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Association between Promoter Polymorphisms of *TFF1*, *TFF2*, and *TFF3* and the Risk of Gastric and Diffuse Gastric Cancers in a Korean Population

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Gastric cancer is one of the most common cancers in the world. The aims of this study were to evaluate the association between polymorphisms in *TFF* gene family, *TFF1*, *TFF2*, and *TFF3* and the risk of gastric cancer (GC) and GC subgroups in a Korean population via a case-control study. The eight polymorphisms in *TFF* gene family were identified by sequencing and genotyped with 377 GC patients and 396 controls by using TaqMan genotyping assay. The rs184432 TT genotype of *TFF1* was significantly associated with a reduced risk of GC (odds ratio, [OR] = 0.45; 95% confidence interval, [CI] = 0.25-0.82; $P = 0.009$), more protective against diffuse-type GC (OR = 0.20; 95% CI = 0.05-0.89; $P = 0.035$) than GC (OR = 0.34; 95% CI = 0.14-0.82; $P = 0.017$) in subjects aged < 60 yr, and correlated with lymph node metastasis negative GC and diffuse-type GC (OR = 0.44; 95% CI = 0.23-0.86; $P = 0.016$ and OR = 0.20; 95% CI = 0.05-0.87; $P = 0.031$, respectively). In addition, a decreased risk of lymph node metastasis negative GC and diffuse-type GC was observed for rs225359 TT genotype of *TFF1* (OR = 0.46, 95% CI = 0.24-0.88; $P = 0.020$ and OR = 0.21, 95% CI = 0.05-0.88; $P = 0.033$, respectively). These findings suggest that the rs184432 and rs225359 polymorphisms in *TFF1* have protective effects for GC and contribute to the development of GC in Korean individuals.

Keywords: Control-case Studies; Diffuse Type; Gastric Neoplasms; Polymorphism; *TFF1* Protein, Human; *TFF2* Protein, Human; *TFF3* Protein, Human

INTRODUCTION

Gastric cancer (GC) is one of the most common cancers affecting people worldwide. In the past few decades, the incidence and mortality rates of GC have steadily declined in several countries. However, these rates continue to be high in Asian countries. Particularly, in Korea, GC is the third-most common cancer, with 34,478 new cases and 7,876 deaths recorded in 2014, as per the report of the Korea National Cancer Center (1-3).

Until date, several studies have reported that genetic alteration including single nucleotide polymorphisms (SNPs) in tumor suppressor genes such as those encoding adenomatous polyposis coli, tumor protein p53, tumor protein p73, deleted in colon cancer, and fragile histidine triad may play an important role in defining susceptibility to GC (4-13).

In addition, trefoil factor 1 (*TFF1*) is a tumor suppressor gene (14) belonging to the *TFF* family. The *TFF* protein family consists of *TFF1*, *TFF2*, and *TFF3*, which are expressed and secreted in the mucous cells of the human stomach and protect the gastrointestinal epithelium (15-17). *TFF* are clustered in a 50-kb region of the chromosome 21q22.3 (18, 19). The abnormal expression levels of *TFF* proteins have been reported to be associated with the progression and development of several cancers such as colon cancer (20, 21), breast cancer (22, 23), prostate cancer (24, 25), and lung cancer (26). Some evidence suggests that *TFF* expression is involved in GC progression. *TFF1*-knockout mice developed antral adenomas, and 30% of them further developed multiple gastric carcinomas (27). *TFF1* was normally expressed in gastric mucosa, but the expression of *TFF1* and *TFF2* was significantly lower in carcinomas than in normal tissues (28, 29).

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Both decreased *TFF1* and *TFF2* expression and increased *TFF3* expression have been reported in gastric carcinoma (30). Furthermore, downregulation of *TFF1* expression and upregulation of *TFF3* expression have also been reported in GC (31). Recently, association studies between polymorphisms of *TFF* and GC susceptibility were reported in two different ethnic groups: a polymorphism in the promoter region of *TFF1* was associated with GC development in an Iranian population, and promoter polymorphisms of *TFF2* and *TFF3* were associated with GC susceptibility in a Chinese population (32, 33). Therefore, we hypothesized that the polymorphisms in *TFF* play a critical role in GC progression and development.

In the present study, we elucidated the relevance of polymorphisms in the coding and promoter regions of the *TFF* family to the risk of GC and GC subgroups in order to clarify our hypothesis in the Korean population.

MATERIALS AND METHODS

Subjects

This case-control study group included 377 patients with GC (267 men, 110 women) with a mean age of 60.1 ± 11.8 yr and 396 healthy controls (132 men, 264 women) with a mean age of 58.7 ± 9.0 yr. The blood samples used in this study were provided by the Chungnam National Hospital Biobank, which is a member of the National Biobank of Korea and is supported and audited by the Ministry of Health and Welfare of Korea. GC patients were recruited from the outpatient clinic at the Chungnam National University Hospital and classified according to Lauren's classification (34). The healthy controls were randomly selected from among healthy volunteers visiting the Chungnam National University Hospital medical center for their annual physical examinations and who had no history of cancer.

DNA preparation and SNP identification

Genomic DNA was extracted from the peripheral blood by using the QIAamp DNA Blood Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's instructions. To identify polymorphic sites in *TFF1*, *TFF2*, and *TFF3*, all exons including intron-exon boundaries, 1.5 kb of the 5′-flanking region, and the 3′-untranslated region (UTR) were amplified by polymerase chain reaction (PCR) with genomic DNA in 24 GC patients and 24 healthy controls. PCR was performed with 50 ng of genomic DNA, Taq DNA polymerase (EF Taq, SolGent, Daejon, Korea), and 0.5 pM of each primer under the following conditions: 30 cycles of denaturation for 10 sec at 98°C, annealing for 30 sec at 65°C, extension for 2 min at 72°C, and a final extension for 10 min at 72°C in a thermocycler (Gene Amp PCR System 9700; Applied Biosystems, Foster, CA, USA). The PCR product was used as a template for sequencing. The SNPs of *TFF1*, *TFF2*, and *TFF3* were detected by a sequence analysis based on the reference sequence of human chromosome 21 (GenBank accession number: NT_011512.12).

Genotyping

Genotyping for the SNPs in *TFF1* (rs184432, rs35448902, rs225359, and rs2156310), *TFF2* (rs3814896, rs13052596, and rs225334), and *TFF3* (rs225362) was performed by using the Applied Biosystems TaqMan SNP Genotyping Assay with the StepOnePlus Real-time PCR System (Applied Biosystems).

Statistical analysis

Chi-square tests were used to estimate the Hardy-Weinberg equilibrium (HWE) of each SNP and to detect age and gender in the GC and control groups. The association between the GC and control groups was analyzed by chi-square test. We used binary logistic regression to estimate the GC risk by odds ratios (OR) and 95% confidence intervals (CI). All statistical analyses were performed by using the SPSS (SPSS Inc., Chicago, IL, USA), version 20.0 for windows. $P < 0.05$ was considered statistically significant.

Ethics statement

All individuals enrolled in this study provided their written informed consent for blood collection and use. The study protocol was approved by the institutional review board of the Chungnam National University Hospital (IRB No. 2013-08-008).

RESULTS

The characteristics of the 377 GC cases and 396 controls are shown in Table 1. No significant difference was noted between GC cases and controls in the distribution of age ($P = 0.063$), whereas the distribution of gender of GC case differed from that of controls ($P < 0.001$). Of the 377 GC cases, 194 (51.5%) were classified as intestinal type, 138 (36.6 %) as diffuse-type, 39 (10.3%) as mixed-type, and 6 (1.6%) were unclassified. GC cases comprised of 264 (30.0%) negative cases and 113 (70%) positive cases for lymph node metastasis.

We conducted sequencing to detect SNPs with a minor allele frequency greater than 5% in 24 GC patients and 24 healthy controls (35). We identified 4 SNPs in *TFF1* (rs184432, rs35448902, and rs225359 in the promoter region and rs2156310 in the 5′UTR), 3 SNPs in *TFF2* (rs3814896 and rs13052596 in the promoter region and rs225334 in the 3′UTR), and 1 SNP in *TFF3* (rs225362 in the promoter region) through gene sequencing. The genotype frequencies of 8 SNPs (rs184432, rs35448902, rs22535, rs2156310, rs3814896, rs13052596, rs225334, and rs225362) were in the HWE in both GC cases and controls ($P > 0.05$; data not shown).

To determine whether *TFF1*, *TFF2*, and *TFF3* variations were associated with the risk of GC or GC subgroups, we analyzed the

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Table 1. Characteristics of gastric cancer patients and controls enrolled in the genetic analyses

Variables	Case N (%)	Control N (%)	*P* value*
All subjects	377 (100)	396 (100)	
Age (yr) (mean ± SD)	60.1 ± 11.8	58.7 ± 9.0	
< 60	169 (44.8)	204 (51.5)	0.063
≥ 60	208 (55.2)	192 (48.5)	
Gender			
Male	267 (70.8)	132 (33.3)	< 0.001
Female	110 (29.2)	264 (66.7)	
Histological type			
Intestinal	194 (51.5)		
Diffuse	138 (36.6)		
Mixed	39 (10.3)		
Unclassified	6 (1.6)		
Lymph node metastasis			
Negative	264 (70.0)		
Positive	113 (30.0)		

*Two-sided chi-square test.

genotypes and allele frequencies of *TFF* SNPs. The genotype and allele frequencies of rs184432 in *TFF1* were significantly associated with a decreased GC risk (OR = 0.45, 95% CI = 0.25-0.82, *P* = 0.009 and OR = 0.75, 95% CI = 0.59-0.94, *P* = 0.012, respectively), whereas the remaining SNPs showed no association (Table 2, Supplementary Table 1).

Furthermore, stratification analyses were performed to evaluate the possible correlation of genetic variations of *TFF1*, *TFF2*, and *TFF3* with the risk of GC or GC subgroups according to the age. Stratified analysis revealed that the genotype and allele frequencies of *TFF1* rs184432 were significantly associated with a decreased risk of GC among subjects aged < 60 yr (OR = 0.34, 95% CI = 0.14-0.82, *P* = 0.017 and OR = 0.69, 95% CI = 0.49-0.96, *P* = 0.028, respectively), but not in subjects aged ≥ 60 yr. In addition, we found that the genotype and allele frequencies of *TFF1* rs184432 were related to a reduced risk of the development of diffuse-type GC in subjects aged < 60 yr (OR = 0.20, 95% CI = 0.05-0.89, *P* = 0.035 and OR = 0.60, 95% CI = 0.38-0.95, *P* = 0.028, respectively), but not in subjects aged ≥ 60 yr (Table 3). We observed the lack of association between *TFF2* (rs3814896, rs13052596, and rs225334) and *TFF3* (rs225362) SNPs, GC risk, and age (data not shown).

In the present study, we investigated whether *TFF* SNPs were related to lymph node metastasis of GC or GC subgroups. The frequencies of CT and TT genotypes and T allele were associated with a decreased risk of GC, indicating negative lymph node metastasis (OR = 0.71, 95% CI = 0.51-1.00, *P* = 0.048; OR = 0.44, 95% CI = 0.23-0.86, *P* = 0.016 and OR = 0.68, 95% CI = 0.52-0.88, *P* = 0.003, respectively). In addition, an association of the *TFF1* rs225359 TT genotype and T allele with a decreased risk of GC was noted, indicating negative lymph node metastasis, as compared to that of the *TFF1* rs225359 CC genotype and C allele, respectively (OR = 0.46, 95% CI = 0.24-0.88, *P* = 0.020 and OR = 0.77, 95% CI = 0.59-0.99, *P* = 0.041, respectively) (Table 4). To estimate the relevance of *TFF* variations and lymph node metastasis in intestinal and diffuse-type GC, we conducted a logistic regression analysis. The *TFF1* rs184432 TT genotype and T allele were related to a decreased risk of diffuse-type GC, indicating negative lymph node metastasis (OR = 0.20, 95% CI = 0.05-0.87, *P* = 0.031 and OR = 0.58, 95% CI = 0.38-0.87, *P* = 0.01, respectively). Further analyses revealed a significant association of *TFF1* rs225359 TT genotype and T allele with a decreased risk of diffuse-type GC, indicating negative lymph node metastasis (OR = 0.21, 95% CI = 0.05-0.88, *P* = 0.033 and OR = 0.64, 95% CI = 0.43-0.96, *P* = 0.030, respectively) (Table 5). No association was observed between *TFF2* (rs3814896, rs13052596, and rs 225334) and *TFF3* (rs225362) SNPs and lymph node metastasis of GC and GC subgroups (data not shown).

Table 2. Genotype and allele frequencies of *TFF1* polymorphisms among gastric cancer patients and controls and their association with gastric cancer risk

SNPs	Genotype	Controls	GC vs. CON		
		N (%)	N (%)	OR (95% CI)	*P* value
TFF1					
rs184432	CC	206 (52.0)	221 (58.6)	1.00 (ref.)	
	CT	153 (38.6)	138 (36.6)	0.84 (0.62-1.13)	0.254
	TT	37 (9.3)	18 (4.8)	0.45 (0.25-0.82)	0.009
Allele	C	565 (71.3)	580 (76.9)	1.00 (ref.)	
	T	227 (28.7)	174 (23.1)	0.75 (0.59-0.94)	0.012
TFF1					
rs35448902	GG	240 (60.6)	228 (60.5)	1.00 (ref.)	
	GA	128 (32.3)	125 (33.2)	1.03 (0.76-1.40)	0.860
	AA	28 (7.1)	24 (6.4)	0.90 (0.51-1.60)	0.726
Allele	G	608 (76.8)	581 (77.1)	1.00 (ref.)	
	A	184 (23.2)	173 (22.9)	0.98 (0.78-1.25)	0.893
TFF1					
rs225359	CC	212 (53.5)	209 (55.4)	1.00 (ref.)	
	CT	145 (36.6)	145 (38.5)	1.01 (0.75-1.37)	0.926
	TT	39 (9.8)	23 (6.1)	0.60 (0.35-1.04)	0.067
Allele	C	569 (71.8)	563 (74.7)	1.00 (ref.)	
	T	223 (28.2)	191 (25.3)	0.87 (0.69-1.09)	0.210
TFF1					
rs2156310	CC	130 (32.8)	130 (34.5)	1.00 (ref.)	
	CT	192 (48.5)	179 (47.5)	0.93 (0.68-1.28)	0.665
	TT	74 (18.7)	68 (18.0)	0.92 (0.61-1.38)	0.686
Allele	C	452 (57.1)	439 (58.2)	1.00 (ref.)	
	T	340 (42.9)	315 (41.8)	0.95 (0.78-1.17)	0.647

SNPs, single nucleotide polymorphism; GC, gastric cancer; CON, controls; OR, odds ratio; CI, confidence interval.

DISCUSSION

Until date, it has been implicated that alteration of *TFF* expression affects the development of several types of cancers. Recently, the association between polymorphisms of *TFF* and the development of GC was reported in Iranian and Chinese populations, but not in a Korean population (32,33). In the present study, we focused on *TFF* polymorphisms. The aim of this study

Table 3. Stratified analysis of *TFF1* polymorphisms in gastric cancer patients and controls by age

SNPs	Genotype	Controls	GC vs. CON			Diffuse-type GC vs. CON		
		N (%)	N (%)	OR (95% CI)	*P* value	N (%)	OR (95% CI)	*P* value
TFF1								
rs184432	CC	109 (53.4)	103 (60.9)	1.00 (ref.)		49 (63.6)	1.00 (ref.)	
Age	CT	73 (35.8)	59 (34.9)	0.86 (0.55-1.32)	0.482	26 (33.8)	0.79 (0.45-1.39)	0.415
< 60	TT	22 (10.8)	7 (4.1)	0.34 (0.14-0.82)	0.017	2 (2.6)	0.20 (0.05-0.89)	0.035
Allele	C	291 (71.3)	265 (78.4)	1.00 (ref.)		124 (80.5)	1.00 (ref.)	
	T	117 (28.7)	73 (21.6)	0.69 (0.49-0.96)	0.028	30 (19.5)	0.60 (0.38-0.95)	0.028
≥ 60	CC	97 (50.5)	118 (56.7)	1.00 (ref.)		31 (50.8)	1.00 (ref.)	
	CT	80 (41.7)	79 (38.0)	0.81 (0.54-1.22)	0.320	23 (37.7)	0.90 (0.49-1.66)	0.736
	TT	15 (7.8)	11 (5.3)	0.60 (0.27-1.37)	0.228	7 (11.5)	1.46 (0.55-3.91)	0.451
Allele	C	274 (71.4)	315 (75.7)	1.00 (ref.)		85 (69.7)	1.00 (ref.)	
	T	110 (28.6)	101 (24.3)	0.80 (0.58-1.09)	0.162	37 (30.3)	1.08 (0.70-1.69)	0.722
TFF1								
rs35448902	GG	119 (58.3)	93 (55.0)	1.00 (ref.)		44 (57.1)	1.00 (ref.)	
Age	GA	72 (35.3)	64 (37.9)	1.14 (0.74-1.75)	0.560	27 (35.1)	1.01 (0.58-1.78)	0.961
< 60	AA	13 (6.4)	12 (7.1)	1.18 (0.52-2.71)	0.694	6 (7.8)	1.25 (0.45-3.49)	0.672
Allele	G	310 (76.0)	250 (74.0)	1.00 (ref.)		115 (74.7)	1.00 (ref.)	
	A	98 (24.0)	88 (26.0)	1.11 (0.80-1.55)	0.526	39 (25.3)	1.07 (0.70-1.65)	0.748
≥ 60	GG	121 (63.0)	135 (64.9)	1.00 (ref.)		41 (67.2)	1.00 (ref.)	
	GA	56 (29.2)	61 (29.3)	0.98 (0.63-1.51)	0.915	16 (26.2)	0.84 (0.44-1.63)	0.612
	AA	15 (7.8)	12 (5.8)	0.72 (0.32-1.59)	0.414	4 (6.6)	0.79 (0.25-2.51)	0.685
Allele	G	298 (77.6)	331 (79.6)	1.00 (ref.)		98 (80.3)	1.00 (ref.)	
	A	86 (22.4)	85 (20.4)	0.89 (0.64-1.25)	0.499	24 (19.7)	0.85 (0.51-1.41)	0.525
TFF1								
rs225359	CC	110 (53.9)	94 (55.6)	1.00 (ref.)		44 (57.1)	1.00 (ref.)	
Age	CT	73 (35.8)	65 (38.5)	1.04 (0.68-1.61)	0.852	30 (39.0)	1.03 (0.59-1.78)	0.923
< 60	TT	21 (10.3)	10 (5.9)	0.56 (0.25-1.24)	0.153	3 (3.9)	0.36 (0.10-1.26)	0.109
Allele	C	293 (71.8)	253 (74.9)	1.00 (ref.)		118 (76.6)	1.00 (ref.)	
	T	115 (28.2)	85 (25.1)	0.86 (0.62-1.19)	0.351	36 (23.4)	0.78 (0.51-1.20)	0.252
≥ 60	CC	102 (53.1)	115 (55.3)	1.00 (ref.)		32 (52.5)	1.00 (ref.)	
	CT	72 (37.5)	80 (38.5)	0.99 (0.65-1.49)	0.945	23 (37.7)	1.02 (0.55-1.88)	0.954
	TT	18 (9.4)	13 (6.3)	0.64 (0.30-1.37)	0.252	6 (9.8)	1.06 (0.39-2.91)	0.906
Allele	C	276 (71.9)	310 (74.5)	1.00 (ref.)		87 (71.3)	1.00 (ref.)	
	T	108 (28.1)	106 (25.5)	0.87 (0.64-1.20)	0.399	35 (28.7)	1.03 (0.66-1.61)	0.904
TFF1								
rs2156310	CC	64 (31.4)	57 (33.7)	1.00 (ref.)		28 (36.4)	1.00 (ref.)	
Age	CT	99 (48.5)	83 (49.1)	0.94 (0.59-1.49)	0.797	35 (45.4)	0.81 (0.45-1.46)	0.478
< 60	TT	41 (20.1)	29 (17.2)	0.79 (0.44-1.44)	0.448	14 (18.2)	0.78 (0.37-1.66)	0.518
Allele	C	227 (55.6)	197 (58.3)	1.00 (ref.)		91 (59.1)	1.00 (ref.)	
	T	181 (44.4)	141 (41.7)	0.90 (0.67-1.20)	0.468	63 (40.9)	0.87 (0.60-1.26)	0.461
≥ 60	CC	66 (34.4)	73 (35.1)	1.00 (ref.)		20 (32.8)	1.00 (ref.)	
	CT	93 (48.4)	96 (46.2)	0.93 (0.60-1.45)	0.757	32 (52.4)	1.14 (0.60-2.16)	0.698
	TT	33 (17.2)	39 (18.7)	1.07 (0.60-1.89)	0.820	9 (14.8)	0.90 (0.37-2.19)	0.817
Allele	C	225 (58.6)	242 (58.2)	1.00 (ref.)		72 (59.0)	1.00 (ref.)	
	T	159 (41.4)	174 (41.8)	1.02 (0.77-1.35)	0.904	50 (41.0)	0.98 (0.65-1.49)	0.934

SNPs, single nucleotide polymorphism; GC, gastric cancer; CON, controls; OR, odds ratio; CI, confidence interval.

was to investigate whether polymorphisms in *TFF* were associated with the risk of GC or GC subgroups in the Korean population. We scanned a Korean-specific polymorphism by sequencing the functional region of *TFF* that directly affect the gene expression, such as an exon, an exon boundary, and a promoter region. However, we did not detect any Korean-specific novel SNP. We finally selected 8 SNPs, 6 SNPs in the promoter region, 1 SNP in 5′UTR, and 1 SNP in 3′UTR after eliminating the SNP in tight LD ($|D'| = 1$ or $r^2 = 1$) for genotyping. The proportion of men in the test cases was higher than that in the control cases, whereas the trend in women was reverse (Table 1). To evaluate whether the difference of the proportion of gender is associated with GC risk, we attempted stratified analysis by gender, but any association between *TFF* SNPs, GC risk, and gender was not observed (data not shown). This result represented that the correlation between *TFF* SNPs and GC risk is not affected by gender. In our study, TT genotype and T allele of rs184432 in the promoter region of *TFF1* was significantly associated with a reduced

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Table 4. Association of genetic polymorphisms in *TFF1* with lymph node metastasis of gastric cancer

SNPs	Genotype	Controls	GC (negative) vs. CON			GC (positive) vs. CON		
		N (%)	N (%)	OR (95% CI)	*P* value	N (%)	OR (95% CI)	*P* value
TFF1								
rs184432	CC	206 (52.0)	164 (62.1)	1.00 (ref.)		57 (50.4)	1.00 (ref.)	
	CT	153 (38.6)	87 (33.0)	0.71 (0.51-1.00)	0.048	51 (45.2)	1.21 (0.78-1.86)	0.398
	TT	37 (9.3)	13 (4.9)	0.44 (0.23-0.86)	0.016	5 (4.4)	0.49 (0.18-1.30)	0.151
Allele	C	565 (71.3)	415 (78.6)	1.00 (ref.)		165 (73.0)	1.00 (ref.)	
	T	227 (28.7)	113 (21.4)	0.68 (0.52-0.88)	0.003	61 (27.0)	0.92 (0.66-1.28)	0.623
TFF1								
rs35448902	GG	240 (60.6)	155 (58.7)	1.00 (ref.)		73 (64.6)	1.00 (ref.)	
	GA	128 (32.3)	94 (35.6)	1.14 (0.81-1.59)	0.451	31 (27.4)	0.80 (0.50-1.28)	0.344
	AA	28 (7.1)	15 (5.7)	0.83 (0.43-1.60)	0.578	9 (8.0)	1.06 (0.48-2.34)	0.892
Allele	G	608 (76.8)	404 (76.5)	1.00 (ref.)		177 (78.3)	1.00 (ref.)	
	A	184 (23.2)	124 (23.5)	1.01 (0.78-1.32)	0.915	49 (21.7)	0.92 (0.64-1.31)	0.625
TFF1								
rs225359	CC	212 (53.5)	155 (58.7)	1.00 (ref.)		54 (47.8)	1.00 (ref.)	
	CT	145 (36.6)	96 (36.4)	0.91 (0.65-1.26)	0.557	49 (43.4)	1.33 (0.85-2.06)	0.209
	TT	39 (9.8)	13 (4.9)	0.46 (0.24-0.88)	0.020	10 (8.8)	1.01 (0.47-2.14)	0.986
Allele	C	569 (71.8)	406 (76.9)	1.00 (ref.)		157 (69.5)	1.00 (ref.)	
	T	223 (28.2)	122 (23.1)	0.77 (0.59-0.99)	0.041	69 (30.5)	1.12 (0.81-1.55)	0.486
TFF1								
rs2156310	CC	130 (32.8)	86 (32.6)	1.00 (ref.)		44 (38.9)	1.00 (ref.)	
	CT	192 (48.5)	127 (48.1)	1.00 (0.70-1.42)	0.999	52 (46.1)	0.80 (0.51-1.27)	0.341
	TT	74 (18.7)	51 (19.3)	1.04 (0.67-1.63)	0.858	17 (15.0)	0.68 (0.36-1.27)	0.227
Allele	C	452 (57.1)	299 (56.6)	1.00 (ref.)		140 (61.9)	1.00 (ref.)	
	T	340 (42.9)	229 (43.4)	1.02 (0.82-1.27)	0.874	86 (38.1)	0.82 (0.60-1.11)	0.190

SNPs, single nucleotide polymorphism; GC, gastric cancer; CON, controls; OR, odds ratio; CI, confidence interval.

Table 5. Association of genetic polymorphisms in *TFF1* with lymph node metastasis of diffuse-type gastric cancer

SNPs	Genotype	Controls	Diffuse-type GC (negative) vs. CON			Diffuse-type GC (positive) vs. CON		
		N (%)	N (%)	OR (95% CI)	*P* value	N (%)	OR (95% CI)	*P* value
TFF1								
rs184432	CC	206 (52.0)	55 (64.7)	1.00 (ref.)		25 (47.2)	1.00 (ref.)	
	CT	153 (38.6)	28 (32.9)	0.69 (0.42-1.13)	0.139	24 (45.3)	1.29 (0.71-2.35)	0.400
	TT	37 (9.3)	2 (2.4)	0.20 (0.05-0.87)	0.031	4 (7.5)	0.89 (0.29-2.71)	0.839
Allele	C	565 (71.3)	138 (81.2)	1.00 (ref.)		74 (69.8)	1.00 (ref.)	
	T	227 (28.7)	32 (18.8)	0.58 (0.38-0.87)	0.010	32 (30.2)	1.08 (0.69-1.68)	0.745
TFF1								
rs35448902	GG	240 (60.6)	50 (58.8)	1.00 (ref.)		35 (66.0)	1.00 (ref.)	
	GA	128 (32.3)	29 (34.1)	1.09 (0.66-1.80)	0.745	14 (26.5)	0.75 (0.39-1.45)	0.390
	AA	28 (7.1)	6 (7.1)	1.03 (0.41-2.61)	0.953	4 (7.5)	0.98 (0.32-2.96)	0.971
Allele	G	608 (76.8)	129 (75.9)	1.00 (ref.)		84 (79.2)	1.00 (ref.)	
	A	184 (23.2)	41 (24.1)	1.05 (0.71-1.55)	0.805	22 (20.8)	0.87 (0.53-1.42)	0.569
TFF1								
rs225359	CC	212 (53.5)	53 (62.4)	1.00 (ref.)		23 (43.4)	1.00 (ref.)	
	CT	145 (36.6)	30 (35.2)	0.83 (0.50-1.36)	0.454	23 (43.4)	1.46 (0.79-2.71)	0.226
	TT	39 (9.8)	2 (2.4)	0.21 (0.05-0.88)	0.033	7 (13.2)	1.65 (0.66-4.12)	0.279
Allele	C	569 (71.8)	136 (80.0)	1.00 (ref.)		69 (65.1)	1.00 (ref.)	
	T	223 (28.2)	34 (20.0)	0.64 (0.43-0.96)	0.030	37 (34.9)	1.37 (0.89-2.10)	0.151
TFF1								
rs2156310	CC	130 (32.8)	28 (32.9)	1.00 (ref.)		20 (37.7)	1.00 (ref.)	
	CT	192 (48.5)	40 (47.1)	0.97 (0.57-1.65)	0.902	27 (51.0)	0.91 (0.49-1.70)	0.776
	TT	74 (18.7)	17 (20.0)	1.07 (0.55-2.08)	0.850	6 (11.3)	0.53 (0.20-1.37)	0.189
Allele	C	452 (57.1)	96 (56.5)	1.00 (ref.)		67 (63.2)	1.00 (ref.)	
	T	340 (42.9)	74 (43.5)	1.03 (0.73-1.43)	0.886	39 (36.8)	0.77 (0.51-1.18)	0.230

SNPs, single nucleotide polymorphisms; GC, gastric cancer; CON, controls; OR, odds ratio; CI, confidence interval.

risk of GC. In addition, in our age-stratified analysis, TT genotype and T allele of rs184432 were associated with a decreased risk of GC and diffuse-type GC in subjects aged < 60 yr. This stratified analysis elucidated that rs184432 SNP is more protective against diffuse-type GC than GC in subjects aged < 60 yr. Furthermore, our stratified study on lymph node metastasis revealed that CT or TT genotypes, and T allele of rs184432 SNP were associated with a reduced risk of lymph node metastasis-negative GC and negative diffuse-type GC. The TT genotype and T allele of rs225359 promoter SNP were associated with a decreased risk of lymph node metastasis-negative GC and negative diffuse-type GC. We demonstrated that the genetic variation at rs184432 and rs225359 may have a protective effect only on lymph node metastasis-negative GC and negative diffuse-type GC. More recently, a study reported that rs3814896 SNP of *TFF2* and rs9981660 SNP of *TFF3* selected from a meta-analysis of the Chinese Han Beijing ethnic group were associated with a decreased risk of GC in a Chinese population (33). Nevertheless, although a positive association of *TFF2* and *TFF3* has been reported in the Chinese population, we did not detect any association between *TFF2* and *TFF3* polymorphisms and the risk of GC in the studied Korean population (Supplementary Table 1). In our haplotype analysis of 8 SNPs of the *TFF* family, no statistical association between haplotypes and the risk of cancer was found ($P > 0.05$) (data not shown).

Our study has some limitations. First, the sample size was inadequate for stratified analysis and for analyzing the association in mixed-type GC patients. Second, although *Helicobacter pylori* is an independent risk factor (36, 37), we did not investigate the relevance of *TFF* polymorphism for *H. pylori* in GC owing to some ethical considerations. Third, we did not investigate whether genetic factors influence smoking, drinking, and diet associated with GC risk due to the lack of data from the GC and control groups. In our future study, the effect of these factors on GC risk will need to be assessed.

In conclusion, our data suggest that single nucleotide change of the rs184432 and rs225359 promoter SNPs of *TFF1* might be associated with the susceptibility of diffuse-type GC in the Korean population. However, further functional studies are necessary to clarify the effect of rs184432 and rs225359 polymorphisms on *TFF1* gene expression and research in other ethnic groups with larger sample size is recommended to confirm our findings.

DISCLOSURE

The authors have no conflicts of interest to disclose.

AUTHOR CONTRIBUTION

Designed the experiment: Jin EH, Lee SI, Hur GM, Hong JH. Performed the experiments and analyzed the data: Jin EH, Lee SI. Contributed reagents/materials/analysis tools: Kim JW, Seo EY, Lee SY, Shin S. Wrote the manuscript and final decision to submit for publication: Jin EH, Lee SI, Hong JH. All authors read and approved the final manuscript.

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Supplementary Table 1. Genotype and allele frequencies of *TFF2* and *TFF3* polymorphisms among gastric cancer patients and controls and their association with gastric cancer risk

SNPs	Genotype	Controls	GC vs. CON		
		N (%)	N (%)	OR (95% CI)	*P* value
TFF2					
rs3814896	TT	288 (72.7)	283 (75.1)	1.00 (ref.)	
	TC	94 (23.8)	84 (22.2)	0.91 (0.65-1.27)	0.581
	CC	14 (3.5)	10 (2.7)	0.73 (0.32-1.66)	0.450
Allele	T	670 (84.6)	650 (86.2)	1.00 (ref.)	
	C	122 (15.4)	104 (13.8)	0.88 (0.66-1.17)	0.370
TFF2					
rs13052596	GG	183 (46.2)	190 (50.4)	1.00 (ref.)	
	GT	166 (41.9)	145 (38.5)	0.84 (0.62-1.14)	0.261
	TT	47 (11.9)	42 (11.1)	0.86 (0.54-1.37)	0.525
Allele	G	532 (67.2)	525 (69.6)	1.00 (ref.)	
	T	260 (32.8)	229 (30.4)	0.89 (0.72-1.11)	0.300
TFF2					
rs225334	CC	208 (52.5)	215 (57.0)	1.00 (ref.)	
	CT	153 (38.7)	142 (37.7)	0.90 (0.67-1.21)	0.478
	TT	35 (8.8)	20 (5.3)	0.55 (0.31-0.99)	0.046
Allele	C	569 (71.8)	572 (75.9)	1.00 (ref.)	
	T	223 (28.2)	182 (24.1)	0.81 (0.65-1.02)	0.073
TFF3					
rs225362	TT	376 (94.9)	363 (96.3)	1.00 (ref.)	
	TC	20 (5.1)	14 (3.7)	0.73 (0.36-1.46)	0.367
	CC	0 (0)	0 (0)	-	-
Allele	T	772 (97.5)	740 (98.1)	1.00 (ref.)	
	C	20 (2.5)	14 (1.9)	0.73 (0.37-1.46)	0.372

SNPs, single nucleotide polymorphisms; GC, gastric cancer; CON, controls; OR, odds ratio; CI, confidence interval.

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