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ORIGINAL ARTICLE

Functional Genetic Variants in *SPHK1* Affect Susceptibility to Gastric Cancer in a Chinese Population

Xiangkun Huan [1, *], Yu He [1, *], Chi Huang [1, *], Younan Wang [1, *], Yi Zhu [2], Zekuan Xu [1], Li Yang [1], Huaxiang Cao [3]

[*] *These authors contributed equally to this work*
[1] *Department of General Surgery, The First Affiliated Hospital of Nanjing Medical University, 210029 Nanjing, China*
[2] *Jiangsu Province Academy of Clinical Medicine, Institute of Tumor Biology, Nanjing, China*
[3] *Department of General Surgery, Wuxi The Fourth People's Hospital, 214062 Wuxi, China*

SUMMARY

Background: **An escalating number of studies have provided identified evidence that sphingosine kinase 1 (*SPHK1*) plays an essential role in carcinogenesis. This present study was devised to seek the possible correlation of two tag single-nucleotide polymorphisms (SNPs) in *SPHK1* (rs3744037 T>C, rs346801 C>T) with the susceptibility to gastric cancer (GC).**

Methods: **This present case-control study was comprised of 710 patients with GC and 710 gender- and age-matched cancer-free individuals. The genotypes of the individuals were acquired by the TaqMan-MGB method. *SPHK1* mRNA level was examined in 60 paired cancerous and noncancerous tissues using quantitative reverse transcription-polymerase chain reaction (qRT-PCR).**

Results: **Our results suggested that the variant genotype T allele of *SPHK1* rs346801 increased the GC risk in the study population [CT vs. CC, odds ratio (OR) = 1.385, 95% confidence interval (CI) = 1.096 - 1.751; TT vs. CC, OR = 2.502, 95% CI = 1.078 - 5.806; CT+TT vs. CC, OR = 1.434, 95% CI = 1.140 - 1.804]. Furthermore, in stratified analyses, rs346801 variant genotypes were associated with a conspicuous risk of GC in younger individuals (< 62 years), females, non-smokers, and individuals from rural areas. In addition, the carriers with variant genotype CT, TT, and CT+TT were observed to possess the higher *SPHK1* messenger RNA levels than those with CC genotype in GC specimens.**

Conclusions: **These results strongly demonstrated that the *SPHK1* rs346801 C>T polymorphism may contribute to the susceptibility to gastric cancer in Chinese population and affect the expression of *SPHK1* and, therefore, may act as a novel biomarker for predicting gastric cancer.**

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Correspondence:
Li Yang
Department of General Surgery
The First Affiliated Hospital of
Nanjing Medical University
Guangzhou Road 300
210029 Nanjing
China
Phone: +86025-83718836
Email: pwkyangli@126.com

Huaxiang Cao
Department of General Surgery
Wuxi The Fourth People's Hospital
Huihe Road 200
214062 Wuxi
China
Phone: +860510-88682999
Email: pwkchx@163.com

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INTRODUCTION

Currently, gastric cancer is considered one of the most recognizable malignant tumors, which remains fifth in tumor incidence and third in cancer-related mortality globally [1]. In spite of a certain amount of the therapeutic advances, the prognosis of patients is often dismal [2,3]. Although the explicit mechanism leading to gastric cancer is still largely unknown, abundant studies have suggested that genetic factors [4], living habit [5], and *Helicobacter pylori* infection [6] may account for developing the probability of suffering gastric carcinoma separately or jointly. Furthermore, our previous studies provided a some evidence to support that genetic polymorphisms as a momentous factor which was implicated in GC development [7-10].

Sphingosine kinase 1 (*SPHK1*) is a member of the SphK isoenzymes which catalyze the process of generating sphingosine-1-phosphate (S1P), a kind of bioactive lipid mediator [11]. S1P was reported to play a key role in cellular functions, such as cell proliferation, survival, and mortality [12]. Recently, multiple lines of studies have indicated that *SPHK1* shows potential correlation with various recognizable malignant tumors, for example gastric cancer [12], breast cancer [13], colorectal cancer [14], and ovarian cancer [15]. As to gastric cancer, *SPHK1* was confirmed to accelerate the progression of gastric cancer via the Akt/Fox O3a signaling pathway and correlated with poor prognosis of patients [12,16]. However, the association between *SPHK1* polymorphisms and malignant tumor including gastric cancer has not been reported up to now.

In view of the vital role of *SPHK1* in carcinogenesis, we hypothesized that an association may exist between the tag SNPs in *SPHK1* and gastric cancer risk. To verify the hypothesis, we carried out a case-control study of selected tag SNPs (rs3744037, rs346801) in *SPHK1* to exam the association between functional *SPHK1* genotypes and GC risk in a Chinese population. In addition, the *SPHK1* mRNA expression of each genotype was compared in both normal tissues and GC tissues to explore the potential cancer risk-associated polymorphism mediated regulation mechanism.

MATERIALS AND METHODS

Subjects

In total, 710 GC patients and 710 cancer-free individuals were selected into our case-control study. All patients were enrolled in this present study successionally from the First Affiliated Hospital of Nanjing Medical University from June 2010 to May 2016. The case group members were diagnosed as GC histopathologically using gastroscopic biopsy or surgical specimens and, in addition, had not received any chemotherapy or radiotherapy preoperatively. In the noncancerous control group, all members matched with gender and age were randomly recruited into groups from the identical hospital as well as during the same period. In this present study, all subjects with secondary recurrent malignancies, who accepted blood transfusion from other individuals, who underwent chemotherapy/radiotherapy, and with genetic diseases were ruled out. There was no genetic relationship between all ethnic Han Chinese residing in Jiangsu Province or its surrounding areas. After signing the informed consent, a 5 mL vein blood sample was collected from each subject for experimental use. A standard questionnaire was used to obtain the data on subjects including age, gender, smoking, hypertension, diabetes, and rural or urban residence.

Enrolled individuals were defined as smokers, if they smoked ≥ 10 cigarettes per day in the pastor present for at least 2 years [7]. The in-patient medical records were read to collect the clinical information, such as tumor size, histological type, tumor lymphatic metastasis according to the7th UICC/AJCC criteria of TNM stage. This study protocol was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University.

SNP selection

The genotype data information for Han Chinese in *SPHK1* was obtained from the HapMap public database (HapMap Data Rel 27 Phasell+III, Feb 09, on NCBI B36 assembly, db SNP b126). Afterwards, Haploview software was used to select the tag SNPs by two criteria, which include minor allele frequency (MAF) ≥ 0.05 and r^2 (linkage disequilibrium (LD) correlation coefficient) > 0.8. In consequence, two SNPs (rs3744037, rs346801) were selected into our present study.

Genotyping

The standard process of genomic DNA extraction from peripheral blood leukocytes were described in our previous study [17]. The whole genotypes of two SNPs (rs3744037 and rs346801) were obtained using TaqMan-MGB allelic discrimination method (Thermo Fisher Scientific, Waltham, MA, USA). The base sequences of the probes and primers applied in genotyping are listed up in Table 1. In total, 0.125 μL probes, 0.25 μL primers, 5 μL 2 x TaqMan Genotyping Master Mix, 2.5 μL double distilled water, and 10 ng genomic DNA constituted the 10 μl reaction mixture. Amplification was performed at 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. On the basis of the MGB probe manufacturer's instructions, the 96-well ABI Step One Plus Real-Time PCR System (Fisher Scientific, Grand Island, NY, USA) was adopted to conduct the amplifications, and allelic discrimination was performed using Stepone v. 2.2.2 software (Fisher Scientific, Grand Island, NY, USA). In order to ensure the genotyping accuracy, each reaction plate included two positive experimental controls with previously known genotype and two negative experimental controls (water). The call rate for each SNP was 100%.

Real-time PCR analysis of *SPHK1*

SPHK1 mRNA expression levels were analysed by qRT-PCR with total RNA extracted from 60 pairs of noncancerous and cancerous gastric tissue samples. The total RNA was extracted from tissues by applying Trizol reagent (Gibco, Grand Island, NY, USA). Afterwards, each isolated RNA was transcribed into first-strand complementary DNA (cDNA) under the action of Prime script RT Reagent (Takara, Otsu, Japan). β-actin was used to normalize the expression levels of *SPHK1* gene and amplified with forward primer 5'-AGAAAATCTGGCACCACACC-3' and reverse primer 5'-TAGCACAGCCTGGATAGCAA-3'. The specific real-time PCR primers for *SPHK1* were listed as follows: forward primer 5'-CTTGCAGCTCTTCCGG AGTC-3'and reverse primer 5'-GCTCAGTGAGCAT CAGCGTG-3'. The process of amplification reactions was conducted in a 10 μL reaction volume consisting of 5 μL Mastermix, 0.2 μL primers, and 100 ngc DNA. The conditions of cycling reaction were set as follows: 95°C for 5 minutes, followed by 40 cycles at 95°C for 10 seconds and 60°C for 30 seconds. One set of Step One Plus Real-Time PCR System (Fisher Scientific, Grand Island, NY, USA) was used to complete the Real-time PCR in triplicate with Fast Start Universal SYBR-Green Master (Vazyme, Nanjing, China). The conventional $2^{-\Delta CT}$ method was applied to appraise the relative *SPHK1* mRNA expression.

Statistical analysis

All statistical analyses were conducted using the SPSS version 22.0 (IBM, Chicago, IL, USA). All p-values in our data analysis were 2-sided and $p < 0.05$ was recognized as statistically significant. Differences in genotype frequencies of two SNPs between two experimental groups and demographic characteristics were analysed using χ^2 tests and Student's *t*-test. The Hardy-Weinberg equilibrium was evaluated for control group members by using the goodness-of-χ^2 test. The numerical values of odds ratios (ORs) and 95% confidence intervals (CIs) were obtained by performing unconditional logistic regression analyses under the adjustments for age, gender, diabetes, hypertension, smoking, and residence. The relative expression levels of *SPHK1* in all tissues were tested using the $2^{-\Delta ct}$ method, compared with the levels ofβ-actin. The associations between the expression levels of *SPHK1* and *SPHK1* polymorphisms were evaluated by one-way ANOVA, post-hoc test.

RESULTS

Baseline characteristics of the study subjects

Overall, 710 GC patients and 710 controls were enrolled in this present research. Table 2 displayed the baseline characteristics of GC cases and controls. No significant differences existed between GC cases and cancer-free controls based on gender and age ($p = 0.057$ and $p = 0.404$, respectively), indicating the frequency was matched adequately. The median age was 62 years for cancer subjects and 60 years for controls. There were no significant differences detected in distributions of diabetes, hypertension, and residence between cases and controls ($p = 0.108$, $p = 0.276$ and $p = 0.957$, respectively); otherwise, smoking status was more distributed among cancer subjects than controls ($p = 0.001$).

Associations of *SPHK1* tag SNPs and GC risk

The genotype distribution of the two tag SNPs rs346801 and rs3744037 in the cases and controls was presented in Table 3. In the control group, the genotype frequency distribution of the two polymorphisms met the demand of Hardy-Weinberg equilibrium ($p = 0.843$ for rs3744 037 and $p = 0.118$ for rs346801).

Concerning our data, we found that the T allele of rs346801 was suggested to have a notably increased risk factor for GC compared with the C allele (T vs. C: $p = 0.001$, OR = 1.402, 95% CI = 1.143 - 1.719). Compared with subjects carrying rs346801 genotype CC, the individuals with the mutated genotypes CT, TT, and (CT+TT) had a significantly elevated risk of GC (CT vs. CC: $p = 0.006$, adjusted OR = 1.385, 95% CI = 1.096 - 1.751; TT vs. CC: $p = 0.033$, adjusted OR = 2.502, 95% CI = 1.078 - 5.806; CT+TT vs. CC: $p = 0.002$, adjusted OR = 1.434, 95% CI = 1.140 - 1.804) after adjustment for gender, age, smoking, diabetes, hypertension, and residence. Nonetheless, no significant correlation between tag SNP rs3744037 and GC risk was observed.

The genotype-phenotype correlation between rs346801 and *SPHK1* expression

Furthermore, with the purpose of investigating the genotype-phenotype correlation between rs346801 and *SPHK1* expression, we analysed the *SPHK1* mRNA expression levels in 60 pairs of GC and normal tissues samples with different genotypes. The relative *SPHK1* mRNA expression levels in samples with CT genotype (0.028 ± 0.004, [n = 17]), TT genotype (0.032 ± 0.002, [n = 3]), and CT+TT genotype (0.029 ± 0.013, [n = 20]) were obviously higher than CC genotype (0.018 ± 0.001, [n = 40]) in GC tissues ($p = 0.004$, $p = 0.046$, and $p = 0.001$, respectively), which was clearly illustrated in Figure 1A. However, in noncancerous tissue samples, we found no significant difference of *SPHK1* expression levels between CT (0.015 ± 0.004, [n = 17]), TT (0.013 ± 0.001, [n = 3]), CT+TT (0.015 ± 0.014, [n = 20]), and CC (0.013 ± 0.002, [n = 40]) genotypes (Figure 1B). Since there was no significant difference observed between rs3744037 and GC risk was, we did not further investigate its allele-specific effect.

Stratified analysis of genetic variants and GC risk

To further explore the non-genetic factors' potential influence on genetic effect, we therefore conducted the stratified analysis of *SPHK1* variant genotypes by age, gender, smoking, and residence. The results of the stratified analysis were presented in Table 4A and 4B. As to

Table 1. Information on primers and probes.

SNPs	Primer sequence (5'-3')	Probe sequence
rs3744037	F-GGCAGGCATATGGAGTATGAATG	T:FAM-CCTACTTGGTATATGTGC-MGB
T>C	R-CAAACACACCTTTCCCATCCTT	C:HEX-CCTACTTGGTATACGTGC-MGB
rs346801	F-GGAGGAAGGGTCCTGCAAGTAGA	C:FAM-ACAGCGCGGCCCAGG-MGB
C>T	R-AGATGCCACCGCCAGAAGAG	T:HEX-ACAGCGTGGCCCAGG-MGB

Table 2. Demographic information.

Characteristics	Cases (n = 710)	Controls (n = 710)	p-value
Age [*](years)	62 (54 - 68)	60 (51 - 70)	0.404
Gender, (n (%))			
Female	200 (28.2)	233 (32.8)	0.057
Male	510 (71.8)	477 (67.2)	
Hypertension, (n (%))			
No	499 (70.3)	480 (67.6)	0.276
Yes	211 (29.7)	230 (32.1)	
Diabetes, (n (%))			
No	641 (90.3)	622 (87.6)	0.108
Yes	69 (9.7)	88 (12.4)	
Smoking, (n (%))			
Ever	168 (23.7)	116 (16.3)	**0.001**
Never	542 (76.3)	594 (83.7)	
Residence, (n (%))			
Rural	404 (56.9)	403 (56.8)	0.957
Urban	306 (43.1)	307 (43.2)	
Tumor differentiation (n (%))			
Well	56 (7.9)		
Moderate	124 (17.5)		
Poor	530 (74.6)		
Depth of tumor infiltration (n (%))			
T1	129 (18.2)		
T2	84 (11.8)		
T3	310 (43.7)		
T4	187 (26.3)		
Lymph node metastasis (n (%))			
Negative	249 (35.1)		
Positive	461 (64.9)		
Localization (n (%))			
Cardia	319 (44.9)		
Noncardia	391 (55.1)		

Notes: The underlined data in the table indicates statistically significant data,[*] - Median (25th - 75th percentiles).

Table 3. Association between SPHK1 gene polymorphisms and risk of gastric cancer.

Genotype	Cases (n (%))	Controls (n (%))	Crude OR (95% CI)	p-value	Adjusted OR (95% CI) *	p-value
overall	710	710				
rs3744037						
TT	239 (33.7)	246 (34.6)	1		1	
CT	353 (49.7)	346 (48.7)	1.050 (0.833 - 1.324)	0.679	1.046 (0.828 - 1.321)	0.706
CC	118 (16.6)	118 (16.6)	1.029 (0.754 - 1.405)	0.856	1.079 (0.786 - 1.481)	0.638
CT+CC	471 (66.3)	464 (65.4)	1.045 (0.839 - 1.301)	0.695	1.050 (0.842 - 1.310)	0.663
Allelic						
T	831	838	1			
C	589	582	0.980 (0.844 - 1.138)	0.790		
HWE	0.84391					
rs346801						
CC	474 (66.8)	527 (74.2)	1		1	
CT	218(30.7)	175 (24.6)	1.385 (1.096 - 1.751)	0.006	1.414 (1.115 - 1.792)	0.004
TT	18 (2.5)	8 (1.1)	2.502 (1.078 - 5.806)	0.033	2.621 (1.122 - 6.120)	0.026
CT+TT	236 (33.2)	183 (25.8)	1.434 (1.140 - 1.804)	0.002	1.467 (1.163 - 1.1.851)	0.001
Allelic						
C	1166	1229	1			
T	254	191	1.402 (1.143 - 1.719)	0.001		
HWE	0.11828					

Notes: The underlined data in the table indicates statistically significant data, * - Adjusted for age, gender, smoking status, residence, hypertension, and diabetes.
Abbreviations: OR - odds radio, CI - confidence interval, HWE - Hardy-Weinberg equilibrium.

Table 4A. Stratified analyses for SPHK1 rs346801genotypes in cases and controls.

Variable	CT+ TT vs. CC for rs346801		Allelic odds ratios and 95% confidence intervals for rs346801	
	Cases, n (%)	Controls, n (%)	Adjusted OR (95% CI) *	p-value
Age (years), median				
≥ 62	118 (16.6)/238 (33.5)	86 (12.1)/237 (33.4)	1.349 (0.961 - 1.896)	0.084
< 62	118 (16.6)/236 (33.2)	97 (13.7)/290 (40.8)	1.536 (1.112 - 2.120)	0.009
Gender				
Females	74 (10.4)/126 (17.7)	59 (8.3)/174 (24.5)	1.777 (1.172 - 2.694)	0.007
Males	162 (22.8)/348 (49.0)	124 (17.5)/353 (49.7)	1.310 (0.987 - 1.738)	0.061
Smoking Status				
Smokers	43 (6.1)/125 (17.6)	22 (3.1)/94 (13.2)	1.407 (0.772 - 2.564)	0.265
Nonsmokers	193 (27.2)/349 (49.2)	161 (22.7)/433 (61.0)	1.476 (1.146 - 1.902)	0.003
Residence				
Rural	144 (20.3)/260 (36.6)	105 (14.8)/298 (42.0)	1.585 (1.168 - 2.149)	0.003
Urban	92 (13.0)/214 (30.1)	78 (11.0)/229 (32.3)	1.327 (0.925 - 1.904)	0.125

Notes: The underlined data in the table indicates statistically significant data.*Adjusted for age, gender, smoking status, residence, hypertension, and diabetes.

Table 4B. Stratified analyses for SPHK1 rs3744037 genotypes in cases and controls.

Variable	CT+ CC vs. TT for rs3744037		Allelic odds ratios and 95% confidence intervals for rs3744037	
	Cases, n (%)	Controls, n (%)	Adjusted OR (95% CI) *	p-value
Age (years), median				
≥ 62	236 (33.2)/120 (16.9)	212 (29.9)/111 (15.6)	1.067 (0.772 - 1.475)	0.693
< 62	235 (33.1)/119 (16.8)	252 (35.5)/135 (19.0)	1.065 (0.784 - 1.447)	0.689
Gender				
Females	130 (18.3)/70 (9.9)	158 (22.3)/75 (10.6)	0.934 (0.620 - 1.407)	0.744
Males	341 (48.0)/169 (23.8)	306 (43.1)/171 (24.1)	1.138 (0.872 - 1.485)	0.341
Smoking Status				
Smokers	111 (15.6)/57 (8.0)	79 (11.1)/37 (5.2)	1.045 (0.620 - 1.761)	0.869
Nonsmokers	360 (50.7)/182 (25.6)	385 (54.2)/209 (29.4)	1.081 (0.845 - 1.382)	0.536
Residence				
Rural	273 (38.5)/131 (18.5)	261 (36.8)/142 (20.0)	1.121 (0.835 - 1.505)	0.447
Urban	198 (27.9)/108 (15.2)	203 (28.6)/104 (14.6)	0.966 (0.690 - 1.354)	0.841

Notes:* - Adjusted for age, gender, smoking status, residence, hypertension, and diabetes.

Table 5A. Associations between SPHK1 rs346801 genotypes and clinicopathologic characteristics of gastric cancer.

Variable	CT+TT, CC for rs346801		Allelic odds ratios and 95% confidence intervals for rs346801	
	CT+TT, n	CC, n	Adjusted OR (95% CI) *	p-value
Tumor differentiation				
Well	19	37	1	
Moderate	43	81	0.836 (0.409 - 1.710)	0.623
Poor	174	356	0.922 (0.510 - 1.668)	0.788
Depth of tumor infiltration				
T1	37	92	1	
T2	31	53	1.635 (0.892 - 2.996)	0.112
T3	105	205	1.319 (0.836 - 2.080)	0.234
T4	63	124	1.262 (0.768 - 2.075)	0.358
Lymph node metastasis				
Negative	75	174	1	
Positive	161	300	1.240 (0.887 - 1.733)	0.208
Localization				
Cardia	103	216	1	
Noncardia	133	258	1.078 (0.783 - 1.483)	0.646

Notes:* - Adjusted for age, gender, smoking status, residence, hypertension, and diabetes.

rs346801, an increased GC risk correlated to the mutant genotypes was observed among younger individuals (age < 62 years) (p = 0.009, adjusted OR = 1.536, 95% CI = 1.112 - 2.120), instead of elder individuals (p = 0.084, adjusted OR = 1.349, 95% CI = 0.961 - 1.896). In terms of female subjects, the variant genotypes were

Table 5B. Associations between *SPHK1* rs3744037 genotypes and clinicopathologic characteristics of gastric cancer.

Variable	CT+CC, TT for rs3744037		Allelic odds ratios and 95% confidence intervals for rs3744037	
	CT+CC, n	TT, n	Adjusted OR (95% CI) *	p-value
Tumor differentiation				
Well	35	21	1	
Moderate	82	42	1.023 (0.509 - 2.056)	0.949
Poor	354	176	1.181 (0.661 - 2.111)	0.574
Depth of tumor infiltration				
T1	85	44	1	
T2	53	31	0.835 (0.463 - 1.504)	0.548
T3	220	90	1.243 (0.796 - 1.942)	0.339
T4	113	74	0.768 (0.477 - 1.235)	0.276
Lymph node metastasis				
Negative	169	80	1	
Positive	302	159	0.906 (0.650 - 1.262)	0.558
Localization				
Cardia	207	112	1	
Noncardia	264	127	1.179 (0.858 - 1.620)	0.309

Notes: * - Adjusted for age, gender, smoking status, residence, hypertension, and diabetes.

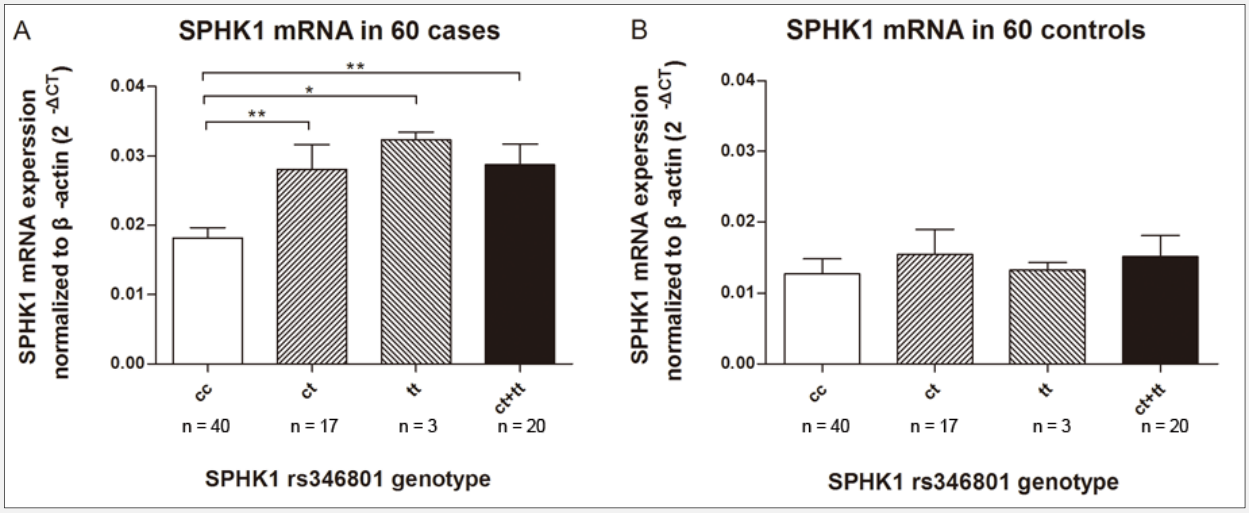

Figure 1. Correlation between rs346801 genotypes and expression of *SPHK1*.

(A) Genotype-phenotype correlation for rs346801 and relative expression levels of *SPHK1* mRNA in 60 GC tissues. Relative *SPHK1* mRNA expression levels were significantly higher for the CT (0.028 ± 0.004) and TT (0.032 ± 0.002) CT+TT (0.029 ± 0.013) than the CC (0.018 ± 0.001) genotype, * - $p < 0.05$, ** - $p < 0.01$. (B) Genotype-phenotype correlation for rs346801 and relative expression levels of *SPHK1* mRNA in 60 non-cancerous tissues. *SPHK1* mRNA expression levels were similar among the three groups with rs346801 CC (0.013 ± 0.002, [n = 40]), CT (0.015 ± 0.004, [n = 17]), TT (0.013 ± 0.001, [n = 3]), and CT+TT (0.015 ± 0.014, [n = 20]) genotypes.

correlated to an increased GC risk (p = 0.007, adjusted OR = 1.777, 95% CI = 1.172 - 2.694), but the statistically significant correlation was not observed in male individuals (p = 0.061, adjusted OR = 1.310, 95% CI = 0.987 - 1.738). In the case of smoking status, the correlation between the relatively rare genotypes and susceptibility to GC was observed among nonsmokers (p = 0.003, adjusted OR = 1.476, 95% CI = 1.146 - 1.902) rather than smokers (p = 0.265, adjusted OR = 1.407, 95% CI = 0.772 - 2.564). In regard to residence, the significant association between polymorphisms and GC risk was observed among subjects from rural areas (p = 0.003, adjusted OR = 1.585, 95% CI = 1.168 - 2.149), but not among urban individuals (p = 0.125, adjusted OR = 1.327, 95% CI = 0.925 - 1.904). However, no significant association with GC susceptibility in stratified analysis was evident in *SPHK1* rs3744037.

We also conducted stratified analyses according to clinicopathological characteristics in GC patients, including depth of tumor infiltration, tumor differentiation, lymph node metastasis, and localization. As a result, no obvious correlation between the mutated genotypes and the clinical features of GC was observed (Table 5A, 5B).

DISCUSSION

To our best knowledge, this present case-control study provided the first investigation of the association between the genetic variants in *SPHK1* (rs3744037, rs34 6801) and susceptibility to GC in a Chinese Han population. In our current findings, the variant genotype CT/TT of *SPHK1* SNP rs346801 was notably correlated to the contributed risk for GC. In stratified analyses, our results revealed that the distribution of mutant genotype CT+TT of rs346801 was profound among younger, women, nonsmoking, and rural subjects. The difference in age could be explained by the cumulative impact of environmental carcinogens attacking the weaker immune system in elder individuals, so that the genetic function may be stronger among younger population [17]. Similarly, in terms of gender, our research suggested the rs346801 polymorphism may possess an important effect in women patients. For female subjects, usually living a lifestyle rarely exposed to tobacco or alcohol compared with male subjects, the mutant genotypes may play a dominant part in the cancer generating process. Nonetheless, more research was needed to seek the conceivable mechanism of the association between *SPHK1* polymorphisms and age or gender. It has been extensively confirmed that tobacco smoking is an essential carcinogenic factor for gastric cancer [18]. The results in this presented study showed the variant genotypes were more distributed among non-smokers, but not among smokers. This phenomenon indicate that long-term smoking situation leads the significant association between polymorphisms and GC apparent in smokers, so that the higher distribution of the mutant genotypes was evident among non-smokers. In addition, a previous study reported that the genetic difference has a stronger effect while environmental pollution is limited [19]. Our results may indicate that the pronounced genetic different effect was observed in less-polluted, rural area rather than more-polluted, urban area. Certainly, all of our conjectures need further forceful research to verify the association between *SPHK1* polymorphisms and smoking status or residence subgroups.

Currently, *SPHK1* has been constantly reported to perform an essential role in many malignant tumors [12-15]. The *SPHK1*/S1P signaling pathway has been confirmed to carcinogenic by modulating the authoritative transcription factor hypoxia-inducible HIF-1α/2α in numerous cancers [20]. Although the clear molecular mechanisms of *SPHK1* carcinogenesis remain largely unknown, a previous study reported the expression of *SPHK1* is critical for cancer cell invasion and adhesion, which may contribute to cancer progression [21]. Furthermore, elevated *SPHK1* expression is associated with unsatisfactory outcomes in cancer patients [16] and also was reported to be related to chemotherapy resistance in GC [22]. Recently, it has been reported that long noncoding RNA HULC may promote cancer angiogenesis by regulating the expression of *SPHK1* positively in liver cancer [23]. As Li et al. claimed that the down-regulated expression of miR-506 could facilitate progression of pancreatic cancer via the *SPHK1*/Akt/NF-κB signaling pathway [24], suggesting that *SPHK1* could be a transit shipment for the oncogenic gene. A similar discovery was also seen in GC where miR-124 plays a crucial role in the suppression of gastric adenocarcinoma through down-regulating *SPHK1* [25]. The detailed information supplied by National Center of Biotechnology Information (NCBI) revealed that the tag SNP rs346801 is located in the 3′ untranslated regions (UTR). As a result, the mutant allele of rs346801 may change the microRNA binding region, leading to the significant correlation between *SPHK1* polymorphisms and the susceptibility to GC. A previous research has confirmed that the SNPs located in 3′ UTRs region of a designated gene can latently regulate microRNA on posttranscriptional regulation, which leads to complex diseases occurring [26]. Several studies have confirmed evidence that SNPs located in a certain region of miRNA binding zone, by influencing the combining of miRNA with its downstream target gene, brought about positive or negative regulation of the translation of target mRNA, which eventually forms the susceptibility to cancer [27-30]. It is also known that the mRNA secondary structure is vital for gene functions and mRNA-miRNA interactions [31]. It is reported that the secondary structure of a certain gene may be changed with the mutation of SNPs [32]. So, it may also explain why the allele of rs346801 could lead to the expression of gene *SPHK1*. Intriguingly, our results revealed that the variant genotype could cause the discrimination of *SPHK1* mRNA expression compared with wild genotype in GC specimens, which caters to the above viewpoints and could explain the potential mechanism of the formation

of the association between rs346801 and GC risk. Our discovery may contribute to providing a novel direction to seek the functional effect in the relation between *SPHK1* polymorphisms and GC risk.

In addition, several limitations should be highlighted in this presented study. Firstly, the selection bias which exists in a case-control study was ultimately unavoidable. Nonetheless, the genotype distribution among individuals in the control group matched the Hardy-Weinberg conditions. Secondly, insufficient statistical power resulting from the comparatively diminutive sample size may lead to the underpowered interactions of genetic and environmental factors in the subgroup analyses. Thirdly, the personal information of enrolled members, for example the data of smoking history, was gathered by questionnaires. Therefore, the information bias and inherent selection bias could not be avoided. Fourthly, partial missing data of clinical information on the subjects, such as alcohol consumption status, prevented further analysis to a certain extent. Moreover, it was not ethical to perform *Helicobacter pylori* tests for each individual, particularly for controls. The information of *Helicobacter pylori* status was deficient in our study. Finally, more attention should be paid to focusing on inferring the association between *SPHK1* SNPs and GC risk to other districts and ethnic groups, except Chinese Han population in our study.

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Declaration of Interest:

No potential conflicts of interest were disclosed.

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