Association of *THBS1* rs1478605 T>C in 5'-untranslated regions with the development and progression of gastric cancer

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Abstract. Thrombospondin 1 (THBS1) plays an important role in angiogenesis and tumor progression. The aim of the present study was to investigate the effects of single-nucleotide polymorphisms (rs1478605 and rs3743125) in the untranslated regions of the *THBS1* gene on the development and progression of gastric cancer. In the case-control study, 275 gastric cancer patients and 275 cancer-free controls were successfully genotyped using polymerase chain reaction-restriction fragment length polymorphism. The data demonstrated that *THBS1* rs1478605 genotypic distributions significantly differed between the patient and control groups (P=0.005). Carriers of the CC genotype exhibited a decreased risk of developing gastric cancer compared to the carriers of the CT and TT genotypes [adjusted odd ratio (OR), 0.56; 95% confidence interval (CI), 0.39-0.79; P=0.001]. The CC genotype of rs1478605 was negatively associated with gastric cancer lymph node metastasis (OR, 0.41; 95% CI, 0.23-0.71; P=0.001) and was associated with a reduced risk of lymph node metastasis in male patients (OR, 0.27; 95% CI, 0.14-0.52; P<0.001). The *THBS1* CT haplotype was associated with a reduced risk of developing gastric cancer (OR, 0.56; 95% CI, 0.33-0.93; P=0.02). By contrast, no association was observed between *THBS1* rs3743125 and the development and progression of gastric cancer. These results suggest that *THBS1* rs1478605 represents a potential molecular marker for gastric cancer.

Introduction

Gastric cancer remains the leading cause of cancer-related mortalities worldwide. In China, the incidence and mortality rate of gastric cancer are higher than the international average. Gastric cancer has a complex multi-step etiology, involving environmental and genetic factors (1-5). The individual variations in cancer risk suggest that gene mutations, in addition to genetic polymorphisms, may contribute to the overall risk of gastric cancer (6). Individual genetic susceptibility may represent a critical factor in the development of gastric cancer.

Thrombospondin 1 (THBS1) is a high molecular weight multi-functional glycoprotein that has been shown to be a potent inhibitor of angiogenesis (7). Previous studies have correlated THBS1 expression to tumor angiogenesis, tumor growth and metastasis (8-12). In gastric cancer, THBS1 may have a pro-angiogenic effect, and elevated THBS1 expression levels have been associated with gastric cancer tumor growth and lymph node metastasis (13-17). However, thus far, genetic evidence of a role for THBS1 in gastric cancer is lacking.

Single-nucleotide polymorphisms (SNPs) have been widely used to search for the association between genetic variations and disease susceptibility. *THBS1* SNPs have been associated with a wide range of diseases (18-21), however, the correlation of *THBS1* SNPs with individual susceptibility to gastric cancer remains unclear, although recently, our previous study found that *THBS1* rs1478604 A>G within the 5'-untranslated region (UTR) of the gene is associated with lymph node metastasis of gastric cancer in a southeast Chinese population (22). To further evaluate a correlation between *THBS1* SNPs and the risk of gastric cancer in a southeast Chinese population, a case-control study was conducted to examine the association of rs1478605 and rs3743125 SNPs in the UTRs of *THBS1* with the development and progression of gastric cancer. *THBS1* rs1478605 is negatively associated with gastric cancer development and lymph node metastasis, while rs3743125 has no significant association with gastric cancer.

Materials and methods

Study population. The study population included 275 patients with gastric carcinoma and 275 cancer-free controls. All the subjects were genetically unrelated ethnic Han Chinese and originated from Fujian (China). Patients were diagnosed with primary incident gastric cancer and were recruited at the Affiliated Hospitals of the Fujian Medical University

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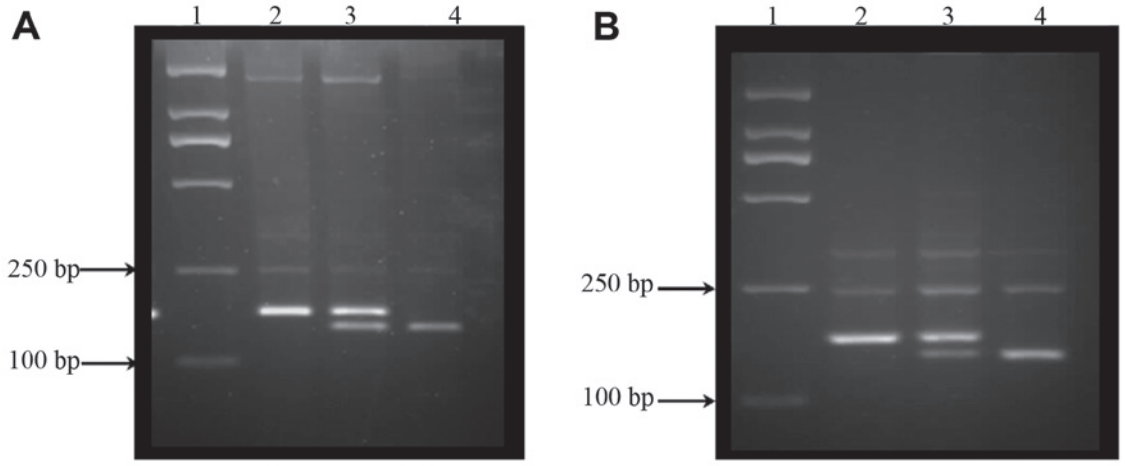

Figure 1. Genotyping patterns of rs1478605 and rs3743125. (A) rs1478605. Lane 1, DNA marker; lane 2, TT genotype [152 base pairs (bp) only]; lane 3, CT genotype (152+132+20 bp); lane 4, CC genotype (132+20 bp). (B) rs3743125: lane 1, DNA marker; lane 2, TT genotype (168 bp only); lane 3, CT genotype (168+145+23 bp); lane 4, CC genotype (145+23 bp).

Table I. Characteristics of the study population.

Variables	Gastric cancer	Controls	P-value[a]
Age, years			
≤60, n (%)	130 (47.3)	112 (40.7)	0.12
>60, n (%)	145 (52.7)	163 (59.3)	
Gender			
Male, n (%)	201 (50.4)	198 (49.6)	0.77
Female, n (%)	74 (49.0)	77 (51.0)	

[a]P-value was calculated by χ^2 test.

(Fujian). All the specimens were histopathologically confirmed gastric cancer cases and had detailed clinicopathological data based on post-operative, histopathological examination. Gastric cancer patients were grouped according to the tumor-node-metastasis staging of the American Joint Commission on Cancer (https://cancerstaging.org). Cancer-free control subjects were selected randomly from local residents, who underwent a routine health check with no history of cancer and other known major diseases. There were no differences in age and gender between gastric cancer patients and control participants (Table I). The study was approved by the Institutional Review Board of the Second Affiliated Hospital of Fujian Medical University subsequent to obtaining written, informed consent.

Genotyping. For control subjects, venous blood samples (5 ml) were collected from each individual. Genomic DNA was extracted from whole blood cell pellets using the Blood Genomic DNA Extraction kit (Takara, Shiga, Japan). DNA from gastric cancer patients was isolated from paraffin-embedded normal stomach tissue adjacent to the tumor (distance >5 cm) using the proteinase K-phenol/chloroformethanol method. DNA concentration was measured by ultraviolet spectrophotometry (NanoVue Plus®; GE Healthcare, Piscataway, NJ, USA) at 260 nm, and quality was determined using the A260/280 ratio. DNA was stored at -20˚C prior to genotypic analysis.

SNP genotypes were determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. Primers used for the amplification of rs1478605 [152 base pairs (bp)] were forward, 5'-GCAGGC CAGCTCGGGCGCCG-3' and reverse, 5'-GGGGGCGGA GAGAGGAGCCCAGAC-3'; and primers used for the amplification of rs3743125 (168 bp) were forward, 5'-GTCAGGGTG GTTTTGTTTGC-3' and reverse, 5'-GGGGGCGGAGAG AGGAGCCCAGAC-3' (Invitrogen, Shanghai, China). PCR was performed in a 25-μl reaction volume containing template DNA (40 ng), primers (5 pmol/μl each) and 2X Taq PCR Master Mix (12.5 μl) (Tiangen, Beijing, China). Amplification was performed using the following cycling conditions: 95˚C for 5 min, and subsequently 35 cycles of 94˚C for 30 sec, 70˚C for 45 sec (for rs1478605) or 55˚C for 45 sec (for rs3743125), 72˚C for 45 sec, and a final extension at 72˚C for 7 min. PCR products were digested overnight at 37˚C with *Sac*II (2 units) or *PvU*II (2 units) restriction enzymes for rs1478605 or rs3743125, respectively (Takara) and separated on 4% agarose gels. The T allele of rs1478605 and rs3743125 contained no restriction site, and the C allele of the two SNPs contained one restriction site and produced two fragments (Fig. 1).

Statistical analysis. Statistical analysis was performed using SPSS 11.5 software (SPSS, Inc., Chicago, IL, USA). Univariate or multivariate analyses were performed to evaluate the association of genotypic distributions with the development of gastric cancer and clinicopathological features. Univariate analysis was performed using the χ^2 test or Fisher's exact test when required. Multivariate analyses were estimated by logistic regression modeling or stratification. Several clinicopathological variables were also dichotomized to avoid the loss of statistical power in logistic regression. All comparisons were two-tailed and P<0.05 was considered to indicate a statistically significant difference. The Haploview 4.2 software (http://www.broad.mit.edu/mpg) was used to measure the

pairwise LD values between SNPs. Haplotype frequency was estimated using Phase software version 2.1 (23).

Results

Association of THBS1 rs1478605 and rs3743125 with the development of gastric cancer. To investigate the association between *THBS1* rs1478605 and rs3743125 SNPs and the development and progression of gastric cancer, SNP genotyping was performed on DNA obtained from 275 patients with gastric cancer and 275 cancer-free individuals. The allelic and genotypic frequencies of the two groups are listed in Tables II and III. The genotypic distributions of the control group were tested for Hardy-Weinberg equilibrium and showed no significant deviations (P=0.99 for the two SNPs).

No significant differences in the genotypic distributions and allele frequencies of rs3743125 were observed between gastric cancer patients and control subjects, indicating that rs3743125 is not associated with gastric cancer development (Table III). However, a significant difference in the CC genotype distribution and C allele frequency of rs1478605 between disease and control groups was identified. These results indicate that carriers of the CC genotype exhibit a decreased risk of developing gastric cancer compared to the carriers with the CT and TT genotypes [adjusted odds ratio (OR), 0.56; 95% confidence interval (CI), 0.39-0.79; P=0.001] (Table II). The risk in C allele carriers was lower than that in the T allele carriers (adjusted OR, 0.71; 95% CI, 0.56-0.92; P=0.008) (Table II). Furthermore, comparison of patients with lymph node metastasis and control subjects revealed that carriers of the CC genotype and C allele had a decreased risk of developing this subgroup of gastric cancer (adjusted OR, 0.43; 95% CI, 0.29-0.63; P<0.0001 and adjusted OR, 0.62; 95% CI, 0.47-0.80; P=0.0003, respectively) (Table II). By contrast, there was no significant association between the CC genotype and C allele frequency in gastric cancer patients with and without lymph node metastasis (P=0.78) (Table II).

Association of THBS1 rs1478605 and rs3743125 SNPs with clinicopathological features of gastric cancer. Associations between the genotypic distribution and overall clinicopathological features were analyzed by univariate analysis and are reported in Table IV. No associations between the genotypic distribution of *THBS1* rs3743125 and overall patient clinicopathological features were observed. However, a significant association between the genotypic distribution of rs1478605 and the status of gastric cancer lymph node metastasis was observed (P=0.005) (Table V). In comparison with CT and TT genotype carriers, CC genotype carriers exhibited a lower risk of lymph node metastasis (OR, 0.41; 95% CI, 0.23-0.71; P=0.001) (Table V).

To confirm the strength of the association between *THBS1* rs1478605 and lymph node metastasis, a multivariate analysis was performed. To identify the potential confounding factors, an analysis of the association between lymph node metastasis and other clinicopathological features was first performed. This analysis revealed that tumor size and tumor invasion depth were significantly associated with lymph node metastasis and were likely to be confounding factors (P=0.002 and P=0.004, respectively) (Table VI). By contrast, the age and gender of

Table II. Association of *THBS1* rs1478605 with the development of gastric cancer.

THBS1 rs1478605	Controls (n=275)	Gastric cancer (n=275)	Adjusted OR (95% CI)[a]	P-value	Gastric cancer without lymph node metastasis (n=73)	Adjusted OR (95% CI)[a]	P-value	Gastric cancer with lymph node metastasis (n=202)	Adjusted OR (95% CI)[a]	P-value
Genotype										
TT, n (%)	28 (10.2)	37 (13.5)	-	-	9 (12.3)	-	-	28 (13.9)	-	-
CT, n (%)	120 (43.6)	148 (53.8)	0.93 (0.54-1.59)	0.78	29 (39.7)	0.75 (0.32-1.77)	0.52	119 (58.9)	0.99 (0.55-1.77)	0.97
CC, n (%)	127 (46.2)	90 (32.7)	0.52 (0.30-0.92)	0.02	35 (48.0)	0.86 (0.37-2.00)	0.73	55 (27.2)	0.42 (0.23-0.78)	0.006
TT+CT, n (%)	148 (53.8)	185 (67.3)	0.56 (0.39-0.79)[b]	0.001	38 (52.1)	1.08 (0.64-1.81)[b]	0.78	147 (72.8)	0.43 (0.29-0.63)[b]	0.00002
CC+CT, n (%)	247 (89.8)	238 (86.5)	1.40 (0.83-2.36)[c]	0.21	64 (87.7)	1.24 (0.55-2.76)[c]	0.61	174 (86.1)	1.44 (0.82-2.52)[c]	0.21
Allele										
C, n (%)	374 (68.0)	328 (59.6)	0.71 (0.56-0.92)	0.008	99 (67.8)	0.99 (0.67-1.47)	0.97	229 (56.7)	0.62 (0.47-0.80)	0.0003
T, n (%)	176 (32.0)	222 (40.4)			47 (32.2)			175 (43.3)		

[a]Adjusted by age and gender. [b]Adjusted OR (95% CI) was calculated by logistic regression modeling, comparing CC with the TT+CT genotype. [c]Adjusted OR (95% CI) was calculated by logistic regression modeling, comparing TT with the CC+CT genotype. *THBS1*, thrombospondin 1; OR, odds ratio; CI, confidence interval.

Table III. Association of *THBS1* rs3743125 with the development of gastric cancer.

THBS1 rs3743125	Gastric cancer (n=275)	Controls (n=275)	Adjusted OR (95% CI)[a]	P-value
Genotype				
TT, n (%)	28 (10.2)	30 (10.9)	-	-
CT, n (%)	133 (48.4)	123 (44.7)	1.14 (0.64-2.02)	0.66
CC, n (%)	114 (41.5)	122 (44.4)	0.98 (0.55-1.75)	0.95
TT+CT, n (%)	161 (58.5)	153 (55.6)	0.88 (0.63-1.24)[b]	0.47
CT+CC, n (%)	247 (89.8)	245 (90.1)	0.94 (0.55-1.63)[c]	0.83
Allele				
C, n (%)	361 (65.6)	367 (66.7)	0.95 (0.74-1.22)	0.70
T, n (%)	189 (34.4)	183 (33.3)		

[a]Adjusted by age and gender. [b]Adjusted OR (95% CI) was calculated by logistic regression modeling, comparing TT+CT with the CC genotype. [c]Adjusted OR (95% CI) was calculated by logistic regression modeling, comparing CC+CT with the TT genotype. *THBS1*, thrombospondin 1; OR, odds ratio; CI, confidence interval.

Table IV. Association of the *THBS1* polymorphisms with the clinicopathological features of gastric cancer.

	Rs1478605 genotype				Rs3743125 genotype			
Clinicopathological features	TT n (%)	CT n (%)	CC n (%)	P-value[a]	TT n (%)	CT n (%)	CC n (%)	P-value[a]
Gender								
Male (n=201)	23 (11.4)	106 (52.7)	72 (35.8)		19 (9.5)	103 (51.2)	79 (39.3)	
Female (n=74)	14 (18.9)	42 (56.8)	18 (24.3)	0.1	9 (12.2)	30 (40.5)	35 (47.3)	0.29
Age (years)								
≤60 (n=130)	16 (12.3)	69 (53.1)	45 (34.6)		11 (8.5)	64 (49.2)	55 (42.3)	
>60 (n=145)	21 (14.5)	79 (54.5)	45 (31.0)	0.79	17 (11.7)	69 (47.6)	59 (40.7)	0.67
Differentiation								
Well and moderate (n=100)	15 (15.0)	51 (51.0)	34 (34.0)		11 (11.0)	46 (46.0)	43 (43.0)	
Poor (n=175)	22 (12.6)	97 (55.4)	56 (32.0)	0.75	17 (9.7)	87 (49.7)	71 (40.6)	0.83
TNM								
IA/IB (n=30)	4 (13.3)	14 (46.7)	12 (40.0)		2 (6.7)	14 (46.7)	14 (46.7)	
II (n=65)	6 (9.2)	32 (56.7)	27 (41.5)		8 (12.3)	30 (46.2)	27 (41.5)	
IIIA/IIIB (n=164)	24 (14.6)	93 (56.3)	47 (28.7)		17 (10.4)	77 (47.0)	70 (42.7)	
IV (n=16)	3 (18.8)	9 (56.3)	4 (25.0)	0.48[b]	1 (6.3)	12 (75.0)	3 (18.8)	0.51[b]
Lymph node metastasis								
Without metastasis (n=73)	9 (12.3)	29 (39.7)	35 (47.9)		7 (9.6)	33 (45.2)	33 (45.2)	
With metastasis (n=202)	28 (13.9)	119 (58.9)	55 (27.2)	0.005	21 (10.2)	100 (48.4)	81 (41.5)	0.75
Location								
Cardia (n=76)	11 (14.5)	42 (55.3)	23 (30.3)		9 (11.8)	33 (43.4)	34 (44.7)	
Corpus (n=73)	7 (9.6)	39 (53.4)	27 (37.0)		10 (13.7)	41 (56.2)	22 (30.1)	
Antrum (n=92)	16 (17.4)	43 (46.7)	33 (35.9)		6 (6.5)	40 (43.5)	46 (50.0)	
Pylorus or other (n=34)	3 (8.8)	24 (70.6)	7 (20.6)	0.28	3 (8.8)	19 (55.9)	12 (41.5)	0.18
Invasion								
Within serosa (n=185)	24 (13.0)	102 (55.1)	59 (31.9)		19 (10.3)	90 (48.6)	76 (41.1)	
Serosa and beyond (n=90)	13 (14.4)	46 (51.1)	31 (34.4)	0.84	9 (10.0)	43 (47.8)	38 (42.2)	0.98
Tumor size								
≤5 cm (n=154)	16 (10.4)	81 (52.6)	57 (37.0)		14 (9.1)	73 (47.4)	67 (43.5)	
>5 cm (n=121)	21 (17.4)	67 (55.4)	33 (27.3)	0.11	14 (11.6)	60 (49.6)	47 (41.5)	0.68

[a]P-value was calculated by the χ^2 test. [b]P-value was calculated by the Fisher's exact test. *THBS1*, thrombospondin 1; TNM, tumor-node-metastasis; OR, odds ratio; CI, confidence interval.

Table V. Association of *THBS1* rs1478605 with gastric cancer lymph node metastasis.

Lymph node metastasis	Genotype				Genotype				Genotype			
	CC n (%)	CT n (%)	TT n (%)	P-value[a]	CT+TT n (%)	CC n (%)	Adjusted OR (95% CI)	P-value	CT+CC n (%)	TT n (%)	Adjusted OR (95% CI)	P-value
Without metastasis (n=73)	35 (47.9)	29 (39.7)	9 (12.3)	0.005[a]	38 (52.1)	35 (47.9)	0.41 (0.23-0.71)[b]	0.001	64 (87.7)	9 (12.3)	1.14 (0.51-2.56)[b]	0.74
							0.37 (0.21-0.66)[c]	0.001			1.13 (0.49-2.56)[c]	0.78
							0.40 (0.23-0.72)[d]	0.002			1.04 (0.45-2.39)[d]	0.93
With metastasis (n=202)	55 (27.2)	119 (58.9)	28 (13.9)		147 (72.8)	55 (27.2)			174 (86.1)	28 (13.9)		

[a]P-value was calculated by χ^2 test. [b]OR (95% CI) was calculated by χ^2 test, comparing CC with TT+CT genotype. [c]OR (95% CI) was calculated using logistic regression modeling adjusted by age, gender and tumor invasion depth, comparing CC with TT+CT genotype. [d]OR (95% CI) was calculated using logistic regression modeling adjusted by age, gender and tumor size, comparing TT with CC+CT genotype. *THBS1*, thrombospondin 1; OR, odds ratio; CI, confidence interval.

patients was included in all the multivariate analyses, as these represented independent variables, and were not associated with lymph node metastasis status (P=0.61 and P=0.49, respectively) (Table VI) or rs1478605 genotypic distribution (P=0.1 and P=0.79, respectively) (Table IV). Following adjustment for tumor invasion depth, patient gender and age, multivariate logistic regression analyses revealed that the homozygous CC genotype was significantly associated with a decreased risk of lymph node metastasis (OR, 0.37; 95% CI, 0.21-0.66; P=0.001) (Table V). Following adjustment for tumor size, patient gender and age, this analysis revealed that the homozygous CC genotype was also associated with a decreased risk of lymph node metastasis (OR, 0.40; 95% CI, 0.23-0.72; P=0.002) (Table V).

To further validate the results from this logistic regression modeling analyses and to investigate the interaction between factors, stratification was applied to analyze the association of rs1478605 with lymph node metastasis, using other clinicopathological features as stratification factors. The majority of these analyses did not yield significant results, including those using age, differentiation status, tumor location, size and invasion depth as stratification factors (data not shown), as each OR value between strata was not significantly different, indicating that the associations were independent of these factors. However, when patient gender was used as the stratification factor, a clear association between rs1478605 and lymph node metastasis was observed in the two strata. There was a synergistic effect of the rs1478605 SNP and male gender on gastric cancer lymph node metastasis without any adjustments (OR, 0.27; 95% CI, 0.14-0.52; P<0.001) (Table VII). By contrast, the effect of the rs1478605 SNP and female gender on lymph node metastasis was not significant (OR, 1.83; 95% CI, 0.46-7.22; P=0.38) (Table VII). A significant difference in the effect of the rs1478605 SNP between the two strata was observed (P=0.01) (Table VII). These results indicate that the CC genotype is associated with a reduced risk of lymph node metastasis in male patients.

Association of THBS1 haplotypes with the development and progression of gastric cancer. *THBS1* rs1478605 and rs3743125 exist in a single block of disequilibrium, with r^2 and D' values of 0.91 and 1, respectively. The four major haplotypes and their frequencies identified in gastric cancer patients and control subjects are shown in Table VIII. A significant difference in the *THBS1* CT haplotype distribution was identified between patient and control groups (OR, 0.56; 95% CI, 0.33-0.93; P=0.02) (Table VIII), indicating that CT haplotype carriers had a lower risk of developing gastric cancer, compared to CC+TT+TC haplotype carriers. However, no association between *THBS1* haplotypes and the overall clinicopathological features of gastric cancer were observed (data not shown).

Discussion

The present study demonstrated that the *THBS1* rs1478605 CC genotype is associated with a decreased risk of developing gastric cancer, particularly gastric cancer with lymph node metastasis. Among the patients with gastric cancer, the rs1478605 CC genotype was negatively associated with lymph node metastasis. The *THBS1* CT haplotype is associated with

Table VI. Associations of the clinicopathological features with gastric cancer lymph node metastasis.

Lymph node metastasis	All cases, n (%)	Cases with no metastasis, n (%)	Cases with metastasis, n (%)	P-value[a]
Gender				
Male	201 (73.1)	55 (75.3)	146 (72.3)	
Female	74 (26.9)	18 (24.7)	56 (27.7)	0.61
Age (year)				
1 (≤60)	130 (47.3)	32 (43.8)	98 (48.5)	
2 (>60)	145 (52.7)	41 (56.2)	104 (51.5)	0.49
Differentiation				
Well and moderate	100 (36.4)	32 (43.8)	68 (33.7)	
Poor	175 (63.6)	41 (56.2)	134 (66.3)	0.12
Location				
Cardia	76 (27.6)	19 (26.0)	57 (28.2)	
Corpus	73 (26.5)	24 (32.9)	49 (24.3)	
Antrum	92 (33.5)	23 (31.5)	69 (34.2)	
Pylorus or other	34 (12.4)	7 (9.6)	27 (13.4)	0.51
Invasion				
Within serosa	185 (67.3)	59 (80.8)	126 (62.4)	
Serosa and beyond	90 (32.7)	14 (19.2)	76 (37.6)	0.004
Tumor size				
≤5 cm	154 (56.0)	52 (71.2)	102 (50.5)	
>5 cm	121 (44.0)	21 (28.8)	100 (49.5)	0.002

[a]P-value was calculated by χ^2 test.

Table VII. Stratified analyses of the associations of rs1478605 with lymph node metastasis.

Gender	Lymph node metastasis	Rs1478605 CT+TT, n (%)	Genotype CC, n (%)	OR (95% CI)[a]	P-value	P-value between strata[b]
Male	Without metastasis (n=55)	23 (41.8)	32 (58.2)	0.27 (0.14-0.52)	0.00005	0.01
	With metastasis (n=146)	106 (72.6)	40 (27.4)			
Female	Without metastasis (n=18)	15 (83.3)	3 (16.7)	1.83 (0.46-7.22)	0.38	
	With metastasis (n=56)	41 (73.2)	15 (26.8)			

[a]OR (95% CI) was calculated by χ^2 test, using gender as the stratification factor. [b]P-value was calculated by Mantel-Haenszel's test. OR, odds ratio; CI, confidence interval.

Table VIII. Correlation between the *THBS1* haplotypes and the development of gastric cancer.

Haplotypes	Gastric cancer, n (%)	Controls, n (%)	P-value	OR (95% CI)[a]
CT	27 (10)	45 (16)	0.02	0.56 (0.33-0.93)
CC	137 (50)	141 (51)		
TT	64 (23)	49 (18)		
TC	47 (17)	40 (15)		

[a]OR (95% CI) was calculated by χ^2 test, comparing CT haplotype with the CC+TT+TC haplotypes. *THBS1*, thrombospondin 1; OR, odds ratio; CI, confidence interval.

a reduced risk of gastric cancer. However, no associations between the *THBS1* rs3743125 genotypic distribution and the development or clinicopathological features of gastric cancer.

In the present study, careful statistical analyses were performed to avoid spurious results caused by artificial bias in the study design and by confounding factors. A multitude of

univariate or multivariate analyses were performed to detect inconsistencies reflecting the presence of biases. The similar results derived from these analyses confirmed and reinforced our findings.

The study focused on *THBS1* rs1478605 and rs37431125 SNPs, which are located in gene UTRs. UTRs are known to play crucial roles in the post-transcriptional regulation of gene expression (22,24-25), which is important for normal cell function, and dysfunctions have been linked to the pathophysiology of numerous diseases (26-30). In recent years, gene polymorphisms in UTRs have also been extensively studied and reported to be associated with cancer susceptibility (31-34). However, there has been relatively little study associated with the correlation of SNPs in *THBS1* UTRs with individual susceptibility to gastric cancer until recently. The present data demonstrate that *THBS1* rs3743125 has no association with the development and progression of gastric cancer, which was similar to our previous study (22). Notably, in the previous study (22), the data showed that the AG and GG genotypes of *THBS1* rs1478604 A>G, the other SNP loci located in 5'-UTRs, was positively associated with lymph node metastasis in gastric cancer. However, our data suggested that the rs1478605 CC genotype was negatively associated with lymph node metastasis. To identify the synergistic effect of the two SNPs, a haplotype-based association study was necessary. However, the data of rs1478604 was unavailable in this study, which requires to be completed in future studies. Despite the limitation, the present study adds to the evidence that SNPs in *THBS1* UTRs may affect the development and progression of gastric cancer.

As non-coding polymorphisms, the SNPs of *THBS1* do not lead to the alteration of the THBS1 protein. However, they may affect the susceptibility to gastric cancer through the following mechanisms: i) The polymorphism locus may change the regulatory sequences of the UTRs, which influence expression of *THBS1* at the level of translation; and ii) other nearby polymorphisms in linkage disequilibrium with the SNPs may have a functional role. Clearly, further studies are required to confirm these hypotheses.

THBS1 has been reported to exert a pro-angiogenic effect in gastric cancer (13-17), which plays an important role in cancer metastasis. Tumor metastasis is well established as a critical event affecting patient prognosis. The present study suggests that the rs1478605 CC genotype is negatively associated with lymph node metastasis, indicating that gastric cancer patients with the *THBS1* rs1478605 CC genotype may have an improved prognosis than patients with CT and TT genotypes.

In conclusion, the present study demonstrates that *THBS1* rs1478605 may be a protective factor in gastric cancer. The study had 89 and 97% power to detect an effect with an OR of 0.56 in the case and control groups under a dominant genetic model and an OR of 0.27 in the lymph node metastasis and non-metastasis groups in male gastric cancer patients. Further studies using larger patient cohorts are required to confirm our findings, and mechanistic studies are required to improve the understanding of the complex mechanisms involved in the development and progression of gastric cancer. Despite several limitations in the present study, our data provide additional information that is necessary for genetic risk assessments, and confirm the important role of *THBS1* in the development and progression of gastric cancer.

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