The *DNMT3B* -579G>T Polymorphism Is Significantly Associated With the Risk of Gastric Cancer but not Lung Cancer in Chinese Population

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Abstract

The -149C>T and -579G>T, 2 single nucleotide polymorphisms in *de novo* methyltransferase *3B* gene promoter, have been previously reported to potentially alter the promoter activity and to influence cancer risk. However, the results from previous studies remain conflicting rather than conclusive. In view of this, we conducted a case–control study and then a meta-analysis to examine the association between these 2 single-nucleotide polymorphisms with risk of lung and gastric cancer in Chinese population. The genotyping was performed by polymerase chain reaction-restriction fragment length polymorphism and confirmed by sequencing. In this case–control study, no significant association with lung or gastric cancer risk was observed for -149C>T, while -579G>T was significantly correlated with the risk of gastric cancer but not lung cancer. Moreover, haplotype analysis showed that haplotype -149T/-579 T, which carried the risk -579 T allele, significantly increased the susceptibility to gastric cancer. However, none of the haplotypes was associated with the risk of lung cancer. The following meta-analysis involved only Chinese population and further confirmed the significant association of -579G>T with gastric cancer but not lung cancer and suggested no significant association between -149C>T and risk of lung or gastric cancer. Collectively, *DNMT3B* -579G>T polymorphism is associated with gastric cancer risk in Chinese population, and the -579G>T may be used as a genetic biomarker to predict the risk of gastric cancer in Chinese population.

Keywords

DNMT3B, single-nucleotide polymorphism (SNP), gastric cancer, lung cancer, meta-analysis, Chinese population

Abbreviations

CI, confidence interval; HWE, Hardy-Weinberg equilibrium; LD, Linkage disequilibrium; OR, odds ratio; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; SNP, single nucleotide polymorphism.

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Introduction

Accumulated evidence demonstrated that DNA methylation, a key epigenetic modificator, plays essential roles in tumorigenesis.[1,2] Indeed, aberrant DNA methylation profiles have been found in almost all types of cancers.[3] Generally, DNA methylation patterns in mammals are established by the *de novo* methyltransferase (DNMT) 3 family (DNMT3A and DNMT3B) and maintained by the maintenance methyltransferase (DNMT1).[1,2] Therefore, the alteration in global DNA methylation patterns may be largely attributed to the dysregulation of *de novo* DNMTs during tumor progression.[4,5] Interestingly, previous studies have suggested that DNMT3B

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Table 1. Primers and Restriction Enzymes of *DNMT3B* Polymorphisms for Genotyping.

Locus	Location	Primer Sequence (5'-3')	Annealing Temperature	Product	Enzyme, Digestion Temperature	Digested Bands
-149C>T	In the transcription start site	Forward: GCCACCCTACCACCTCTATTC Reverse: GGACACTCACTGGGGCTTAG	58°C	153 bp	*BfaI*, 37°C	C allele: 153bp T allele: 131bp + 22bp
-579G>T	In the exon 1B transcription start site	Forward: GCCAACCAAAGGTGGAACA Reverse: GAGGCACAGGCAGAAAGC	57°C	169 bp	*PvuII*, 37°C	G allele: 169bp T allele: 104bp+65bp

promotes tumorigenesis, and abnormal expression of DNMT3B contributes to the aberrant DNA methylation in carcinogenesis.[6]

Lung and gastric cancers have been the leading cancer diagnosed and are the cause of cancer death for many years in Hubei province of China, and the incidences still increase rapidly.[7-9] More seriously, most patients with lung and gastric cancers are detected in advanced stage, during which period the tumors are unresectable anymore.[10,11] Thus, it is no doubt that discovery of genetic biomarkers and their application accompanied with traditional diagnosis may be more efficiency for risk prediction and early diagnosis of lung and gastric cancer.

On the other side, it had been proved that certain functional single-nucleotide polymorphisms (SNPs) in the 5'-untranslated regions of genes could influence promoter activity and then expression of genes.[12] Therefore, identification of functional SNPs in *DNMT3B* gene would lead to a better understanding of how DNMT3B contributes to individuals' susceptibility to cancer. Recently, the -149C>T (rs2424913) and -579G>T (rs1569686) polymorphisms in *DNMT3B* gene promoter, which may be able to alter promoter activity, have been widely studied for their association with cancer susceptibility.[13-22] However, none of the studies has been conducted in Hubei Chinese population. Moreover, the results from previous studies remain conflicting rather than conclusive. In view of this, a case–control study was performed to evaluate the association between the -149C>T and -579G>T polymorphisms and susceptibility to lung and gastric cancer in a Chinese population of Hubei province with larger sample size. Next, a meta-analysis combining the current study and previously published studies was further conducted to clarify the real impact of *DNMT3B* -149C>T and -579G>T polymorphisms on the risk of lung and gastric cancer in Chinese population.

Material and Methods

Participants

A total of 550 patients with lung cancer, 460 patients with gastric cancer, and 800 normal controls were recruited in the current study. All participants were biologically unrelated Chinese living in Hubei province. Nowadays, more and more Chinese are inclined to have a physical examination every year. The normal controls were selected from cancer-free individuals who visited Wuhan Xinzhou District People's Hospital for regular physical examinations between September 2014 and December 2016 or who volunteered to participate in the epidemiology survey during the same period. It was required that the normal controls passed all annual physical examinations in the latest 3 years. Patients with lung and gastric cancer were confirmed histopathologically and volunteers recruited from Hubei Cancer Hospital and Wuhan Xinzhou District People's Hospital between January 2015 and December 2016. This study was approved by the Ethical Committees of Wuhan University of Technology, and written informed consent for the genetics analysis was obtained from all participants or their guardians.

The Genotyping of DNMT3B Polymorphisms

Samples were collected into blood vacuum tubes containing EDTA and stored at 4°C. Genomic DNA was extracted within 1 week of sample collection by proteinase K digestion as described previously.[23] Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to detect the -149C>T and -579G>T polymorphisms of *DNMT3B* gene. The primers, length of PCR products, related restriction endonuclease as well as digested bands are shown in Table 1. The PCR reaction was performed in a total of 15 μL containing 50 ng genomic DNA, 1.5 μL 10 × Taq Buffer (Mg^{2+} Plus), 0.2 μL 10 mmol/L deoxy-ribonucleoside triphosphate, 1-μL 1 mmol/L primers, and 0.5 U Taq polymerase (Takara Biotechnology Co Ltd, Dalian, China). The PCR products were then digested with 10-unit restriction enzymes following the manufacturer's instructions (Takara Biotechnology Co Ltd, Dalian, China). Digested fragments were separated by electrophoresis on 3% agarose gel and visualized under ultraviolet light with Gel-Red staining. For quality control, genotyping analysis was performed blind, with respect to case/control status, and repeated twice for all participants. The results of genotyping were 100% concordant. In order to confirm the genotyping results, 20% randomly selected PCR-amplified DNA samples were examined by DNA sequencing, and the results were also 100% concordant.

Statistical Analysis

The chi-square test was used to compare the difference in age, gender, smoking status, and alcohol status between patients and

Table 2. Characteristics of Patients With Lung Cancer, Patients With Gastric Cancer, and Normal Controls.

Variables		Patients With Lung Cancer	Patients With Gastric Cancer	Normal Controls	P Value[a]	P Value[b]
Age, years	≤60	306 (55.6%)[c]	252 (54.8%)	434 (54.3%)	.615	.855
	>60	244 (44.4%)	208 (45.2%)	366 (45.7%)		
Gender	Male	373 (67.9%)	323 (70.3%)	558 (69.7%)	.451	.862
	Female	177 (32.1%)	137 (29.7%)	242 (30.3%)		
Smoking status	Ever	150 (27.3%)	132 (28.8%)	209 (26.1%)	.639	.323
	Never	400 (72.7%)	328 (71.2%)	591 (73.9%)		
Alcohol status	Ever	170 (31.0%)	148 (32.1%)	237 (29.6%)	.613	.344
	Never	380 (69.0%)	312 (67.9%)	563 (70.4%)		

[a]Age, gender, smoking status, and alcohol status distributions of patients with lung cancer and normal controls were compared using 2-sided χ^2 test.
[b]Age, gender, smoking status, and alcohol status distributions of patients with gastric cancer and normal controls were compared using 2-sided χ^2 test.
[c]Values are represented as number (percentage).

normal controls. Genotypic frequency of -149C>T and -579G>T polymorphisms were tested for departure from Hardy-Weinberg equilibrium (HWE) using the χ^2 test. To evaluate the association between *DNMT3B* polymorphisms and cancer risk, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by logistic regression analysis. Linkage disequilibrium (LD) plot was performed to test the -149C>T and -579G>T polymorphisms using *D'* as the measure of LD. SHEsis software (http://analysis.bio-x.cn/myAnalysis.php)[24] was used to test a possible association of statistically inferred haplotypes with cancer risk by a global test and haplotype-specific test, and haplotype frequencies were compared between the patients and the controls with Fisher exact test and logistic regression analysis. Statistical significance was established at *P* <.05, and a Bonferroni correction for multiple testing was applied. The statistical analyses were performed using SPSS 15.0 software (SPSS, Chicago, Illinois).

Meta-Analysis

We searched the all publications updated to March 2017 from the PubMed, EMBASE, ISI Web of Science, China National Knowledge Infrastructure, and WanFang databases without language restriction. The following words were searched: "DNMT3B or DNA methyltransferase 3B", "rs2424913/-149C>T", "rs1569686/-579G>T", "lung cancer or gastric cancer" and "Chinese population". References listed in retrieved articles were also checked for missing information. Next, studies were eligible for inclusion in the meta-analysis if they met the following criteria: (1) studies on humans; (2) investigation of the *DNMT3B* -149C>T polymorphism or *DNMT3B* -579G>T polymorphism and the risk of lung cancer or gastric cancer; (3) case-control study design; (4) valid data were accessible to estimate the OR and its 95% CI; (5) HWE equilibrium should be established in control groups.

We calculated the departure from the HWE for the control group in each study using Pearson goodness-of-fit χ^2 test. The analyses were conducted in Review Manager 5.3 (Cochrane Collaboration, Oxford, United Kingdom). The overall strength of an association between *DNMT3B* polymorphisms and cancer risk was assessed by crude ORs together with their

corresponding 95% CIs. Heterogeneity was evaluated by the χ^2 test of heterogeneity and the inconsistency index (I^2). By heterogeneity test, heterogeneity was considered significant when *P* value ($P_{\text{heterogeneity}}$) < .1 was consistent with possible substantial heterogeneity. If $P_{\text{heterogeneity}} \geq .1$ we used the fixed-effect model to calculate the combined OR (the Mantel-Haenszel method),[25] otherwise, random-effects model (Der Simonian and Laird method) was conducted.[26] The significance of combined OR was determined by the Z test.

Results

Table 2 showed us the frequency distributions of patients with lung cancer, patients with gastric cancer, and normal controls. There were no significant difference in the frequency distributions of age stratification, gender, smoking status, and drinking status between patients with lung cancer and normal controls as well as between patients with gastric cancer and normal controls, suggesting that matching based on these 4 variables were adequate.

The -149C>T and -579G>T polymorphisms were successfully genotyped in a total of 1810 participants. No significant deviations from HWE were observed for -149C>T and -579G>T in normal controls (*P* > .05). The allele and genotype distributions of *DNMT3B* polymorphisms and their association with risk of lung and gastric cancer are presented in Table 3. No association was found between -149C>T and risk of lung or gastric cancer as well as between -579G>T and lung cancer risk. However, it was showed that the frequency of -579 T allele was significantly higher among patients with gastric cancer than normal controls (*P* = .001, OR = 1.58, 95% CI = 1.22-2.04) after Bonferroni correction for multiple testing (0.05/12 = 0.004), indicating -579 T allele was associated with an increased risk of gastric cancer. Concordantly, we also found a significant association between -579TT genotype with increased risk of gastric cancer in 2 genetic models: TT vs TG (*P* = .001, OR = 1.62, 95%CI = 1.22-2.17) and TT vs TG+GG (*P* = .001, OR = 1.65, 95% CI = 1.25-2.19), and the association remained significant after Bonferroni correction for multiple testing (0.05/12 = 0.004). These results indicated that the *DNMT3B* -579G>T polymorphism significantly increases the risk of gastric cancer.

Table 3. Genotype and Allele Distributions of *DNMT3B* -149C>T and -579G>T Polymorphisms, and Their Association With the Risk of Lung and Gastric Cancer.

DNMT3B Polymorphisms	I. Patients With Lung Cancer	II. Patients With Gastric Cancer	III. Normal Controls	HWE[a]	Logistic Regression, P^b, OR (95% CI)[c]		
					Genetic Model	I vs III	II vs III
-149C>T							
T	1038 (94.4%)	884 (96.1%)	1522 (95.1%)		T vs C	.381, 0.86 (0.61-1.21)	.264, 1.26 (0.84-1.88)
C	62 (5.6%)	36 (3.9%)	78 (4.9%)				
TT	490 (89.1%)	425 (92.3%)	724 (90.4%)	0.940	TT vs TC	.427, 0.86 (0.60-1.24)	.257, 1.28 (0.84-1.95)
TC	58 (10.6%)	34 (7.5%)	74 (9.3%)		TT vs CC	.697, 0.68 (0.10-4.82)	.896, 1.17 (0.11-13.0)
CC	2 (0.3%)	1 (0.2%)	2 (0.3%)		TC vs CC	.810, 0.78 (0.11-5.73)	.946, 0.92 (0.08-10.5)
					TT vs TC+CC	.398, 0.86 (0.60-1.23)	.255, 1.28 (0.84-1.94)
					TT+TC vs CC	.707, 0.69 (0.10-4.90)	.909, 1.15 (0.10-12.7)
-579G>T							
T	953 (86.7%)	829 (90.1%)	1364 (85.3%)		T vs G	.311, 1.12 (0.90-1.40)	.001, 1.58 (1.22-2.04)
G	147 (13.4%)	91 (9.9%)	236 (14.7%)				
TT	413 (75.1%)	374 (81.4%)	580 (72.5%)	0.693	TT vs TG	.302, 1.14 (0.89-1.48)	.001, 1.62 (1.22-2.17)
TG	127 (23.1%)	81 (17.7%)	204 (25.5%)		TT vs GG	.749, 1.14 (0.51-2.54)	.161, 2.06 (0.75-5.68)
GG	10 (1.8%)	5 (1.0%)	16 (2.0%)		TG vs GG	.993, 1.00 (0.44-2.26)	.651, 1.27 (0.45-3.58)
					TT vs TG+GG	.289, 1.14 (0.89-1.47)	.001. 1.65 (1.25-2.19)
					TT+TG vs GG	.811, 1.10 (0.50-2.45)	.230, 1.86 (0.68-5.10)

Abbreviations: CI, confidence interval; HWE, Hardy-Weinberg equilibrium; OR, odds ratio.
[a]Genotypic frequency of *DNMT3B* polymorphisms in normal controls were tested for departure from Hardy-Weinberg equilibrium (HWE) using the χ^2 test.
[b]The *P* value was calculated using 2-sided χ^2 test.
[c]OR (95% CI) was estimated by logistic regression analysis.

Table 4. Association Between *DNMT3B* Haplotypes With Risk of Lung and Gastric Cancer.

Haplotype[a]	I. Patients With Lung Cancer	II. Patients With Gastric Cancer	III. Normal Controls	I vs III			II vs III		
				χ^2	P^b	OR (95% CI)[c]	χ^2	P^b	OR (95% CI)[c]
-149C/-579G	0.024	0.013	0.024	0.001	.985	1.00 (0.60-1.65)	3.434	.068	0.54 (0.28-1.05)
-149C/-579T	0.033	0.026	0.032	0.015	.902	1.03 (0.67-1.59)	0.675	.411	0.81 (0.50-1.33)
-149T/-579G	0.091	0.086	0.110	2.582	.108	0.81 (0.63-1.05)	3.722	.054	0.76 (0.58-1.00)
-149T/-579T	0.852	0.875	0.834	1.591	.207	1.15 (0.93-1.42)	7.697	.006	1.40 (1.10-1.77)
Global test	1.000	1.000	1.000	2.450	.484		8.563	.036	

Abbreviations: CI, confidence interval; OR, odds ratio.
[a]The haplotype structure was -149C>T/-579G>T. All haplotypes with frequency <0.01 in both case and control groups would be ignored in analysis.
[b]The *P* value was calculated using 2-sided χ^2 test.
[c]OR (95% CI) was estimated by logistic regression analysis.

The LD analysis revealed a low LD between -149C>T and -579G>T in patients with gastric cancer ($D' = 0.26$), patients with lung cancer ($D' = 0.35$), and normal controls ($D' = 0.41$), which were consistent with the results from the Han Chinese data set of the International HapMap Consortium. Since the haplotype analysis could enhance the statistical power in the mapping of human complex trait loci,[27] the analysis of haplotypes consisting of -149C>T/-579G>T was performed to assess *DNMT3B* gene with lung and gastric cancer susceptibility in this study. As presented in Table 4, none of the haplotypes was significantly associated with risk of lung cancer. However, when comparing patients having gastric cancer to normal controls, it showed a strong, significant difference in the overall distribution (global, $P = .036$). The frequency of haplotype -149T/-579 T was significantly higher in patients with gastric cancer than in normal controls (87.5% vs 83.4%, $P = .006$) at the significant level $P < .013$ (0.05/4) using the Bonferroni correction, and logistic regression analysis indicated that haplotype -149T/-579 T increased the risk of gastric cancer (OR = 1.40, 95% CI = 1.10-1.77).

According to inclusion criteria, 9 previous studies were finally selected in the following meta-analysis.[14-22] Table 5 showed the main features of the current and previous studies that evaluated the association between -149C>T or -579G>T and lung or gastric cancer risk. In Table 6, no association was observed between -149C>T and risk of lung or gastric cancer as well as between -579G>T and risk of lung cancer. In contrast, the -579G>T was significantly associated with an increased risk of gastric cancer in 3 genetic models (T vs G, $P < 1 \times 10^{-5}$, OR = 1.70, 95% CI = 1.36-2.13; TT vs TG, $P < 1 \times 10^{-5}$, OR = 1.77, 95% CI = 1.38-2.28; TT vs TG+GG, $P < 1 \times 10^{-5}$, OR = 1.80, 95% CI = 1.41-2.29) after

Table 5. Characteristics of Current and Previous Studies in Chinese Population.

References	Region	Cancer Type	Case (n)			Control (n)			Matching Y/N	Quality Control[a] Y/N	HWE[b]
-149C>T			Total	T/C	TT/TC/CC	Total	T/C	TT/TC/CC			
Wang et al[14]	Hebei	Gastric cancer	212	417/7	205/7/0	294	573/15	279/15/0	Y	Y	0.654
Zhang[18]	Jiangsu	Gastric cancer	156	309/3	154/1/1	156	311/1	155/1/0	Y	Y	0.968
Hu et al[15]	Jiangsu	Gastric cancer	259	516/2	257/2/0	262	521/3	259/3/0	Y	Y	0.926
Qiu et al[22]	Jiangsu	Gastric cancer	233	462/4	229/4/0	208	412/4	204/4/0	Y	Y	0.889
Current study	Hubei	Gastric cancer	460	884/36	425/34/1	800	1522/78	724/74/2	Y	Y	0.940
Yang[17]	Jilin	Lung cancer	52	99/5	47/5/0	55	107/3	52/3/0	Y	Y	0.835
Zhang et al[19]	Heilongjiang	Lung cancer	50	97/3	48/1/1	60	118/2	58/2/0	Y	Y	0.896
Current study	Hubei	Lung cancer	550	1038/62	490/58/2	800	1522/78	724/74/2	Y	Y	0.940
-579G>T			Total	T/G	TT/TG/GG	Total	T/G	TT/TG/GG			
Hu et al[15]	Jiangsu	Gastric cancer	259	487/31	230/27/2	262	461/63	203/55/4	Y	Y	0.901
Zhang et al[21]	Heilongjiang	Gastric cancer	50	93/7	43/7/0	60	108/12	48/12/0	Y	Y	0.389
Current study	Hubei	Gastric cancer	460	829/91	374/81/5	800	1364/236	580/204/16	Y	Y	0.693
Liu et al[16]	Heilongjiang	Lung cancer	174	327/21	154/19/1	135	244/26	109/26/0	Y	Y	0.216
Zhang et al[20]	Heilongjiang	Lung cancer	98	175/21	77/21/0	105	185/25	80/25/0	Y	Y	0.166
Current study	Hubei	Lung cancer	550	953/147	413/127/10	800	1364/236	580/204/16	Y	Y	0.693

Abbreviation: HWE, Hardy-Weinberg equilibrium
[a]Quality control was conducted when sample of cases and controls was genotyped.
[b]Genotypic frequencies of -149C>T and -579G>T in normal controls were tested for departure from Hardy-Weinberg equilibrium (HWE) using the χ^2 test.

Table 6. Pooled ORs and 95% CIs in the Meta-Analysis.

Genetic Model	Heterogeneity Test			Summary OR (95% CI)	Hypothesis Test		Studies (n)
	Q	P	I^2		Z	P	
-149C>T and gastric cancer							
T vs C	1.61	.81	0%	1.25 (0.89-1.76)	1.28	.20	5
TT vs TC	0.27	.99	0%	1.31 (0.92-1.88)	1.48	.14	5
TT vs CC	0.38	.54	0%	0.72 (0.12-4.35)	0.36	.72	2
TC vs CC	0.19	.67	0%	0.68 (0.09-5.04)	0.37	.71	2
TT vs TC+CC	0.85	.93	0%	1.29 (0.90-1.83)	1.39	.16	5
TT+TC vs CC	0.37	.54	0%	0.71 (0.12-4.30)	0.38	.71	2
-149C>T and lung cancer							
T vs C	0.53	.77	0%	0.83 (0.60-1.15)	1.15	.25	3
TT vs TC	0.65	.72	0%	0.85 (0.601.21)	0.90	.37	3
TT vs CC	0.22	.64	0%	0.52 (0.10-2.65)	0.79	.43	2
TC vs CC	0.39	.53	0%	0.57 (0.10-3.17)	0.64	.52	2
TT vs TC+CC	0.35	.84	0%	0.83 (0.59-1.17)	1.03	.30	3
TT+TC vs CC	0.23	.63	0%	0.52 (0.10-2.66)	0.79	.43	2
-579G>T and gastric cancer							
T vs G	1.45	.48	0%	1.69 (1.36-2.10)	4.73	$<1\times10^{-5}$	3
TT vs TG	1.51	.47	0%	1.76 (1.38-2.24)	4.57	$<1\times10^{-5}$	3
TT vs GG	0.01	.93	0%	2.11 (0.88-5.05)	1.68	.09	2
TG vs GG	0.06	.80	0%	1.19 (0.49-2.91)	0.39	.70	2
TT vs TG+GG	1.46	.48	0%	1.78 (1.41-2.25)	4.79	$<1\times10^{-5}$	3
TT+TG vs GG	<0.01	.94	0%	1.89 (0.79-4.51)	1.43	.15	2
-579G>T and lung cancer							
T vs G	1.46	.48	0%	1.17 (0.96-1.43)	1.56	.12	3
TT vs TG	2.27	.32	12%	1.22 (0.98-1.52)	1.74	.08	3
TT vs GG	0.28	.60	0%	1.07 (0.50-2.32)	0.18	.86	2
TG vs GG	0.67	.41	0%	0.90 (0.41-1.97)	0.26	.79	2
TT vs TG+GG	1.90	.39	0%	1.21 (0.97-1.50)	1.71	.09	3
TT+TG vs GG	0.32	.57	0%	1.03 (0.48-2.22)	0.08	.93	2

Abbreviations: CI, confidence interval; OR, odds ratio.

Bonferroni correction for multiple testing (0.05/6 = 0.008). Interestingly, these results of the meta-analysis confirmed our current findings.

Discussion

Various studies have described the roles of -149C>T and -579G>T in different types of cancer including gastric cancer[14,15,18,21,22] and lung cancer.[13,16,17,19,20] Of the 10 studies that attempted to evaluate the association between -149C>T or -579G>T and susceptibility to lung cancer or gastric cancer, 9 studies focused on Chinese[14-22] and 1 on Korean.[13] However, none of the studies has been performed in Hubei Chinese population. Therefore, we analyzed the distribution of -149C>T and -579G>T and assessed their association with risk of gastric cancer and lung cancer in a Chinese population of Hubei province.

In this study, it was demonstrated that the -579 T allele was a harmful effect potentially exhibited by -579G>T polymorphism in gastric tumorigenesis, which was consistent with the finding of Hu *et al*[15] but not Zhang *et al*[21] On the other hand, previous studies suggested a significant association between -579G>T and lung cancer risk in Northeastern Chinese population[16] and Korean population,[13] but this association did not remain statistically in Zhang *et al* study[20] and the present study. One possibility for the discrepancy may be attributed to different environments, lifestyles, and genetic backgrounds among different ethnic populations. Admittedly, the Chinese populations from different geographic regions and small sample size may also contribute to the difference of the results. Of note, alongside previous findings,[14,15,17-19,22] our present results consistently suggested that -149C>T was not associated with the risk of gastric or lung cancer in Chinese population.

To solve the discrepancies and the problem of inadequate statistical strength among previous studies,[28] a meta-analysis was further conducted to systematically evaluate the impacts of DNMT3 -149C>T and -579G>T polymorphisms on individuals' susceptibility to gastric and lung cancer in Chinese population. Interestingly, the pooled results further confirmed that -579G>T was significantly associated with the risk of gastric cancer but not lung cancer, while -149C>T was irrelevant to the risk of gastric and lung cancer. However, additional independent studies with larger sample sizes in Chinese populations across different geographical areas are still needed to validate or further reinforce our present findings.

The recent successful completion of the HapMap project suggested that haplotype analysis would enhance the statistical power in the mapping of human complex trait loci, with the potential of reducing the sample size of association studies.[27,29,30] Our study also included a haplotype study to assess the potential combined effect of -149C>T and -579G>T on risk of lung and gastric cancer. The LD analysis of -149C>T and -579G>T indicated a low LD with each other, suggesting that -149C>T and -579G>T might be sufficient to capture some of the haplotype structures in *DNMT3B* gene.[31] The haplotype -149T/-579 T was significantly associated with an increased risk of gastric cancer, which suggested that -149C>T and -579G>T might act together to affect gastric tumorigenesis. Since -579 T allele was associated with an increased risk of gastric cancer, it was speculated that the -579G>T might be used as a risk biomarker for gastric cancer prediction in Chinese population. To our knowledge, this is the first report of a significant association between haplotype -149T/-579 T of *DNMT3B* gene and gastric cancer, which needs to be further confirmed.

Collectively, our results demonstrated that the *DNMT3B* -579G>T polymorphism is significantly associated with an increased risk of gastric cancer but not lung cancer in Chinese population. In addition, the haplotype -149T/-579 T, carrying the risk -579 T allele, significantly increases the susceptibility of individuals to gastric cancer in Chinese population. Besides, the *DNMT3B* -149C>T polymorphism does not contribute to the risk of lung or gastric cancer in Chinese population. These reported findings may initiate novel prediction and prevention strategy for lung and gastric cancer in Chinese population. However, further confirmatory studies should be undertaken in other ethnic populations because the present observations involved only Chinese population.

Authors' Note

Bifeng Chen and Jingdong Wang contributed equally to this work.

Declaration of Conflicting Interests

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