39	0.21-0.75	0.003
GA + AA	135 (33.95%)	326 (33.96%)	0.89	0.69-1.14	0.348
Allele					
G	713 (82.91%)	1529 (79.64%)	Reference		
A	147 (17.09%)	391 (20.36%)	0.81	0.65-0.99	0.043

belonged to base on interaction coefficient (γ , $\gamma = \beta_{eg}/\beta_e$) [19]. Survival curve was plotted using the Kaplan Meier method and comparison of survival rate was evaluated by the Log-Rank test. The Cox Proportional Hazards model was used to assess independent factors for GC prognosis. P values < 0.05 were considered as statistical significant. Data were analyzed with SPSS Software package 19.0 (SPSS Inc., Chicago, IL, USA).

Results

Clinical characteristics of GC patients and healthy controls

GC patients and healthy controls were well matched in age ($P = 0.415$) and sex ratio ($P = 0.952$; Table 2). Such environmental hazards as smoking status, family history, intake of processed food and *H. pylori* infection all imposed significant impacts on susceptibility to GC ($P < 0.05$). Moreover, the pathological types of GC samples were primarily made up of adenocarcinoma (70.70%), mucoid carcinoma (8.60%) and signet-ring cell carcinoma (11.39%), and the prevalence of patients with well-, moderately- and poorly- differentiated GC appeared as 3.26%, 38.84% and 51.63%, respectively. Additionally, in accordance with GC tumor node metastasis (TNM) staging criteria enacted by union for international cancer control/American joint committee on cancer (UICC/AJCC), 430 GC patients were categorized into IIIA (3.02%), IIIB (11.86%), IIIC (52.33%) and IV (32.79%) clinical stages [20]. The

Table 4. Association of *PTPN11* SNPs (rs12229892, rs7958372 and rs2301756) with chemotherapy responses of gastric cancer

SNPs	N	Four cycles		OR	95%CI	P value*	Two cycles		OR	95%CI	P value*
		CR + PR	SD + PD				CR + PR	SD + PD			
rs12229892											
GG	104	46	58			Reference	47	57			Reference
GA	255	134	121	0.97	0.71-1.36	0.153	138	117	0.70	0.44-1.11	0.125
AA	71	39	32	1.08	0.64-1.81	0.164	42	29	0.57	0.31-1.05	0.070
GA+AA	326	173	153	0.70	0.45-1.09	0.117	170	156	0.76	0.49-1.18	0.217
rs7958372											
TT	317	150	167			Reference	142	175			Reference
TC	106	59	47	0.72	0.46-1.11	0.137	55	50	0.74	0.47-1.15	0.177
CC	7	4	3	0.67	0.15-3.06	0.607	4	4	0.81	0.20-3.30	0.770
TC+CC	113	63	50	0.71	0.46-1.10	0.124	59	54	0.74	0.48-1.14	0.175
rs2301756											
GG	284	110	174			Reference	92	192			Reference
GA	123	108	15	0.09	0.05-0.16	< 0.001	101	30	0.14	0.09-0.23	< 0.001
AA	12	10	2	0.13	0.03-0.59	0.002	11	4	0.17	0.05-0.56	0.001
GA+AA	146	128	18	0.09	0.05-0.15	< 0.001	112	34	0.14	0.09-0.23	< 0.001

Table 5 The layer analysis on interaction between genotypes of *PTPN11* SNPs (i.e. rs12229892 and rs2301756) and susceptibility to gastric cancer

Genotype		Case (n, %)	Control (n, %)	OR	95% CI	P value
rs12229892	rs2301756					
GG	GG	71 (16.51%)	214 (22.30%)	0.69	0.51-0.93	0.014
GG	GA	30 (6.98%)	88 (9.17%)	0.74	0.48-1.14	0.176
GG	AA	3 (0.70%)	22 (2.29%)	0.30	0.09-1.01	0.039
GA	GG	175 (40.70%)	301 (31.35%)	1.50	1.19-1.90	<0.001
GA	GA	73 (16.98%)	124 (12.92%)	1.38	1.01-1.89	0.045
GA	AA	7 (1.63%)	31 (3.23%)	0.49	0.22-1.23	0.087
AA	GG	49 (11.40%)	120 (12.50%)	0.83	0.59-1.19	0.314
AA	GA	20 (4.65%)	49 (5.10%)	0.91	0.53-1.55	0.719
AA	AA	2 (0.47%)	12 (1.25%)	0.37	0.08-1.66	0.176

number of GC patients diagnosed as N2-3 axillary lymph node metastasis was nearly 3.44 folds more than that confirmed as N0-1 node metastasis. Furthermore, GC populations with *in vivo* carcinoembryonic antigen (CEA) contents of <5 ng/mL and ≥5 ng/mL were roughly equal in size (58.30% vs. 41.70%).

Difference in PTPN11 SNP genotype distributions and allele frequencies

Genotype frequencies of rs12229892, rs7958372 and rs2301756 in the control group all complied with HWE (all $P > 0.05$). As for rs12229892 (Table 3), subjects carrying heterozygote GA were more prone to develop GC than ones carrying homozygote GG (OR = 1.75, 95% CI = 1.33-2.29, $P < 0.001$). The dominant model also implied that genotypes GA+AA were at greater risk of GC than homozygote GG (OR = 1.60, 95% CI = 1.23-2.07, $P < 0.001$). Besides, it could be drawn from the allelic model of rs2301756 (Table 3) that GC patients possessed notably lower frequency of A allele than that of G allele (OR = 0.81, 95% CI = 0.65-0.99, $P = 0.043$). And carriers with homozygote AA appeared to suffer from GC less readily than those with genotype GG (OR = 0.39, 95% CI = 0.21-0.75, $P = 0.003$). However, with respect to rs7958372, no statistically significant distinctions were observed in both genotype and allele frequencies between GC patients and healthy controls (all $P > 0.05$).

Table 6. The conditional logistic regression analysis on interaction between *PTPN11* SNPs (i.e. rs12229892 and rs2301756) and smoking status

Genotype	Smoking	Case (n, %)	Control (n, %)	OR	95%CI	β_e	β_{eg}	γ
rs12229892								
GG	No	49 (11.39%)	181 (18.85%)					
GA	No	120 (27.91%)	254 (26.46%)	1.75	1.19-2.56			
AA	No	33 (7.67%)	101 (10.52%)	1.21	0.73-2.00			
GG	Yes	55 (12.79%)	143 (14.89%)	1.42	0.91-2.21	0.35		
GA	Yes	135 (31.40%)	201 (20.94%)	2.48	1.69-3.64	0.35	0.91	2.59
AA	Yes	38 (8.84%)	80 (8.34%)	1.76	1.07-2.89	0.35	0.57	1.61
rs2301756								
GG	No	139 (32.33%)	353 (36.77%)					
GA	No	58 (13.49%)	145 (15.10%)	1.02	0.71-1.46			
AA	No	6 (1.39%)	36 (3.75%)	0.42	0.17-1.03			
GG	Yes	156 (36.28%)	281 (29.27%)	1.41	1.07-1.86	0.34		
GA	Yes	65 (15.12%)	116 (12.08%)	1.42	0.99-2.04	0.34	0.35	1.02
AA	Yes	6 (1.39%)	29 (3.02%)	0.53	0.21-1.25	0.34	-0.63	-1.84

Table 7. The conditional logistic regression analysis on interaction between *PTPN11* SNPs (i.e. rs12229892 and rs2301756) and family history

Genotype	Family history	Case (n, %)	Control (n, %)	OR	95%CI	β_e	β_{eg}	γ
rs12229892								
GG	No	78 (18.14%)	276 (28.75%)					
GA	No	190 (44.19%)	387 (40.31%)	1.74	1.28-2.36			
AA	No	53 (12.33%)	154 (16.04%)	1.22	0.82-1.81			
GG	Yes	26 (6.05%)	48 (5.00%)	1.92	1.12-3.28	0.65		
GA	Yes	65 (15.12%)	68 (7.08%)	3.38	2.22-5.16	0.65	1.22	1.87
AA	Yes	18 (4.17%)	27 (2.82%)	2.36	1.23-4.51	0.65	0.86	1.32
rs2301756								
GG	No	220 (51.16%)	540 (56.25%)					
GA	No	92 (21.39%)	222 (23.13%)	1.02	0.76-1.36			
AA	No	9 (2.09%)	55 (5.73%)	0.40	0.20-0.83			
GG	Yes	75 (17.44%)	94 (9.79%)	1.96	1.39-2.76	0.67		
GA	Yes	31 (7.21%)	39 (4.06%)	1.95	1.19-3.21	0.67	0.66	0.99
AA	Yes	3 (0.71%)	10 (1.04%)	0.74	0.20-2.70	0.67	-0.30	-0.44

Association between *PTPN11* polymorphisms and GC patients' response to chemotherapy

Either heterozygote GA or homozygote AA was tightly correlated with favorable short-term efficacy of chemotherapy (CR+PR) in comparison with homozygote GG, whether 4-cycle (GA: OR = 0.09, 95% CI = 0.05-0.16, $P < 0.001$; AA: OR = 0.13, 95% CI = 0.03-0.59, $P = 0.002$) or 2-cycle chemotherapeutic responses (GA: OR = 0.14, 95% CI = 0.09-0.23, $P < 0.001$; AA: OR = 0.17, 95% CI = 0.05-0.56, $P = 0.001$) were considered (Table 4). Meanwhile, results of both 4-cycle and 2-cycle chemotherapies indicated that the effective rate for GA+AA of rs2301756 was significantly higher than that for GG genotype with ORs of 0.09 (95% CI = 0.05-0.16, $P < 0.001$) and 0.14 (95% CI = 0.09-0.23, $P < 0.001$), respectively (Table 4). No significant associations were observed between rs12229892 or rs7958372 and effects of 2-cycle/4-cycle chemotherapy on GC patients.

Table 8. The conditional logistic regression analysis on interaction between *PTPN11* SNPs (i.e. rs12229892 and rs2301756) and intake of processed food

Genotype	Intake of processed food	Case (n, %)	Control (n, %)	OR	95%CI	β_e	β_{eg}	γ
rs12229892								
GG	No	35 (8.14%)	169 (17.60%)					
GA	No	85 (19.77%)	238 (24.79%)	1.72	1.11-2.68			
AA	No	24 (5.58%)	95 (9.89%)	1.22	0.68-2.17			
GG	Yes	69 (16.05%)	155 (16.15%)	2.15	1.36-3.41	0.77		
GA	Yes	170 (39.53%)	217 (22.60%)	3.78	2.50-5.73	0.77	1.33	1.74
AA	Yes	47 (10.93%)	86 (8.96%)	2.64	1.59-4.39	0.77	0.97	1.27
rs2301756								
GG	No	98 (22.79%)	332 (34.58%)					
GA	No	41 (9.53%)	136 (14.17%)	1.02	0.67-1.55			
AA	No	4 (0.93%)	34 (3.54%)	0.40	0.14-1.51			
GG	Yes	197 (45.81%)	302 (31.46%)	2.21	1.66-2.95	0.79		
GA	Yes	82 (19.07%)	125 (13.02%)	2.22	1.55-3.18	0.79	0.80	1.01
AA	Yes	8 (1.87%)	31 (3.23%)	0.87	0.39-1.96	0.79	-0.14	-0.18

Table 9. The conditional logistic regression analysis on interaction between *PTPN11* SNPs (i.e. rs12229892 and rs2301756) and *H. pylori* infection

Genotype	<i>H. pylori</i> infection	Case (n, %)	Control (n, %)	OR	95%CI	β_e	β_{eg}	γ
rs12229892								
GG	No	31 (7.21%)	165 (17.19%)					
GA	No	77 (17.91%)	231 (24.06%)	1.77	1.12-2.81			
AA	No	21 (4.88%)	92 (9.58%)	1.22	0.66-2.24			
GG	Yes	73 (16.98%)	159 (16.56%)	2.44	1.52-3.92	0.89		
GA	Yes	178 (41.40%)	224 (23.33%)	4.23	2.75-6.51	0.89	1.44	1.62
AA	Yes	50 (11.62%)	89 (9.27%)	2.99	1.78-5.02	0.89	1.10	1.23
rs2301756								
GG	No	89 (20.70%)	322 (33.54%)					
GA	No	37 (8.60%)	133 (13.85%)	1.01	0.65-1.55			
AA	No	4 (0.93%)	33 (3.44%)	0.44	0.15-1.27			
GG	Yes	206 (47.91%)	312 (32.50%)	2.39	1.78-3.20	0.87		
GA	Yes	86 (20.00%)	128 (13.33%)	2.43	1.70-3.49	0.87	0.89	1.02
AA	Yes	8 (1.86%)	32 (3.34%)	0.90	0.40-2.03	0.87	-0.11	-0.12

Correlation between combined effects of PTPN11 polymorphisms (rs12229892 and rs2301756) and susceptibility to GC

Compared with other genotype combinations (Table 5), homozygote GG of rs12229892 coupled with homozygote GG or AA of rs2301756 seemed to confer protective effects on GC risk (OR = 0.69, 95% CI = 0.51-0.63, $P = 0.014$; OR = 0.30, 95% CI = 0.09-1.01, $P = 0.039$). On the contrary, heterozygote GA of rs12229892 combined with either GA or GG genotype of rs2301756 could be more frequently found among GC populations with their ORs of 1.38 (95% CI = 1.01-1.89, $P = 0.045$) and 1.50 (95% CI = 1.19-1.90, $P < 0.001$), respectively.

Interaction between PTPN11 polymorphisms (rs12229892 and rs2301756) and environmental hazards (i.e. smoking status, family history, intake of processed food and H. pylori infection)

Even though smoking (OR_e = 1.42) or homozygote AA of rs12229892 (OR_g = 1.21) alone might not significantly serve as predisposing factors for GC development, their

Table 10. Association of variables with GC patients' overall survival

Variable	Univariate analysis			Multivariate analysis		
	OR	95%CI	P value	OR	95%CI	P value
Pathological subtype						
Adenocarcinoma	Reference					
Mucoid carcinoma	0.68	0.46-1.00	0.053	0.73	0.49-1.08	0.118
Signet-ring cell carcinoma	0.86	0.61-1.21	0.380	0.81	0.58-1.15	0.246
Others	1.22	0.86-1.72	0.262	1.23	0.86-1.76	0.268
Differentiation degree						
Well differentiated	Reference					
Moderately differentiated	16.42	2.92-117.55	0.005	10.86	1.50-78.36	0.018
Poorly differentiated	53.87	7.53-385.26	< 0.001	26.35	3.64-190.60	0.001
Others	27.51	3.71-204.26	0.001	15.98	2.12-120.62	0.007
Clinical stages						
IIIA	Reference					
IIIB	2.62	0.93-7.39	0.069	0.80	0.28-2.29	0.676
IIIC	5.49	2.04-14.78	0.001	1.31	0.48-3.57	0.604
IV	6.02	2.22-16.32	< 0.001	1.59	0.58-4.36	0.367
Axillary lymph node metastasis						
N0-1	Reference					
N2-3	4.38	3.07-6.26	< 0.001	2.60	1.80-3.77	< 0.001
CEA						
< 5 ng/mL	Reference					
≥ 5 ng/mL	1.30	1.05-1.60	0.014	1.14	0.92-1.41	0.222
rs12229892						
GG	Reference					
GA+AA	2.47	1.88-3.25	< 0.001	1.91	1.44-2.53	< 0.001
rs2301756						
GG	Reference					
GA+AA	1.91	1.54-2.37	< 0.001	0.57	0.44-0.72	< 0.001

additive effects were strikingly correlated with increased GC risk ($OR_{eg} = 1.76$, 95% CI = 1.07-2.89) (Tables 6-9). Since their γ value was equivalent to 1.61 (> 1), rs12229892 AA and smoking were inferred to be positively interacted in driving occurrence of GC, indicating their mutually amplified effects in aggravating GC risk. Moreover, as 1.76 was more than the product of 1.42 and 1.21, the interaction mechanism regarding AA of rs12229892 and smoking was considered as the super-multiplicative model. Similarly, the underlying mechanism concerning rs12229892 AA and three other environmental factors were also confirmed as super-multiplicative model (family history, $OR_{eg} 2.36 > OR_e 1.92 \times OR_g 1.22$; intake of processed food, $OR_{eg} 2.64 > OR_e 2.15 \times OR_g 1.22$; *H. pylori* infection, $OR_{eg} 2.99 > OR_e 2.44 \times OR_g 1.22$).

As far as rs2301756 was concerned, smoking ($OR_e = 1.41$, 95% CI = 1.07-1.86) alone was closely related with incremental susceptibility to GC, but the co-existence of smoking and homozygote AA made this tight correlation less distinct and even the harmful tendency was reversed to a protective trend ($OR_{eg} = 0.53$). Taking $\gamma = -1.84$ (< 0) and $\beta_e = 0.34$ (> 0) into account, smoking and AA genotype interacted negatively concerning their combined effects on GC risk and homozygote AA could largely inhibit incidence of GC. Besides, the inequality ($OR_{eg} 0.53 < OR_e 1.41 \times OR_g 0.42$) implied that the reciprocal action of smoking and AA genotype tallied with the sub-multiplicative model. Analogously, the joint impacts of

Fig. 1. Kaplan-Meier survival curve relevant to gastric cancer patients with various differentiation degrees (i.e. well differentiation, moderate differentiation, poor differentiation and others) ($P < 0.001$).

Fig. 2. Kaplan-Meier survival curve relevant to gastric cancer patients with distinct degrees of axillary lymph node metastasis (i.e. N0-1 and N2-3) ($P < 0.001$).

family history/intake of processed food/*H. pylori* infection and AA genotype on GC risk were indicated as the sub-multiplicative model (family history, $OR_g 0.74 < OR_e 1.96 \times OR_g 0.40$; intake of processed food, $OR_g 0.87 < OR_e 2.21 \times OR_g 0.40$; *H. pylori* infection, $OR_g 0.90 < OR_e 2.39 \times OR_g 0.44$). The corresponding γ values (all < 0) and β_e values (all > 0) also showed that homozygote AA could greatly compete against the effects imposed by family history/intake of processed food/*H. pylori* infection on GC development.

Association between *PTPN11* polymorphism and prognosis of GC

Totally 430 GC cases were followed up with the median survival time of 46 months, and 67 cases (15.58%) survived to the end. As was displayed in Table 10, type of GC (i.e. adenocarcinoma, mucoid carcinoma, signet-ring cell carcinoma and others) seemed not be the determining factor of OS through judgement of either univariate or multivariate analyses (all $P > 0.05$). Nonetheless, poorly-differentiated GC was obviously associated with the least survival time of investigated subjects (Long-Rank $P < 0.001$) (Fig. 1) and moderately-differentiated GCs might anticipate shorter life span than well-differentiated GCs regardless of pathological subtype, clinical stage, axillary lymph node metastasis and other elements ($OR = 10.86$, 95% CI = 1.50-78.36, $P = 0.018$). Additionally, axillary lymph node metastasis (N2-3 vs. N0-1), rs12229892 (GA + AA vs. GG) and rs2301756 (GA + AA vs. GG) were also independently associated with dramatically altered mortality of GC patients based on multivariate analyses ($OR = 2.60$, 95% CI = 1.80-3.77, $P < 0.001$; $OR = 1.91$, 95% CI = 1.44-2.53, $P < 0.001$; $OR = 0.57$, 95% CI = 0.44-0.72, $P < 0.001$) (Fig. 2-4).

Fig. 3. Kaplan-Meier survival curve of gastric cancer patients carrying diverse genotypes (i.e. GG, GA and AA) of rs12229892 ($P < 0.001$).

Fig. 4. Kaplan-Meier survival curve of gastric cancer patients carrying diverse genotypes (i.e. GG, GA and AA) of rs2301756 ($P < 0.001$).

Fig. 5. Kaplan-Meier survival curve relevant to gastric cancer patients in various clinical stages (i.e. IIIA, IIIB, IIIC and IV stages) ($P < 0.001$).

Furthermore, comparisons of OS among IIIA, IIIB, IIIC and IV stages indicated that the more advanced the clinical stage, the higher death rate of GC patients (Long-Rank $P < 0.001$) (Fig. 5), though the significant tendency did not persist after adjustment of multivariate analyses (all $P > 0.05$). Similar circumstances also appeared on the indicator of CEA, which demonstrated that GC cases with *in vivo* CEA content of ≥ 5 ng/mL were more likely to obtain

Fig. 6. Kaplan-Meier survival curve relevant to gastric cancer patients with distinct carcinoembryonic antigen (CEA) levels (i.e. < 5 ng/ml and $\geq$ 5 ng/ml) ($P = 0.012$).

pessimistic prognosis than those carrying CEA content of < 5 ng/mL (Long-Rank $P = 0.012$) (Fig. 6). However, the overwhelming differentiation (OR = 1.30, 95% CI = 1.05-1.60, $P < 0.014$) was not replicated after eliminating impacts of extra parameters ($P = 0.106$; Table 10).

Discussion

Human GC is still the primary lethal cancer among all malignant neoplasms despite current therapeutic approaches including surgery, radiation and chemotherapy have improved the overall survival status of GC patients. Therefore, the association of *PTPN11* with GC risk was explored in this study to find possible treatment targets for GC, which indicated that remarkable differences in genotype and allelic distributions existed between case and control groups for rs12229892 and rs2301756. In addition, both 2-cycle and 4-cycle chemotherapy were more effective in individuals with mutation gene A (GA+AA) of rs2301756, and genotypes GA/AA of rs2301756 are independent protective factors for GC prognosis.

Individuals infected by *H. pylori*, which carries CagA, are associated with significantly higher risk of GC [21]. Particularly, once CagA bound to SHP2, the encoded protein of *PTPN11*, cell motility along with a highly stretched cell shape were elevated [22], which actually counted much in forming gastric carcinoma [23]. Several studies conducted in Japanese populations have reported a correlation between *PTPN11* rs2301756 in the intron 3 and gastric atrophy identified in subjects with *H. pylori* infections, providing evidence that Japanese individuals who have A allele of the *PTPN11* rs2301756 were associated with a significantly lower risk of severe gastric atrophy which is a precursory phase of GC [12]. The present study also considered G allele of *PTPN11* rs2301756 as one susceptible element for GC when compared with A allele. Conversely, a case-control study among the Uzbekistan population indicated that G allele of *PTPN11* rs2301756 could augment the onset risk of gastric atrophy [24]. The inconsistency might be ascribed to distinctions in the genetic distribution among Japanese, Chinese and Uzbeks. It is hypothesized that rs2301756 might be involved in shaping mRNA splicing variants of *PTPN11*, owing to the fact that this SNP is situated merely 223 base pairs upstream from exon 4, which partly determines encoding of C-terminal src homology 2 (SH2) domain of SHP-2 [12]. Of note, it is the SH2 domain that binds to tyrosine phosphorylated CagA, in which way conformational alterations of SHP-2 are induced and SHP-2 phosphatase is thus activated [25, 26]. Hence, it is quite likely that homozygote AA or allele A of rs2301756 is crucial for prevention of GC development with homozygote GG or allele G as respective controls. Meanwhile, rs12229892 variant A-allele was previously revealed to be associated with a reduced risk of GC in subjects without *H. pylori* infection but an increased risk of GC in individuals with *H. pylori* infection [27]. The current

study also certified the association of rs12229892 with GC, yet the underlying mechanism remained vague. Accordingly, two G/A SNPs of *PTPN11* (rs2301756 and rs12229892) have been considered as candidate polymorphisms for this Chinese population [18, 24]. However, both functional analysis and larger-scale studies should be undertaken to confirm the relationship between *PTPN11* polymorphisms and GC.

Besides, cisplatin and 5-fluorouracil (5-Fu) were essential drugs in the process of applying chemotherapies for gastrointestinal tumors due to their capacities of inhibiting cell proliferation [28]. Specifically, the metabolites of 5-Fu mainly interfered with DNA synthesis through acting on enzymes that were necessary for DNA synthesis to form stable covalent complexes, while cisplatin principally formed intra-chain/inter-chain DNA adducts to hinder DNA replication [29, 30]. In contrast, SHP2 usually served to enhance cell proliferation and motility via binding to multiple molecules, so as to modify certain pathways in numerous tumors such as laryngeal and breast cancer [31, 32]. For instance, SHP-2 could interact with scaffolding/adaptor protein (i.e. Gab2) to accelerate hyperproliferation of mammary cells [33]. In addition, SHP2 was deemed to basally regulate Jak/STAT signaling induced by IL-6, which commonly functioned in cancer cells [34, 35]. SHP2 also mediates GC progression under the influence of PTEN/AKT signaling and promotes growth of laryngeal cancer through the Ras/Raf/Mek/Erk pathway [36]. To sum up, DNA synthesis and replications affected by SHP-2 might be accelerated before they were blocked by cisplatin and 5-Fu. As a result, it was inferred that GC patients with dysfunctional SHP-2 would suffer from more undesirable prognosis than those with normal SHP-2, since the degree of identical cisplatin and 5-Fu to restrain in-vivo tumor proliferation for GC patients with aberrant SHP-2 appeared lower than that for GC patients with normal SHP-2. Therefore, under the effects of SHP-2-induced cell hyperproliferation, it was acceptable that GC patients carrying homozygote GG of rs2301756 were associated with poorer short-term efficacies after chemotherapies than those with GA/AA genotypes. However, whether SHP-2 directly acted on target DNA still demanded further investigations.

In addition, smoking and intake of processed food were eventually concluded to be two independent risk factors for GC development. The explanations might be that smoking could trigger intestinal metaplasia and mal-development of gastric mucosa, which would progressively develop into GC [37]. To be specific, risks of intestinal metaplasia and mal-developed gastric mucosa would be increased to 118 and 316 folds, respectively, for subjects smoking > 10 cigarettes per day when compared with non-smokers [38]. Moreover, smoking was deduced to genetically incapacitate organisms from cancer-suppression by bringing down active P53 contents and enhancing c-myc expressions [39]. Similarly, processed food also injured gastric mucosa and even advanced genetic mutations that could be relevant to GC, but based on a different mechanism that nitrates and nitrites within processed food could be transformed to harmful N-nitrosamides with aid of stomach bacterial actions [40, 41].

Despite the above results, several defects still existed. First of all, the studied populations were of one single ethnic background (i.e. Han Chinese), so the conclusion was less convincing if it was generalized to other ethnicities. Besides, the selected SNPs were not full-scale enough and extra potentially functional SNPs could still not be identified. Moreover, though the sample size seemed considerable, a larger sample size would be more reliable. Finally, finite elements relevant to GC prognosis were investigated, for instance, psychological pressure could indeed aggravate GC prognosis for that patients with advanced GC suffered from more severe anxiety and depression than those at the initial stage of GC [42]. Hence, to confirm *PTPN11* genetic polymorphisms as independent factors for undesirable GC prognosis, the effects of mental pressure should be excluded.

In summary, this study conducted on a Chinese population concluded that rs2301756 in the *PTPN11* which encodes SHP-2 was associated with a reduced risk of GC. Since *PTPN11* polymorphism in the intron 3 region is a sensitive indicator which evaluates the effect of chemotherapy on GC, it may be considered as an independent predictor of GC prognosis. As a result, this study may provide additional information for both GC diagnosis and prognosis.

Abbreviations

GC (gastric cancer); PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism); ORs (Odds ratios); CIs (confidence intervals); *H. pylori* (*Helicobacter pylori*); SHP-2 (signaling protein); SNPs (single-nucleotide polymorphisms); EDTA (ethylene diamine tetraacetic acid); RECIST (response evaluation criteria in solid tumors); CR (complete response); PR (partial response); SD (stable disease); PD (progressive disease); HWE (Hardy-Weinberg equilibrium); GEI (gene-environment interaction).

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Disclosure Statement

The authors have no conflicts of interest to disclose.

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