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ORIGINAL RESEARCH

A Variant of *Leptin* Gene Decreases the Risk of Gastric Cancer in Chinese Individuals: Evidence from a Case–Control Study

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Background: A host of studies have explored the potential connection between *leptin* (*LEP*) G19A polymorphism and the risk of cancers, but the relationship between gastric cancer (GC) susceptibility and *LEP* G19A polymorphism was not revealed before. The aim of this study was to investigate this relationship in Chinese Han population.

Methods: Thus, this case–control study with 380 GC cases and 465 controls was designed to unearth the link between *LEP* G19A polymorphism and GC susceptibility. Genotyping was accomplished by a custom-made 48-Plex SNP scanTM kit. Relative *LEP* gene expression was detected by real-time reverse transcription-polymerase chain reaction.

Results: *LEP* G19A polymorphism was shown to relate with a decreased risk of GC. Subgroup analyses uncovered significant connections in the males, nondrinkers, and those at age <60 years. G19A polymorphism was also linked with tumor size and location and pathological type of GC. Last, *LEP* gene expression in gastric tissues was considerably less than in control tissues.

Conclusion: This study shows that G19A polymorphism of *LEP* gene is linked with a lower risk of GC in the tested Chinese Han individuals.

Keywords: *LEP*, gastric cancer, G19A polymorphism, case-control study

Introduction

Gastric cancer (GC) is the 3th dominant cause of cancer mortality and has the 5th highest incidence among cancers worldwide.[1] The GC incidence and mortality rates are the highest in East Asia, and about 679,100 new GC patients and 498,000 cancer-associated deaths were reported in 2015 in China.[2] GC has two anatomical types: gastro-oesophageal-junction adenocarcinoma and true gastric adenocarcinoma,[3] and is histologically separated into diffuse and intestinal types. GC is a multi-step complex disorder due to both environmental and genetic factors.[4] Tobacco smoking, alcohol consumption, *Helicobacter pylori* (HP) infection, and unhealthy dietary habits including low consumption of vegetables and fruits can induce the risk of GC.[5–7] Genetic factors are also critical in GC progression. Genome-wide association research has recognized several gene polymorphisms are linked with the risk of GC.[8–10]

Leptin (LEP), a hormone of energy expenditure, is secreted by white adipose tissues and is associated with endocrinologic metabolism.[11] It is expressed in the hypothalamus and regulates appetite and energy expenditure.[12] LEP is engaged in energy homeostasis, insulin signaling, immune response, and inflammation.[13–15]

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Reduced sensitivity to LEP may result in the development of metabolic disorders, such as cancers.[16] LEP and its receptor are involved in various carcinogenesis-related signal pathways, including MAPK, PI3K, mTOR, and JAK/STAT.[17,18] Increased LEP level is involved in the development of many malignancies.[19–21] As for GC, LEP plays an important role in GC via stimulating the proliferation of GC cells through activating the ERK1/2 and STAT3 pathways.[22] LEP can also enhance GC cell migration by increasing ICAM-1.[23] Recombinant human LEP could induce apoptosis and inhibit growth in human GC cell lines.[24] Gastric LEP performed diverse functions including nutrient absorption and tumorigenesis in the gastrointestinal system.[25] LEP could induce the expression of tumorigenic genes in the gastric mucosa of male rats.[26] Dietary fat-accelerating LEP signaling was shown to promote protumorigenic gastric environment in mice.[27] Overexpression of LEP was linked with the development of GC in humans and murine.[25,28] Serum LEP levels were related with insulin resistance in GC patients[29] and may be a valuable diagnostic indicator.[30] LEP is connected with chemotherapy resistance and therapy-independent prognosis of GC.[31] Besides, LEP was correlated with the progression and prognosis of GC patients. [32]

G19A polymorphism, a SNP in the 5′-untranslated region of *LEP* gene, may impact RNA transcription, translation and steadiness, and change LEP protein expression. This polymorphism was correlated to cancer risk.[33–45] However, the existing findings in different types of cancers are discrepant. Furthermore, no researchers have studied the relationship between G19A polymorphism and the risk of GC. To explore such potential relationships, we performed this study to explore this relationship in Chinese Han subjects.

Patients and Methods

Subjects

Totally 380 newly identified GC cases and 465 age- and gender-matched controls were enrolled from Danyang People's Hospital (Jiangsu, China) from March 2016 to January 2020. All GC patients received surgery treatment. GC tissues and normal tissues were obtained during surgery, which were stored at −80°C after surgery. GC was diagnosed according to pathological examination results. Exclusion criteria for GC patients included: 1) a history of other cancers; 2) patients with gastritis; 3) patients with gastric ulcers; 4) patients not providing enough data and written informed consent. The age and gender-matched control individuals receiving health examinations were recruited from the hospital at the same period. Exclusion criteria for controls were as follows: patients 1) with a history of cancers; 2) with metabolic diseases; 3) with autoimmune diseases. 4) receiving no gastroscopy or having symptoms of gastritis. The demographic characteristics and lifestyle habits of all individuals were collected by a structured questionnaire. Medical records provided the clinical data of GC patients. All subjects completed a C-urea breath test to measure whether they had HP infection. The gastric tissues for RT_qPCR were not fresh because these tissues did not be used immediately after surgery. All individuals signed written informed consent in this study. Approval was obtained from the Ethics Committee of this Hospital (number: 20,200,618), and the Declaration of Helsinki was followed.

Genotyping

The blood samples of participants were stored at −80°C immediately. DNA sample was from peripheral leukocytes using a TIANamp blood DNA kit (Qiagen, Hilden, Germany). *LEP* G19A polymorphism was genotyped using a custom-by-design 48-Plex SNP scanTM kit (Genesky Biotechnologies Inc., Shanghai, China). Each PCR system (50 μL) contained 5 μL of 10×PCR buffer for KOD-Plus-Neo, 1 μL of upstream and downstream primers, 34 μL of ddH_2O, 1 μL of temple, 2 mM dNTPs (5 μL), 1 μL of cDNA, 25 mM MgSO4 (3 μL), and 1 μL of KOD-Plus-Neo (TOYOBO, Japan). Reaction conditions were 95 ºC, 5 min; 94 ºC, 30 s, 50 ºC, 30 s; 72 ºC, 1 min, 35 cycles; extension at 72 ºC, 10 min, cooling to 4 ºC. The PCR products were digested with BglI (New England Biolabs, Beverly, MA) at 37°C for 5 h and then sent to 2% agarose gel electrophoresis. About 10% of the samples were repeatedly genotyped, and the concordance of genotypes was 100%.[46,47]

Reverse-Transcription Polymerase Chain Reaction (RT-PCR)

The gastric tissues of participants were stored at −80°C. Total RNA was isolated from the gastric tissues from all subjects using the Trizol reagent (Invitrogen, CA, USA) and was reverse-transcribed with a relevant kit (TaKaRa, Shiga, Japan) as instructed by the manufacturers. The SYBR Green PCR master mix (TaKaRa, Otsu, Shiga, Japan) was used for real-time PCR. Forward and reverse

primers used for PCR were: 5′-CGTTAAGGGAAG GAACTCTGG-3′, 5′-TTGATGGCTGAAGACCTTGG-3′ (LEP); 5′-GATGAGATTGGCATGGCTTT-3′, 5′-GTCAC CTTCACCGTTCCAGT-3′ (β-actin). The PCR condition was: an initial 94°C for 2 min; 35 cycles, 94°C, 30 s, 60°C, 25 s, and 72°C, 30 s; final extension at 72°C, 10 min. The relative expression of *LEP gene* was calculated using the $2^{-\Delta\Delta CT}$ method.

Statistical Analysis

Descriptive variables of GC patients and controls were shown as the mean and standard deviations for continuous variables, and frequencies and percentages for categorical variables. The Chi-square test was applied to compare the case–control differences in the distributions of genotypic frequencies and categorical variables. Student's *t*-test was adopted for continuous variables. The Hardy–Weinberg equilibrium (HWE) test was examined among the controls for *LEP* G19A polymorphism. The odds ratios (ORs) and 95% confidence interval (CI) were computed via logistic regression.[48] Five genetic models including dominant model, homozygote model, recessive model, heterozygote model, and allele model were utilized in this study. Stratified analyses by sex, age, smoking and alcohol were done. We addressed the association of *LEP* G19A polymorphism with clinical features of GC patients. $P < 0.05$ was the significance level. All data analyses were finished on SPSS 22.0 (SPSS Inc., USA).

Results

Population Characteristics

A flowchart of patient enrollment is shown in Figure 1. Totally 380 GC patients and 465 controls were involved. Clinical data are listed in Table 1. Data showed no remarkable discrepancy between cases and controls in age, sex, alcohol, or smoking. The percentage of HP infection was higher in GC patients than the controls. Additionally, *LEP* gene level in gastric cancer tissues versus normal tissues was significantly lower (Figure 2).

Association of *LEP* G19A Polymorphism with GC Risk

LEP G19A polymorphism was in line with HWE ($P > 0.05$). Data found that the AA genotype (OR: 0.50; 95% CI: 0.26–0.97; $P = 0.040$) or A allele (OR: 0.73; 95% CI: 0.58–0.93; $P = 0.009$) of G19A polymorphism showed a less risk for GC (Table 2). These associations hold true even after adjusting for age and sex. Stratified analyses demonstrated that this polymorphism was linked to a lower risk of GC patients among the non-drinkers, males, and those at age <60 years (Table 3). No significant associations were obtained in subgroup analysis of smoking and HP status.

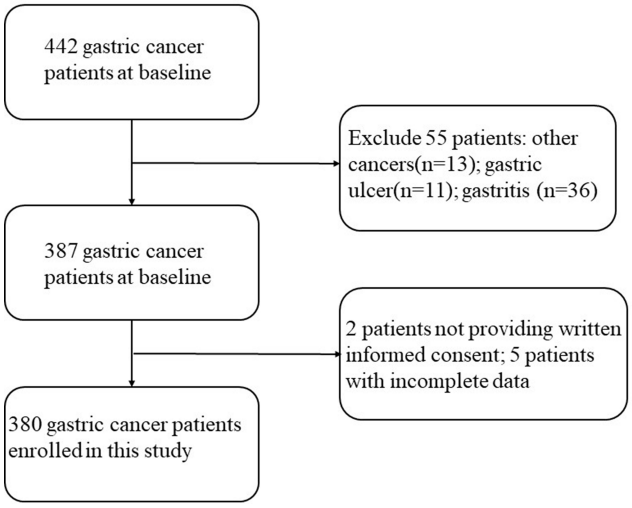

Figure 1 The flowchart of patient enrollment.

Relationship Between *LEP* G19A Polymorphism and Clinical Features of GC

Last, we addressed the relationship between G19A polymorphism of *LEP* gene and GC clinical features (Table 4). The SNP was related to tumor size, cardia GC and adenocarcinoma, but not to histological grade, R classification, or TNM stage.

Discussion

In this case–control study, we observed that *LEP* G19A polymorphism was correlated with less risk for GC in Chinese subjects. Next, this polymorphism can lower the risk of GC patients among non-drinkers, males, and those at age <60 years. In addition, *LEP* G19A polymorphism showed connection with tumor size, cardia GC and adenocarcinoma. Last, *LEP* gene level in gastric tissues was remarkably lower than in normal tissues.

Some recent case–control studies have probed into the possible relationship between *LEP* G19A polymorphism and the risk of various cancers. Tsilidis et al observed no association between this locus and CRC susceptibility in a Caucasian population from the USA.[33] Later, a study with 1567 cases and 1965 controls from the USA indicated

Table 1 Patient Demographics and Risk Factors for Gastric Cancer

Characteristics	Case (N=380)	Control (N=465)	*P*
Age	57.51 (34–89)	55.94 (31–92)	0.064
Sex			0.893
Male	196(51.6%)	242(51.6%)	
Female	184(48.4%)	223(48.4%)	
Smoking			0.817
Yes	191(50.3%)	230(36.6%)	
No	189(49.7%)	235(63.4%)	
Alcohol			0.361
Yes	213(56.1%)	246(42.0%)	
No	167(43.9%)	219((58.0%)	
H. pylori			**<0.001**
Seronegative	68(17.9%)	191(44.3%)	
Seropositive	312(82.1%)	274(55.7%)	
R classification			
R0	101(26.6%)		
R1	174(45.8%)		
R2	105(27.6%)		
Lauren classification			
Intestinal	142(37.4%)		
Diffuse	220(57.9%)		
Mixed	18(4.7%)		
Histological grade			
Well differentiated	66(17.4%)		
Moderately differentiated	197(51.8%)		
Poorly differentiated	117(30.8%)		
Location			
Cardia	131(34.5%)		
Non-cardia	249(65.5%)		
TNM			
I+II	129(33.9%)		
III+IV	251(66.1%)		
Tumor size			
>4 cm	248(65.3%)		
≤4 cm	132(34.7%)		
Histology			
Adenocarcinoma	345(90.8%)		
Not Adenocarcinoma	35(9.2%)		

Note: Bold values are statistically significant ($P < 0.05$).
Abbreviations: TNM, tumor node metastasis; R0, no cancer infiltration at the margin; R1, microscopic cancer infiltration; R2, macroscopic cancer infiltration.

that G19A polymorphism of *LEP* gene was related with lower risk for colon cancer.[34] Partida-Perez et al suggested that this SNP was associated with colorectal cancer risk in

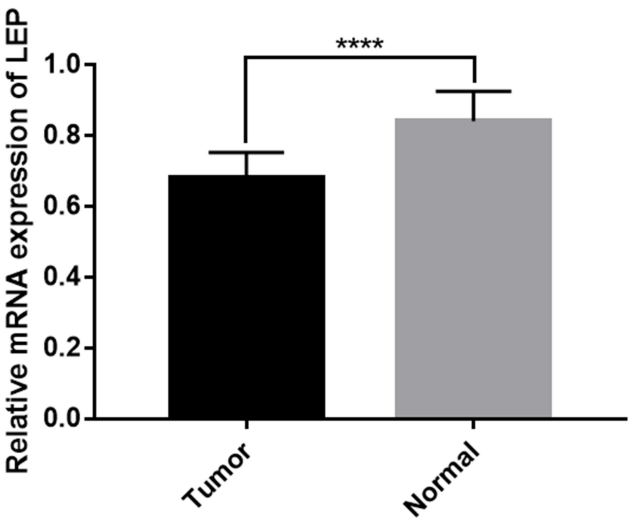

Figure 2 The *LEP* mRNA expression in gastric tissues and normal tissues. ***The *LEP* mRNA expression in gastric tissues was significantly lower than those in normal tissues ($P < 0.001$).
Abbreviation: LEP, leptin.

Mexican patients.[35] As for esophageal cancer, an Australian study showed *LEP* G19A polymorphism was inactive for esophageal carcinogenesis,[36] which was in line with the findings of a Chinese study.[37] However, another Chinese study indicated that *LEP* G19A polymorphism contributed to less risk for esophagogastric junction adenocarcinoma.[38] As for prostate cancer, two studies obtained it was not heavily associated with *LEP* G19A polymorphism.[39,40] Next, several studies addressing the relationship between non-Hodgkin's lymphoma (NHL) risk and this polymorphism yielded conflicting findings. Willett et al suggested that G19A polymorphism was a protective factor for NHL;[41] Skibola et al found this polymorphism increased the risk of NHL.[42] Nevertheless, a Chinese study revealed that G19A polymorphism was not associated with susceptibility to NHL.[43] In addition, Kim et al[44] observed no connection between G19A polymorphism and breast cancer risk and Zhang et al showed this SNP was associated with the risk of hepatocellular carcinoma.[45] Obviously, these studies obtained inconsistent results in different cancers regarding *LEP* G19A polymorphism. Some factors may account for paradoxical results. First, genetic heterogeneity existed in different cancers, and *LEP* G19A polymorphism may be a specific locus for some cancers. Second, clinical heterogeneity and third, the varying sample sizes may contribute to it. Fourth, different races are neglectable. Fifth, exposure factors and grade malignancy of cancers are different.

Table 2 Genotype Frequencies of *LEP* G19A Polymorphism in Cases and Controls

Models	Genotype	Case (n, %)	Control (n, %)	OR (95% CI)	*P*-value	*OR (95% CI)	**P*-value
Co-dominant	GG	245(64.6%)	263(56.8%)	1.00	-	1.00	-
Heterozygote	GA	120(31.7%)	170(36.7%)	0.76(0.57–1.01)	0.062	0.75(0.56–1.01)	0.058
Homozygote	AA	14(3.7%)	30(6.5%)	**0.50(0.26–0.97)**	**0.040**	**0.49(0.26–0.96)**	**0.036**
Dominant	GG	245(64.6%)	263(56.8%)	1.00	-	1.00	-
	AA+GA	134(35.4%)	200(43.2%)	0.55(0.29–1.06)	0.074	0.55(0.29–1.05)	0.069
Recessive	GA+GG	365(96.3%)	433(93.5%)	1.00	-	1.00	-
	AA	14(3.7%)	30(6.5%)	**0.72(0.54–0.95)**	**0.021**	**0.72(0.54–0.95)**	**0.019**
Allele	G	610(80.5%)	696(75.2%)	1.00	-	-	-
	A	148(19.5%)	230(24.8%)	**0.73(0.58–0.93)**	**0.009**	-	-

Notes: Bold values are statistically significant ($P < 0.05$); The genotyping was successful in 380 cases and 465 controls; *Adjust age and sex.
Abbreviations: OR, odds ratio; CI, confidence interval.

Table 3 Stratified Analyses Between *LEP* G19A Polymorphism and the Risk of Gastric Cancer

Variable	(Case/Control)			GA vs GG	AA vs GG	AA vs GG+GA	AA+GA vs GG
	GG	GA	AA	OR (95% CI); *P*	OR (95% CI); *P*	OR (95% CI); *P*	OR (95% CI); *P*
Sex							
Male	127/134	61/86	8/21	0.75(0.50–1.13); 0.036	**0.40(0.17–0.94); 0.036**	0.45(0.19–1.03); 0.059	0.68(0.46–1.00); 0.052
Female	118/129	59/84	6/9	0.77(0.51–1.16); 0.214	0.73(0.25–2.11); 0.560	0.80(0.28–2.30); 0.168	0.76(0.51–1.14); 0.191
Smoking							
Yes	120/127	61/84	10/17	0.77(0.51–1.16); 0.212	0.62(0.27–1.41); 0.257	0.69(0.31–1.54); 0.359	0.74(0.50–1.10); 0.140
No	125/136	59/86	4/13	0.75(0.50–1.13); 0.163	0.34(0.11–1.05); 0.061	0.37(0.12–1.16); 0.088	0.69(0.47–1.03); 0.071
Alcohol							
Yes	130/135	74/98	9/13	0.78(0.53–1.15); 0.217	0.72(0.30–1.74); 0.464	0.79(0.33–1.89); 0.597	0.78(0.54–1.12); 0.183
No	115/128	46/72	5/17	0.71(0.46–1.11); 0.135	**0.33(0.12–0.92); 0.033**	0.37(0.13–1.01); 0.053	**0.64(0.42–0.98); 0.039**
Age (years)							
<60	149/169	66/109	5/17	0.69(0.47–1.00); 0.051	**0.33(0.12–0.93); 0.035**	0.38(0.14–1.05); 0.061	**0.64(0.44–0.92); 0.016**
≥60	96/94	54/61	9/13	0.88(0.55–1.38); 0.546	0.68(0.28–1.66); 0.395	0.72(0.30–1.72); 0.445	0.83(0.54–1.30); 0.418
H. pylori							
Seropositive	200/158	99/100	12/15	0.78(0.55–1.11); 0.166	0.63(0.29–1.39); 0.253	0.69(0.32–1.50); 0.349	0.76(0.55–1.07); 0.112
Seronegative	45/105	21/70	2/15	0.70(0.38–1.28); 0.244	0.31(0.07–1.42); 0.131	0.35(0.08–1.59); 0.175	0.63(0.35–1.13); 0.119

Note: Bold values are statistically significant ($P < 0.05$).
Abbreviations: OR, odds ratio; CI, confidence interval.

We firstly found that *LEP* G19A polymorphism was connected to decreased risk for GC. Data suggested that AA genotype or A allele carriers decreased the risk of GC. However, another study observed that AA genotype was a risk factor for other cancers.[45] We assumed that *LEP* G19A polymorphism may play diverse roles in different cancers. To our knowledge, this is the first study to uncover the significant link between this SNP and GC susceptibility. Savino et al indicated *LEP* G19A polymorphism was linked with LEP down-expression.[49] In addition, serum LEP downregulation may be a protective factor and predict good prognosis for breast cancer patients.[50] Thereby, we assume that lower serum LEP levels related to genotypes of *LEP* G19A polymorphism may contribute to decreased risk for GC. However, further studies are needed to verify this assumption. Furthermore, subgroup analysis found that *LEP* G19A polymorphism was linked with a less risk for GC patients

Table 4 The Associations Between *LEP* G19A Polymorphism and Clinical Characteristics of Gastric Cancer

Characteristics	Genotype Distributions			
	GG	**GA**	**AA**	**GA+AA**
Histological grade WD/PD OR (95% CI); *P*-value	41/76 1.0 (reference)	24/35 1.27(0.67–2.42); 0.465	1/6 0.31(0.036–2.65); 0.260	25/41 1.13(0.61–2.11); 0.701
Histological grade MD/PD OR (95% CI); *P*-value	128/76 1.0 (reference)	61/35 1.04(0.63–1.71); 0.894	7/6 0.69(0.22–2.14); 0.521	80/129 0.91(0.54–1.55); 0.729
R classification R1/R2 OR (95% CI); *P*-value	114/64 1.0 (reference)	55/36 0.86(0.51–1.44); 0.563	5/5 0.56(0.16–2.01); 0.370	60/41 0.82(0.50–1.36); 0.442
R classification R0/R2 OR (95% CI); *P*-value	67/64 1.0 (reference)	29/36 0.77(0.42–1.40);0.389	4/5 0.76(0.20–2.97); 0.697	33/41 0.77(0.43–1.36); 0.368
Location Cardia/Non-cardia OR (95% CI); *P*-value	96/149 1.0 (reference)	34/86 **0.61(0.38–0.98); 0.042**	1/13 **0.12(0.02–0.93); 0.016**	35/99 **0.55(0.35–0.87); 0.011**
TNM I+II/III+IV OR (95% CI); P-value	85/160 1.0 (reference)	40/80 0.94(0.59–1.49); 0.800	4/10 0.75(0.23–2.47); 0.639	44/90 0.92(0.59–1.44); 0.715
Tumor size >4 cm/≤4 cm OR (95% CI); P-value	161/84 1.0 (reference)	83/37 1.17(0.73–1.87); 0.510	4/10 **0.21(0.06–0.69); 0.005**	87/47 0.97(0.62–1.50); 0.877
Metastasis M1/M0 OR (95% CI); P-value	29/216 1.0 (reference)	14/106 0.98(0.50–1.94); 0.962	3/11 2.03(0.54–7.71); 0.289	17/117 1.08(0.57–2.05); 0.809
Histology Adenocarcinoma/NOT OR (95% CI); P-value	230/15 1.0 (reference)	104/16 **0.42(0.20–0.89); 0.020**	10/4 **0.16(0.05–0.58); 0.002**	114/20 **0.37(0.18–0.75); 0.005**

Note: Bold values are statistically significant ($P < 0.05$).

Abbreviations: OR, odds ratio; CI, confidence interval; TNM, tumor node metastasis; PD, poorly differentiation; MD, moderately differentiation; WD, well differentiation; R0, no cancer infiltration at the margin; R1, microscopic cancer infiltration; R2, macroscopic cancer infiltration.

among the males, non-drinkers, and those aged <60 years, suggesting that those individuals are less susceptible to GC. On the contrary, the females, drinkers, and those aged >60 years may be prone to GC. From the view of GC management or prevention, those groups need to receive gastroscopy or other early examinations, which could help with early intervention on the occurrence of GC. Analyses concerning the relationship between *LEP* G19A polymorphism and clinical characteristics of GC patients found that this SNP was linked with the tumor size, cardia GC and adenocarcinoma. Furthermore, AA genotype was a protective factor for GC patients with smaller tumor size, non-cardia, and non-adenocarcinoma.

Limitations of this study existed. First, the relatively small sample size may yield false-positive results. Second, we did not investigate other SNPs of *LEP* gene. Third, gene-environment or gene–gene interaction was ignored. Fourth, the underlying mechanisms of the *LEP* G19A polymorphism in GC risk should be discovered. Fifth, we did collect the follow-up data of GC patients previously. Thus, we could not explore the correlation between GC prognosis and *LEP* G19A polymorphism at recent stage. Last, our findings should be validated in other populations in China and other races.

In sum, *LEP* G19A polymorphism is linked with a lower risk of GC in Chinese individuals. Overall, our

findings may be generally helpful for early screening of individuals at high-risk of GC in Chinese Han population. Further studies are needed, which will help to comprehensively elucidate the potential role of *LEP* G19A polymorphism in the pathogenesis of GC.

Disclosure

The authors report no conflicts of interest in this work.

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