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The Significant Contribution of Interleukin-16 Genotypes, Smoking, Alcohol Drinking, and *Helicobacter Pylori* Infection to Gastric Cancer

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Abstract. *Background/Aim: Elevated serum interleukin-16 (IL-16) levels have been reported in gastric cancer (GC) tissues; however, the role of IL-16 genotypes in GC susceptibility remains largely unexplored. This study aimed to investigate the contribution of IL-16 genotypes to GC susceptibility and to assess their interactions with smoking, alcohol drinking, and Helicobacter pylori (H. pylori) infection. Materials and Methods: Polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP) methodology was employed to determine IL-16 rs4778889, rs11556218, and rs4072111 genotypic characteristics in 161 patients with GC and 483 controls. Results: Significant differences were observed in the distribution of genotypic (p=0.0009) and allelic (p=0.0002) frequencies of IL-16 rs11556218 among cases and controls. Specifically, the frequencies of TG and GG genotypes of IL-16 rs11556218 were 37.3% and 6.8% among patients with GC, respectively, which were higher than those among the controls (26.7% and 2.7%). In contrast, no significant differences were found concerning IL-16 rs4778889 or rs4072111. Notably, individuals with IL-16 rs11556218 TT genotypes exhibited significant protective effects against GC when exposed to risk factors, such as smoking, alcohol drinking, and H. pylori infection. Conclusion: IL-16 rs11556218 T allele was associated with reduced susceptibility to GC. Furthermore, carriers of the TT genotype showed protection against GC risk factors, including smoking, alcohol drinking, and H. pylori infection. These findings provide valuable insights into the potential role of IL-16 genotypes in GC development and their interactions with lifestyle and infectious factors.*

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In spite of its declining trends in incidence and mortality rates, gastric cancer (GC) remains the fifth most common cancer worldwide (1, 2). Approximately two-thirds of GC cases occur in developing countries, with up to three-quarters of cases concentrated in Asia (3, 4). The detailed mechanisms of gastric carcinogenesis remain largely unknown but are believed to be complex. Generally, Asian countries exhibit higher incidence and mortality rates compared to other global regions, with over 60% of GC cases recently reported in Eastern Asia (5). Common risk factors for GC include Helicobacter pylori (*H. pylori*) infection, genetic alterations, dietary habits, smoking behaviors, and heavy alcohol consumption (6, 7). However, the role of inherited genetic variations in determining individual susceptibility to GC remains largely unrevealed (8-11). In recent decades, with the decoding of the human genome, numerous studies have been conducted to investigate the associations of specific genes with the risk of GC (10, 12-16).

Interleukin-16 (IL-16) is a cytokine known as lymphocyte chemoattractant factor (17). It is encoded by the IL-16 gene located on chromosome 15q26.3 and consists of 631 amino acids. Caspase 3 cleaves IL-16 into its active form, comprising the C-terminal 121 amino acids (18-20). When IL-16 binds to the CD4 protein, it activates CD4+ T cells, monocytes, macrophages, eosinophils, and dendritic cells, leading to the secretion of inflammatory cytokines, such as IL-1β (21), TNF-α, and IL-15 (22). Interestingly, elevated IL-16 has been observed in various types of cancer tissues both *in vitro* and *in vivo* (23-28), including GC (29). In 2017, elevated serum IL-16 levels were found in patients with GC compared to healthy controls, and these elevated levels were significantly associated with tumor recurrence and poor prognosis (30). Furthermore, a genome-wide association study reported that *IL-16* genotype might serve as a practical marker for prostate cancer prediction (31). In 2009, another study indicated that the *IL-16* rs11556218 T/G variant was significantly associated with the risk of colorectal cancer and GC in both male and female patients (29). In the same study, female carriers of the T allele at *IL-16* rs4072111 had a lower risk of developing colorectal cancer and GC compared to those carrying the C allele (29). Additionally, in 2013, *IL-16* rs4778889 CC and rs11556218 GG genotypes were found to be associated with an increased GC risk in another Chinese population (32). However, *IL-16* rs4072111 or rs1131445 genotypes were not associated with GC risk in the same study (32). In 2016, *IL-16* rs4778889 were found not to associate with GC risk in another Chinese population (33). On the contrary, in the same year, the rs1131445 and rs4072111 variants of *IL-16* were significantly associated with an increased risk of GC in an Iranian population (34).

As shown above, despite the significance of serum IL-16 as a potential marker in GC pathogenesis, there have been limited investigations into the association between *IL-16* genotypes and GC. In light of these observations, this study aimed to evaluate whether the rs4778889, rs11556218, and rs4072111 polymorphisms of *IL-16* are associated with the individual risk of GC in a representative Taiwanese population. Additionally, we also examined the combined effect of smoking, alcohol consumption, *H. pylori* infection status, and *IL-16* genotypes on GC risk.

Table I. *Selected characteristics of the control and gastric cancer groups.*

Characteristic	GC Cases (n=161)	Controls (n=483)	*p*-Value[a]
Age (SD)	53.7 (12.4)	52.0 (8.6)	0.3519
Sex (female/male)	72/89	215/267	1.0000
BMI average (SD)	25.4 (2.7)	25.3 (1.9)	0.5015
Cigarette consumption			
Non-smokers	102 (63.4)	400 (82.8)	
Smokers (%)	59 (36.6)	83 (17.2)	0.0001*
Heavy smokers (%)[c]	18 (11.2)	14 (2.9)	0.0001*
Alcohol consumption			
Non-drinkers (%)	106 (65.8)	382 (79.1)	
Drinkers (%)	55 (34.2)	101 (20.9)	0.0010*
Heavy drinkers (%)[b]	20 (12.4)	19 (3.9)	0.0001*
H. pylori infection			
Not infected (%)	49 (30.5)	256 (53.0)	
Infected (%)	112 (69.5)	227 (47.0)	0.0001*
Tumor location			
Upper (%)	23 (14.2)		
Middle (%)	69 (42.9)		
Lower (%)	69 (42.9)		

[a]*p*-value based on χ^2 test; [b]Drunk more than twice weekly or more than 100 ml per day for at least half year; [c]More than 1 pack per day for at least half year. BMI: Body mass index. *Statistically significant.

Materials and Methods

Investigated GC controls and cases. A total of 161 patients diagnosed with GC were recruited from the outpatient clinics of General Surgery at the China Medical University Hospital (CMUH), as previously reported (14, 15, 35). Briefly, all participants voluntarily provided peripheral blood samples. For comparison, 483 non-cancer healthy individuals matched for age and sex were selected from the Health Examination Cohort of CMUH. The demographic characteristics, including age, sex, body mass index (BMI), smoking, alcohol drinking, *H. pylori* infection status, and histological types, are presented in Table I.

IL-16 genotyping conditions. Genomic DNA from peripheral blood leukocytes of each patient with GC and control subject was extracted using the QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan, ROC) (36, 37). Subsequently, the DNA samples were subjected to standard polymerase chain reaction (PCR) procedures, as previously described in our publications (38, 39). The primer sequences, restriction endonucleases (New England BioLabs, Ipswich, MA, USA), and PCR products after enzyme digestion for *IL-16* genotyping are summarized in Table II. Agarose gel (3%) was

Table II. *Sequences of the primers, restriction endonucleases, and adduct fragments for IL-16 rs4778889, rs11556218, and rs4072111 polymorphic genotyping in gastric cancer.*

Polymorphism	5' to 3' primer sequences	Restriction enzymes	Allelic type and adduct size (bp) after digestion
rs4778889	CTCCACACTCAAAGCCCTTT	*Ahd I*	T: 280
	CCATGTCAAAACGGTAGCCT		C: 246+34
rs11556218	GCTCAGGTTCACAGAGTGTT	*Nde I*	G: 171
	TGTGACAATCACAGCTTGCC		T: 147+24
rs4072111	CACTGTGATCCCGGTCCAGT	*BsmA I*	C: 164
	TTCAGGTACAAACCCAGCCA		T: 140+24

freshly prepared using 0.5X Tris-Borate-EDTA (TBE) buffer for electrophoresis under conditions of 100 Volts for 25 min. For the genotyping analysis, three researchers, as mentioned in the Acknowledgments, conducted the analysis independently and blindly. The results were found to be 100% consistent among the different researchers.

Statistical analyses. One hundred and sixty-one GC cases and four hundred and eighty-three controls, all of whom had complete genotypic and demographic information, were included in the final analysis. The Student's *t*-test was employed to assess the difference in age between the case and control groups. For comparisons of the distribution of *IL-16* genotypes between the case and control groups, the Pearson's Chi-square test was used. Odds ratios (ORs) and their corresponding 95% confidence intervals (CIs) were calculated to estimate the associations between *IL-16* genotypes and GC risk. Statistical significance was considered at $p<0.05$ for any comparison outcome.

Results

Table I presents the demographic characteristics of the 644 participants, comprising 161 patients with GC and 483 non-cancer healthy controls, who were included in this study. Age and sex were matched, and no significant differences were observed in age distribution ($p=0.3519$) or sex distribution ($p=1.0000$) between the two groups. However, significant differences were found in the distributions of smoking ($p<0.0001$), alcohol drinking ($p<0.0001$), and *H. pylori* infection status ($p<0.0001$) between the GC and non-cancer control groups, indicating that these factors may act as risk factors for GC in the Taiwanese population. In terms of the pathological tumor location, the majority of patients with GC (60.9%) had tumors located in the upper (14.2%), middle (42.9%), and lower (42.9%) regions, as shown in Table I.

Table III presents the distribution of genotypic frequencies for rs4778889, rs11556218, and rs4072111 in *IL-16* among all study participants. First, all genotypic frequencies for the three SNPs were in agreement with the Hardy-Weinberg equilibrium in the control group (all $p>0.05$). Second, regarding *IL-16* rs4778889, there was no significant difference observed among the genotypes (p for trend=0.7674). Specifically, the frequencies of the *IL-16* rs4778889 heterozygous variant CT and homozygous variant CC were 26.1% and 3.7% among the GC patients, respectively, which were not significantly different from those among the non-cancer controls (28.8% and 3.1%, OR=0.88 and 1.16, 95%CI=0.59-1.32 and 0.44-3.07, $p=0.6053$ and 0.9582, respectively) (Table III, top panel). Third, there was a significant difference in the distribution of *IL-16* rs11556218 genotypic frequencies between patients with GC and non-cancer controls (p for trend=0.0008) (Table III, middle panel). Specifically, the frequency of the *IL-16* rs11556218 heterozygous variant TG and homozygous variant GG among patients with GC were 37.3% and 6.8%, respectively, significantly higher than those among the non-cancer controls (26.7% and 2.7%, OR=1.76 and 3.21, 95%CI=1.20-2.59 and 1.39-7.40, $p=0.0050$ and 0.0090, respectively). When the TG+GG variants at *IL-16* rs11556218 were combined, the elevated risk for GC still existed in these combined genotypes compared with the wild-type TT genotype (OR=1.89, 95%CI=1.31-2.74, $p=0.0008$) (Table III, middle panel). Finally, for *IL-16* rs4072111, no significant difference was observed among the genotypes (p for trend=0.6595). Specifically, the frequencies of the *IL-16* rs4072111 heterozygous variant CT and homozygous variant TT among GC patients with GC were 28.6% and 4.3%, respectively, which were not significantly different from those among the non-cancer controls (28.2% and 3.3%, OR=1.04 and 1.34, 95%CI=0.70-1.54 and 0.54-3.35, $p=0.9404$ and 0.7015, respectively) (Table III, bottom panel).

The allelic frequencies of *IL-16* rs4778889, rs11556218, and rs4072111 were analyzed to further validate the findings presented in Table III. Consistent with the conclusive results in Table III, the variant G allele at *IL-16* rs11556218 was found to be significantly associated with an elevated risk of GC when compared with the wild-type allele T (OR=1.79, 95%CI=1.32-2.42, $p=0.0002$) (Table IV). The frequencies of the T and G alleles of *IL-16* rs11556218 were 74.5% and 25.5% among patients with GC, respectively, whereas they

Table III. *Distribution of IL-16 rs4778889, rs11556218, and rs4072111 genotypes among the 161 gastric cancer cases and 483 controls.*

Genotype	Cases		Controls		Odds ratio (95%CI)	*p*-Value[a]
	n	%	n	%		
rs4778889						
TT	113	70.2%	329	68.1%	1.00 (reference)	
CT	42	26.1%	139	28.8%	0.88 (0.59-1.32)	0.6053
CC	6	3.7%	15	3.1%	1.16 (0.44-3.07)	0.9582
CT+CC	48	29.8%	154	31.9%	0.91 (0.62-1.34)	0.6949
p_{trend}						0.7674
p_{HWE}						0.9455
rs11556218						
TT	90	55.9%	341	70.6%	1.00 (reference)	
TG	60	37.3%	129	26.7%	1.76 (1.20-2.59)	0.0050*
GG	11	6.8%	13	2.7%	3.21 (1.39-7.40)	0.0090*
TG+GG	71	44.1%	142	29.4%	1.89 (1.31-2.74)	0.0008*
p_{trend}						0.0009*
p_{HWE}						0.8487
rs4072111						
CC	108	67.1%	331	68.5%	1.00 (reference)	
CT	46	28.6%	136	28.2%	1.04 (0.70-1.54)	0.9404
TT	7	4.3%	16	3.3%	1.34 (0.54-3.35)	0.7015
CT+TT	53	32.9%	152	31.5%	1.07 (0.73-1.56)	0.8071
p_{trend}						0.8159
p_{HWE}						0.6595

[a]Based on Chi-square with Yate's correction test; p_{trend}: *p*-Value for trend analysis; p_{HWE}: *p*-Value for Hardy-Weinberg Equilibrium; *Statistically significant.

Table IV. *Distributions of IL-16 rs4778889, rs11556218, and rs4072111 allelic frequencies among the gastric cancer cases and controls.*

Allele	Cases	%	Controls	%	Odds ratio (95%CI)	*p*-Value[a]
rs4778889						
Allele T	268	83.2%	797	82.5%	1.00 (reference)	
Allele C	54	16.8%	169	17.5%	0.95 (0.68-1.33)	0.8316
rs11556218						
Allele T	240	74.5%	811	84.0%	1.00 (reference)	
Allele G	82	25.5%	155	16.0%	1.79 (1.32-2.42)	0.0002*
rs4072111						
Allele C	262	81.4%	798	82.6%	1.00 (reference)	
Allele T	60	18.6%	168	17.4%	1.09 (0.79-1.51)	0.6734

[a]Based on Chi-square with Yate's correction test; *Statistically significant.

were 84.0% and 16.0% among non-cancer controls (Table IV). On the other hand, neither the variant C allele of *IL-16* rs4778889 nor the variant T allele of *IL-16* rs4072111 showed any association with altered GC risk (Table IV). The allelic frequencies of *IL-16* rs4778889 and rs4072111 did not differ significantly between patients with GC and non-cancer controls (Table IV).

As GC is one of the smoking-related types of cancer, we conducted further analysis to investigate the potential interaction between the genotype of *IL-16* rs11556218 and personal smoking behavior within the study population. The results indicated that non-smokers carrying variant TG or GG genotypes at *IL-16* rs11556218 did not exhibit any altered risk for GC (OR=0.72, 95%CI=0.46-1.14, *p*=0.1974) (Table V). In contrast, ever smokers who carried the TG or GG genotypes at *IL-16* rs11556218 were found to have a significantly elevated risk of GC (OR=4.74, 95%CI=2.47-9.09, *p*=0.0001) (Table V). However, forever smokers carrying the TT genotype at *IL-16* rs11556218, no significant difference in risk was observed between the patients with GC and non-cancer control groups (OR=1.35, 95%CI=0.75-2.42, *p*=0.3993) (Table V). No joint effect was found between *IL-*

Table V. *Association of IL-16 rs11556218 genotype and cigarette consumption for gastric cancer risk.*

IL-16 rs11556218 TT carrier	Cigarette consumption	Controls/ Cases	Odds ratio (95%CI)	*p*-Value[a]
(–)	(–)	120/38	1.0 (reference)	
(+)	(–)	280/64	0.72 (0.46-1.14)	0.1974
(–)	(+)	22/33	4.74 (2.47-9.09)	0.0001*
(+)	(+)	61/26	1.35 (0.75-2.42)	0.3993

CI: Confidence interval; [a]*p*-Value based on Chi-square test. *Statistically significant.

Table VI. *Association of IL-16 rs11556218 genotype and alcohol consumption for gastric cancer risk.*

IL-16 rs11556218 TT carrier	Alcohol consumption	Controls/ Cases	Odds ratio (95%CI)	*p*-Value[a]
(–)	(–)	117/41	1.0 (reference)	
(+)	(–)	265/65	0.70 (0.45-1.09)	0.1471
(–)	(+)	25/30	3.42 (1.81-6.49)	0.0002*
(+)	(+)	76/25	0.94 (0.53-1.67)	0.9447

CI: Confidence interval; [a]*p*-Value based on Chi-square test. *Statistically significant.

16 rs4778889 or rs4072111 genotypes and smoking on the determination of GC risk (data not shown).

In Table I, the data also indicated that alcohol drinking may be a significant environmental risk factor for GC. Hence, we further explored the genetic-environment interaction between *IL-16* rs11556218 and alcohol drinking behavior in relation to the risk of GC (Table VI). Among individuals who were non-alcohol drinkers, carriers of the variant TG or GG genotype at *IL-16* rs11556218 did not show any altered risk of GC (OR=0.70, 95%CI=0.45-1.09, p=0.1471) (Table VI). Conversely, among alcohol drinkers who carried the variant TG or GG genotypes at *IL-16* rs11556218, there was a significantly elevated risk of GC (OR=3.42, 95%CI=1.81-6.49, p=0.0002) (Table VI). However, forever alcohol drinkers carrying the TT genotype at *IL-16* rs11556218, no significant difference in risk was observed between the GC patient and non-cancer control groups (OR=0.94, 95%CI=0.53-1.67, p=0.9447) (Table VI). No joint effect was found between *IL-16* rs4778889 or rs4072111 genotypes and alcohol consumption on the determination of GC risk (data not shown).

The most intriguing aspect of our findings is the combined effects of *H. pylori* infection status and *IL-16* rs11556218 genotypes on GC risk. Among non-infected individuals, the presence of *IL-16* rs11556218 genotype was not associated with any significant alteration in the risk of GC (OR=1.68, 95%CI=0.82-3.47, p=0.2052). However, in individuals with *H. pylori* infection, there was a remarkable increase in the risk of GC among those carrying the variant TG or GG genotypes at *IL-16* rs11556218 (OR=7.90, 95%CI=3.83-16.31, p<0.0001). Notably, H. pylori-infected individuals with the TT genotype of *IL-16* rs11556218 exhibited a lower level of elevated GC risk compared to those with the TG or GG genotypes (OR=2.50, 95%CI=1.23-5.04, p=0.0223). No joint effect was observed between *IL-16* rs4778889 or rs4072111 genotypes and alcohol consumption in the determination of GC risk (data not shown).

Discussion

In the current study, we examined the association between *IL-16* genotypes and the risk of GC in a Taiwanese population, consisting of 161 patients with GC and 483 non-cancer healthy controls (Table I). The genotyping results revealed that carriers of the *IL-16* rs11556218 T allele had a reduced risk of GC (Table III and Table IV). Specifically, the variant TG and GG genotypes of *IL-16* rs11556218 served as diagnostic biomarkers for predicting an elevated risk of GC in Taiwan. Moreover, we found compelling evidence indicating that the variant genotypes of *IL-16* rs11556218 were linked to an increased GC risk among subgroups of smokers, alcohol drinkers, and individuals infected with *H. pylori* (Table V, Table VI, and Table VII). Interestingly, for the subgroups carrying the *IL-16* rs11556218 TT wild-type genotype, the risk of GC was significantly lower compared to those carrying the *IL-16* rs11556218 variant TG and GG genotypes (Table V, Table VI, and Table VII).

Among the three polymorphic sites, rs11556218 is a missense variant responsible for encoding amino acids. The wild-type T allele encodes Asn, while the variant G allele

Table VII. *Association of IL-16 rs11556218 genotype and H. pylori infection for gastric cancer risk.*

IL-16 rs11556218 TT carrier	H. pylori infection	Controls/ Cases	Odds ratio (95%CI)	*p*-Value[a]
(–)	(–)	84/11	1.0 (reference)	
(+)	(–)	172/38	1.68 (0.82-3.47)	0.2052
(–)	(+)	58/60	7.90 (3.83-16.31)	0.0001*
(+)	(+)	169/52	2.50 (1.23-5.04)	0.0223*

CI: Confidence interval; [a]*p*-Value based on Chi-square test. *Statistically significant.

encodes Lys. In a study conducted by Gao and colleagues in 2009, it was reported that the expression level of IL-16 was higher in patients with colorectal cancer and GC (29). However, to date, there is no conclusive evidence regarding the genotype-phenotype correlation, and the exact role of IL-16 in GC etiology remains unclear. Furthermore, we have not measured the serum expression levels of IL-16 in patients with GC and non-cancer controls. Determining the expression level of IL-16 could provide valuable insights into understanding the role of IL-16 in GC carcinogenesis.

In the present study, we identified the TG and GG genotypes of *IL-16* rs11556218 as potential diagnostic biomarkers for GC in Taiwan (Table III and Table IV). Our findings align with the report by Zhang *et al*. (32), which also showed a positive association between *IL-16* rs11556218 genotypes and increased GC risk. Notably, similar associations with elevated risk have been observed in several other types of cancer, including lung cancer (39), colorectal cancer (29), HBV-related hepatocellular carcinoma (40), and nasopharyngeal carcinoma (29).

In contrast to the findings of Zhang *et al*. (32), our study showed a negative association between the C allele of *IL-16* rs4778889 and GC risk (Table III and Table IV). This result is in line with the study conducted by Wang in 2016 (33), which also found no significant association. Interestingly, *IL-16* rs4778889 genotypes have been reported to be associated with renal cell carcinoma (41). Moreover, a meta-analysis examining the *IL-16* genotype and its association with several types of cancer, including renal cell carcinoma, revealed that the C allele at *IL-16* rs4778889 significantly correlates with an increased risk of renal cell carcinoma, particularly among Asian ethnicities (42).

The role of the variant T allele of *IL-16* rs4072111 in GC risk is still controversial. In 2009, it was suggested to contribute to a decreased risk of GC in a Chinese population (29). However, in 2016, a study reported that the T allele was associated with an elevated risk of GC in an Iranian population (34). In our current study, we found that the T allele of rs4072111 seemed to be neither associated with an elevated nor a decreased risk of GC (Table III and Table IV). This finding is consistent with a report from Zhang *et al*. who investigated another Chinese population (32). Another polymorphic site, rs1131445, has also been investigated for its contribution to GC risk. However, the results are conflicting, with one study suggesting a positive association (34), whereas another found no association with GC risk (32).

Smoking has been previously reported to positively contribute to GC (43, 44). In this study, we further analyzed the interaction between the genotype of *IL-16* rs11556218 and cigarette smoking status. The results revealed that the risk of GC was significantly elevated among smokers without TT genotypes of *IL-16* rs11556218 (Table V). Conversely, smokers carrying the protective TT genotypes of *IL-16* rs11556218 exhibited a non-significant level of GC risk, with the odds ratios (ORs) decreasing from 4.74 to 1.35 (Table V). Similar findings were observed in alcohol drinkers (Table VI). However, the most intriguing finding is the interaction between *IL-16* rs11556218 genotype and *H. pylori* infection status. The data demonstrated that individuals infected with *H. pylori* carrying *IL-16* rs11556218 TT genotype had an extremely high OR (7.90) of GC risk, which was significantly reduced to 2.5 among individuals infected with *H. pylori* carrying the TT genotype. Unlike smokers or alcohol drinkers, the protective effects of *IL-16* rs11556218 TT genotype were not strong enough to mitigate the risk to a non-risk level for individuals infected with *H. pylori*. These results indicate that *IL-16* rs11556218 genotype not only determines personal susceptibility to GC but also regulates the risk for smokers, alcohol drinkers, and those infected with *H. pylori* with respect to GC. The mechanisms underlying the role of IL-16 in personal immunology are intriguing and warrant further investigation.

In conclusion, this study provides compelling evidence that the variant TG and GG genotypes of *IL-16* rs11556218 are associated with an increased risk of GC among Taiwanese individuals, particularly among smokers, alcohol drinkers, and those infected with *H. pylori*. However, further investigations are warranted to explore the protective role of *IL-16* rs11556218. Additionally, conducting more genotyping studies on *IL-16* could help to validate our findings and potentially establish *IL-16* rs11556218 T allele as a protective marker for GC risk.

Conflicts of Interest

All the Authors declare no conflicts of interest regarding this study.

Authors' Contributions

Research design: Fu CK, Mong MC, Bau DT; patient and questionnaire summaries: Fu CK, Yang MD, Tzeng HE, Hsia TC, Hsia NY, Chen CP; experimental supervision: Chang WS, Tsai CW; data recognition: Fu CK, Tsai CW, Chen CP; statistical analysis: Mong MC, Tzeng HE, Chen JC; manuscript writing: Chen CP, Bau DT; review & revision: Bau DT, Chang WS, Tsai CW.

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