

Research Article

PLCE1 Polymorphisms and Risk of Esophageal and Gastric Cancer in a Northwestern Chinese Population

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The reported risk susceptibility between *phospholipase C epsilon 1 (PLCE1)* polymorphisms and esophageal cancer (EC) and gastric cancer (GC) remained inconsistent and controversial, especially on variants other than rs2274223. The relationship between *PLCE1* polymorphisms and gene expression is also unclear. Here we conducted a case-control study from northwest China, genotyped seven tag single nucleotide polymorphisms (SNPs) in *PLCE1* with multiplexed SNP MassARRAY assay. Stratified analysis was carried out and *PLCE1* expression was evaluated in specified groups with the method of qRT-PCR and immunohistochemistry. Results showed that the minor alleles of rs3765524, rs2274223, and rs10509670 were associated with increased risk of EC and GC. Linkage disequilibrium analysis revealed protective haplotypes of CCAAGTC and CCAA. By stratification, a more significant association was found in subgroups of male, age ≥ 54 , tumor stages of I-II and tumor size ≤ 5 cm, EC and cardia cancer (CC) of stomach, and moderate to well differentiated squamous carcinoma. In addition, a significant association for rs3765524 with noncardia cancer (NCC) and adenocarcinoma which is predominant in China was also observed. Further expression analysis identified that *PLCE1* was downregulated in NCC tissues comparing to their adjacent noncancerous tissues, and its protein expression was higher in genotype rs3765524 CT/TT than in rs3765524 CC. In summary, our study suggests that *PLCE1* polymorphisms may affect its gene expression and are associated with not only EC and CC, but also, to some extent, NCC risk in this study population.

1. Introduction

Esophageal cancer (EC) and gastric cancer (GC) are the two most common cancers originating from digestive tract around world [1], especially in China [2, 3]. There are many differences between EC and GC, such as genetic background, histological type, and *Helicobacter pylori* infection, while they are both known to be the results of complex interactions between inherited and environmental factors [4, 5].

Phospholipase C epsilon 1 (PLCE1) gene was reported to locate at 10q23, encoding a member of the human phosphoinositide-specific phospholipase C family [6]. It has

been involved in the regulation of cell growth, differentiation, and oncogenesis [7]. Genome wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs), mostly rs2274223 A>G, and rs3765524 C>T in *PLCE1* gene as shared susceptibility loci for EC and GC [8–10].

Although several independent candidate-gene studies have confirmed the association between EC, GC, and *PLCE1* SNPs subsequently, there is more limited data on variants other than rs2274223, especially for GC [11–24]. Moreover, whether these loci are associated with noncardia cancer in addition to cardia and esophageal is not clear; whether or not *PLCE1* polymorphisms affect gene expression and protein

function were only reported in few contradictory results [9, 14, 15, 23, 25–28].

To further explore the association between *PLCE1* polymorphisms and risk of EC and GC or their subtypes, we collected blood samples from Chinese northwestern population and used multiplexed SNP MassARRAY assay to sequence a panel of tag SNPs (tSNPs) of *PLCE1* in a case-control study. We completed a comprehensive analysis by logistic regression and stratification method and examined the expression of *PLCE1* in tissue samples.

2. Material and Methods

2.1. Study Population. A total of 324 GC or EC patients and 357 control volunteer individuals without known malignancies in the Xijing hospital of the Fourth Military Medical University in Xi'an city, China, during 2009 to 2012 were enrolled in the study. The cases had no previous history of other cancers, or prior chemotherapy or radiotherapy. All of the chosen subjects were Chinese Han living in Xi'an city and its surrounding areas. Generally, subjects with chronic diseases and conditions involving vital organs (heart, lung, liver, kidney, and brain) and severe endocrinological, metabolic, and nutritional diseases were excluded from this study. The purpose of the above exclusion procedures was to minimize the known environmental and therapeutic factors that influence the variation of human complex diseases. Peripheral blood samples from GC and EC patients were collected before or after surgery. Formalin-fixed, paraffin-embedded cancer and paired adjacent noncancerous tissues were collected after surgery from part of the GC patients. Patients' clinical data and postoperative pathological reports including the pathological types, pTNM and clinical stages, and the degrees of tumor differentiation were indexed from medical records. The study was approved by the Ethical Committee of Xijing Hospital (Xi'an, China), and this study complied with the World Medical Association Declaration of Helsinki. Informed consent was given by all the subjects for participation in this study.

2.2. DNA Isolation and Genotyping Assays. A panel of seven tSNPs of rs3765524, rs3818432, rs2274223, rs10509670, rs11187852, rs3781264, and rs11187866 in *PLCE1* gene were included in this study. All the SNPs have a disequilibrium (D') threshold of 0.8 and minor allele frequency (MAF) > 0.05 in the HapMap Chinese Han population. Genomic DNA was extracted from peripheral blood using a Blood DNA Extraction Kit (TIANGEN, China), quantified with NanoDrop 2000 (Thermo, USA) and stored at -20°C until use. Primers were designed in a multiplexed SNP MassEXTEND assay with the Sequenom MassARRAY Assay Design 3.0 Software. SNP genotyping was performed by Sequenom MassARRAY RSI000 as reported previously [29]. Data management was conducted and analyzed by Sequenom Typer 4.0 Software.

2.3. Quantitative Real-Time PCR. Total RNA was extracted from tissue samples with E.Z.N.A.TM FFPE RNA Kit (OMEGA, USA). The protocol of total RNA isolation, cDNA

preparation, and qRT-PCR was as reported previously by using the PrimeScriptTM RT Master Mix (Takara, Japan) on a 7500 fast real-time PCR system (Applied Biosystems) [29]. We used the following primers covering the two *PLCE1* spliceosomes, respectively: *PLCE1A*, forward 5'-ATC-ATAGAGACAGGCAGAGCACA-3' and reverse 5'-ATGCCACATAGTTTTCTTTTGC-3'; *PLCE1B*, forward 5'-GATTAATGGTTTCAGAAGGAAGTGC-3' and reverse 5'-CTCCAGCATCCACATCCATCC-3'. Human β -actin was used as an endogenous control. For each sample, we calculated the difference in threshold cycles for each *PLCE1* copy by the $2^{-\Delta\text{CT}}$ method.

2.4. Immunohistochemistry Staining. The procedure of immunohistochemistry staining has been described in our previous publication [29]. Paraffin-embedded tissue specimens were deparaffinized in xylene and then soaked in ethanol and then PBS. We performed antigen retrieval in 100 mM sodium citrate buffer at 100°C for 20 min. Subsequently, we blocked endogenous peroxidase activity in 3% hydrogen peroxide in methanol for 15 min and then blocked nonspecific binding in 5% normal goat serum overnight at 4°C . We incubated sections for 2 hours at room temperature with rabbit anti-*PLCE1* (SIGMA, HPA015598, 1:20 dilution) antibody, and then with alkaline phosphatase conjugated anti-rabbit IgG antibody. We visualized *PLCE1* protein by HistostainTM-Plus Kits (ZYMED, SP-9001). At least three experienced pathologists examined the staining using the following criteria: strong positive (signal in the cancer cells is stronger than the normal gastric gland), positive (signal in the cancer cells is as strong as that in a normal gastric gland), weak positive (signal between positive and negative), and negative (signal is no more than the background signal in the surrounding stromal cells).

2.5. Statistical Analysis. We performed statistical analysis using Microsoft Excel and SPSS 16.0 statistical package (SPSS, Chicago, IL). All P values in this study were two-sided. We considered $P \leq 0.05$ the threshold for statistical significance. We tested genotypic frequencies in control subjects for each SNP for departure from HWE using an exact test. We compared genotype frequencies of case and control subjects using the Chi^2 test. We calculated OR and 95% CI by unconditional logistic regression analysis. There were two factors of age and gender adjusted for the analysis. We used the Haploview program to estimate the pairwise LD between markers and partition haplotype blocks. We inferred haplotypes using the Haploview software package (version 4.2).

3. Results

3.1. Overall Association between the *PLCE1* tSNPs and the Risk of EC and GC. The characteristics of all cases and controls included in the study were listed in Table 1. Seven tSNPs in *PLCE1* gene were genotyped and all of the tSNPs were in Hardy-Weinberg equilibrium (HWE) in the control population ($P > 0.05$).

TABLE 1: Characteristics of case and control.

Variables	Case (324)		Control (357)	
	No.	(%)	No.	(%)
Age, mean years (SD)	57.4	(11.4)	50.4	(12.7)
Sex				
Male	252	(77.8)	212	(59.4)
Female	72	(22.2)	145	(40.6)
Tumor site				
EC	48	(14.8)		
CC	35	(10.8)		
NCC	241	(74.4)		
Pathology				
Adenocarcinoma	223	(68.8)		
Squamous carcinoma	38	(11.7)		
Unspecified	63	(19.4)		
Differentiation				
Poor	117	(36.1)		
Moderate to well	117	(36.1)		
Unspecified	90	(27.8)		
Tumor size				
≤5cm	153	(36.2)		
>5cm	71	(16.8)		
Unspecified	199	(47.0)		
Tumor stage				
I-II	174	(53.7)		
III-IV	62	(19.1)		
Unspecified	88	(27.2)		

EC, esophageal cancer; CC, cardia cancer; NCC, noncardia cancer.

After genotyping, we conducted logistic regression to evaluate the association of each tSNP with the risk of EC and GC (Table 2). Results showed that there were three tSNPs (rs3765524, rs2274223, and rs10509670) associated with the risk of EC and GC: rs3765524 (CT vs CC, OR = 1.66, 95% CI 1.16-2.38, $P = 0.006$; CT/TT vs CC, OR = 1.65, 95% CI 1.17-2.34, $P = 0.004$); rs2274223 (AG vs AA, OR = 1.57, 95% CI 1.10-2.26, $P = 0.014$; AG/GG vs AA, OR = 1.55, 95% CI 1.10-2.20, $P = 0.013$); rs10509670 (AG vs AA, OR = 1.54, 95% CI 1.07-2.21, $P = 0.019$; AG/GG vs AA, OR = 1.54, 95% CI 1.09-2.18, $P = 0.014$).

3.2. Linkage Disequilibrium and Haplotype Evaluation for the *PLCE1* tSNPs. Linkage disequilibrium (LD) analysis revealed that the seven tSNPs of *PLCE1* linked with each other (Figure 1). Haplotype “CCAAGTC” accounted for 71.5% of the whole haplotypes in EC and GC cases. This is a protective haplotype against the risk of EC/GC (OR = 0.72; 95% CI = 0.53-0.97; $P = 0.029$) (Table 3). Further analysis revealed that the LD block could be divided into two subblocks (Figure 1(a)). Subblock 1 ($r^2 > 0.79$) was composed of four tSNPs of rs3765524, rs3818432, rs2274223, and rs10509670, where the three risk SNPs identified above were included. Subblock 2 ($r^2 > 0.87$) included the later three tSNPs of rs11187852, rs3781264, and rs11187866. In subblock 1, “CCAA” accounted for 72.4% of the whole haplotypes in EC/GC

cases and was found to be the protective haplotype against the risk of EC/GC (OR = 0.67; 95% CI = 0.49-0.91; $P = 0.009$).

3.3. Stratified Analysis for the Clinicopathologic Data of Patients. Anatomically, gastric cancer includes cardia cancer (CC) and noncardia cancers (NCC). Pathologically, gastric cancer has adenocarcinoma and squamous carcinoma. Then we performed a stratified analysis to determine the association between the three tSNPs (rs3765524, rs2274223, and rs10509670) and clinicopathologic data in dominant model (Table 4). Significant association between the three tSNPs and risk of EC and GC was observed for subgroup patients of male, age ≥ 54 , tumor stages of I-II and tumor size ≤ 5 cm, EC and cardia cancer (CC), and moderate to well differentiated squamous carcinoma. In addition, a significant association for rs3765524 with noncardia cancer (NCC) and adenocarcinoma was also observed.

3.4. Expression Distribution of *PLCE1* Protein in Stomach Tissue. Now that the association between *PLCE1* polymorphisms and GC risk exhibited disparity according to the tumor subsites, we then evaluated the expression distribution of *PLCE1* protein in human GC and adjacent noncancer tissues (ANC) by tissue microarray. In the ANC tissue,

TABLE 2: Logistic regression of candidate tSNPs in *PLCE1* and their association with EC and GC risk.

ID	Genotype	Case (n = 324)		Control (n = 357)		Crude OR		P	Adjusted OR		P	HWE-P
		No.	(%)	No.	(%)	(95%CI)	(95%CI)					
rs3765524	CC	168	(51.9)	223	(62.6)	1			1			0.896
	CT	136	(42.0)	117	(32.9)	1.54	(1.12-2.12)	0.008	1.66	(1.16-2.38)	0.006	
	TT	20	(6.2)	16	(4.5)	1.66	(0.83-3.30)	0.149	1.60	(0.72-3.55)	0.247	
	CT/TT	156	(48.1)	133	(37.4)	1.56	(1.15-2.11)	0.005	1.65	(1.17-2.34)	0.004	
rs3818432	CC	104	(57.5)	193	(65.6)	1			1			0.856
	CA	73	(40.3)	91	(31.0)	1.49	(1.01-2.20)	0.045	1.47	(0.97-2.23)	0.073	
	AA	4	(2.2)	10	(3.4)	0.74	(0.23-2.42)	0.622	0.87	(0.24-3.09)	0.826	
	CA/AA	77	(42.5)	101	(34.4)	1.41	(0.97-2.07)	0.074	1.41	(0.94-2.13)	0.098	
rs2274223	AA	177	(54.8)	229	(64.1)	1			1			0.457
	AG	126	(39.0)	111	(31.1)	1.47	(1.06-2.03)	0.019	1.57	(1.10-2.26)	0.014	
	GG	20	(6.2)	17	(4.8)	1.52	(0.77-2.99)	0.223	1.43	(0.66-3.14)	0.367	
	AG/GG	146	(45.2)	128	(35.9)	1.48	(1.08-2.01)	0.013	1.55	(1.10-2.20)	0.013	
rs10509670	AA	178	(54.9)	229	(64.5)	1			1			0.391
	AG	124	(38.3)	109	(30.7)	1.46	(1.06-2.02)	0.021	1.54	(1.07-2.21)	0.019	
	GG	22	(6.8)	17	(4.8)	1.66	(0.86-3.23)	0.132	1.54	(0.72-3.32)	0.268	
	AG/GG	146	(45.1)	126	(35.5)	1.49	(1.10-2.03)	0.011	1.54	(1.09-2.18)	0.014	
rs11187852	GG	122	(67.4)	212	(72.1)	1			1			0.506
	GA	56	(30.9)	77	(26.2)	1.26	(0.84-1.90)	0.263	1.33	(0.85-2.07)	0.212	
	AA	3	(1.7)	5	(1.7)	1.04	(0.24-4.44)	0.955	1.68	(0.34-8.18)	0.522	
	GA/AA	59	(32.6)	82	(27.9)	1.25	(0.84-1.87)	0.276	1.34	(0.87-2.08)	0.185	
rs3781264	TT	120	(66.3)	206	(70.1)	1			1			0.511
	TC	58	(32.0)	82	(27.9)	1.21	(0.81-1.82)	0.347	1.24	(0.80-1.92)	0.331	
	CC	3	(1.7)	6	(2.0)	0.86	(0.21-3.49)	0.831	1.07	(0.23-5.00)	0.932	
	TC/CC	61	(33.7)	88	(29.9)	1.19	(0.80-1.77)	0.390	1.23	(0.80-1.89)	0.340	
rs11187866	CC	120	(66.7)	210	(71.4)	1			1			0.723
	CG	56	(31.1)	76	(25.9)	1.29	(0.85-1.95)	0.226	1.33	(0.85-2.08)	0.210	
	GG	4	(2.2)	8	(2.7)	0.88	(0.26-2.97)	0.830	1.13	(0.30-4.23)	0.857	
	CG/GG	60	(33.3)	84	(28.6)	1.25	(0.84-1.86)	0.274	1.31	(0.85-2.03)	0.218	

Notes. The adjusted OR is derived from the correction for age and gender. The results were in bold, if the *P* value < 0.05. EC, esophageal cancer; GC, gastric cancer.

TABLE 3: Haplotypes of *PLCE1* and their association with EC and GC risk.

ID	Block	Haplotype	Frequency ^a		OR	(95%CI)	Chi ²	P ^b
			Case	Control				
1	Global	CCAAGTC	71.5%	77.8%	0.72	(0.53-0.97)	4.742	0.029
2		TAGGACG	16.8%	14.6%	1.18	(0.83-1.69)	0.861	0.354
3		TAGGGTC	4.7%	2.9%	1.66	(0.83-3.28)	2.115	0.146
4		TCAAGTC	3.0%	1.2%	2.49	(0.97-6.41)	3.834	0.050
5		TCGGGTC	2.2%	1.2%	1.82	(0.66-5.03)	1.381	0.240
6	Sub-1	CCAA	72.4%	79.7%	0.67	(0.49-0.91)	6.756	0.009
7		TAGG	22.1%	17.6%	1.33	(0.96-1.84)	2.859	0.091
8		TCAA	3.0%	1.2%	2.48	(0.95-6.42)	3.752	0.053
9		TCGG	2.2%	1.2%	1.81	(0.65-5.04)	1.331	0.249
10	Sub-2	GTC	81.5%	83.2%	0.89	(0.63-1.25)	0.436	0.509
11		ACG	17.1%	14.8%	1.19	(0.83-1.70)	0.921	0.337

Notes. (A) Only haplotypes with frequencies of $\geq 3\%$ are shown. (B) The results were in bold, if the *P* value < 0.05. EC, esophageal cancer; GC, gastric cancer.

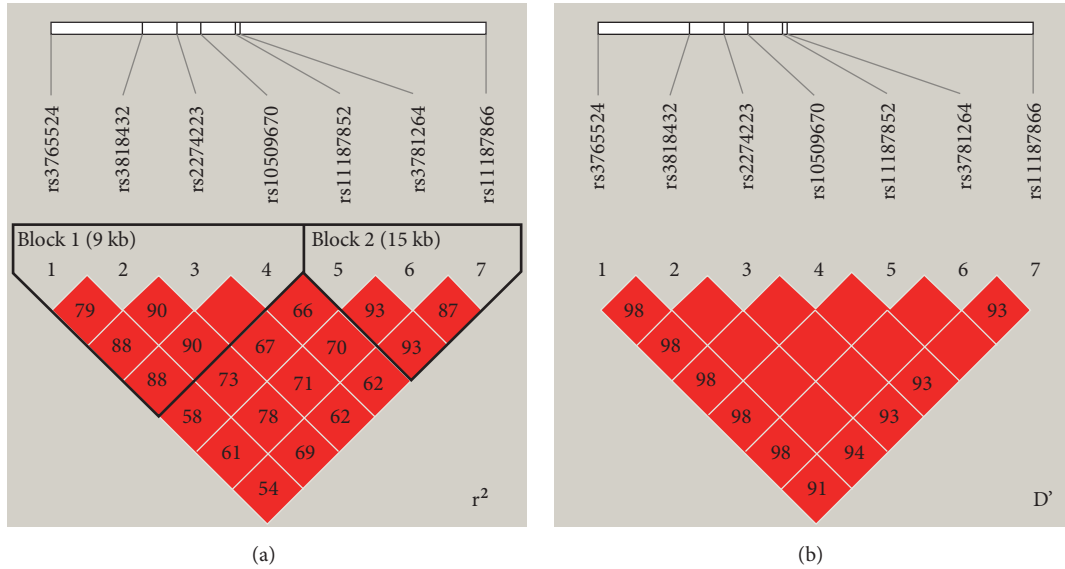


FIGURE 1: Linkage disequilibrium (LD) analysis of *PLCE1* tSNPs. (a) r^2 of LD analysis, which showed that the seven tSNPs are linked to each other and could be divided into two subblocks. (b) D' of LD analysis.

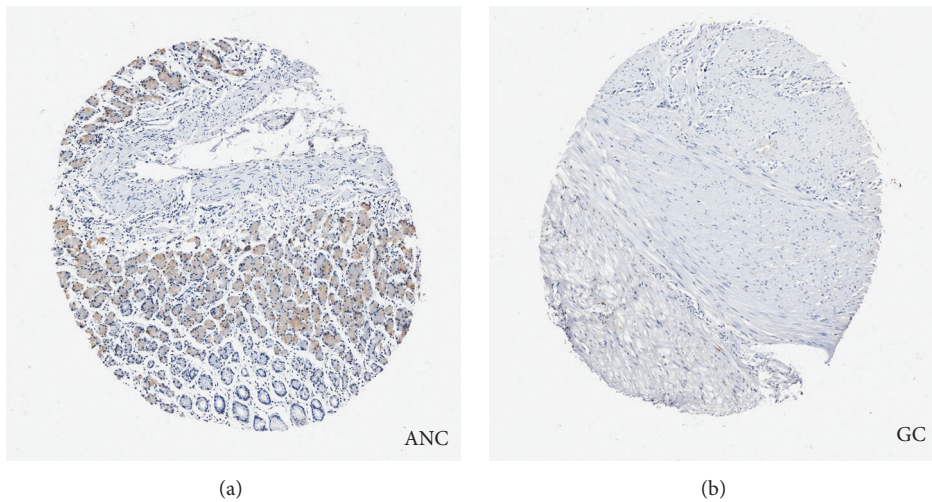


FIGURE 2: Representative images of *PLCE1* expression distribution in gastric cancer (GC) and adjacent noncancerous (ANC) tissues by tissue microarray. (a) ANC tissues. *PLCE1* protein was expressed in the cytoplasm of columnar epithelial cells and mainly distributed in the junction of cardia and gastric fundus glands (200 \times). (b) GC tissues. Structure distortion and confusion of tubular glands, heterogeneity of epithelial cells with irregular nuclear staining, and lower expression of *PLCE1* were observed (200 \times).

PLCE1 protein expression was positive in the cytoplasm of columnar epithelial cells and mainly distributed in the junction of cardia and gastric fundus glands (Figure 2(a)). In the GC tissue, the structure distortion and confusion of tubular glands, obvious heterogeneity of epithelial cells with irregular nuclear staining, and lower expression of *PLCE1* protein were observed (Figure 2(b)). These suggested, together with the results of rs3765524 genotyping by stratified analysis, that *PLCE1* protein may be involved in carcinogenesis of NCC and adenocarcinoma, although more significant association has been found with EC, CC, and squamous carcinoma.

3.5. Effect of *PLCE1* Polymorphisms on Its Expression in NCC and ANC Tissues. Because NCC and adenocarcinoma have been the predominant subtype of gastric cancer in China, we then examined the expression of *PLCE1* in adenocarcinoma of NCC and their ANC tissues with different genotypes of rs3765524. For mRNA transcription, we identified two *PLCE1* processing units, *PLCE1A* and *PLCE1B*, by quantitative real-time PCR (qRT-PCR). As presented in Figures 3(a)(i) and 3(a)(ii), compared with ANC tissues, the two processing units were both downregulated in NCC tissues (*PLCE1A*: 22 of 28, 78.57%, $P = 0.034$; *PLCE1B*: 21 of 28, 75.00%, $P = 0.021$), while there was no significant difference between genotype

TABLE 4: Stratified analysis for the association between tSNPs in *PLCE1* and EC and GC risk.

Variables	rs3765524 (Case/Control)		Adjusted OR (95%CI)	P	rs2274223 (Case/Control)		Adjusted OR (95%CI)	P	rs10509670 (Case/Control)		Adjusted OR (95%CI)	P
	CC	CT/TT			AA	AG/GG			AA	AG/GG		
Age, years												
≤ 53	58/159	55/109	1.34	0.203	62/161	50/107	1.18	0.464	63/161	50/105	1.18	0.478
>53	110/64	101/24	2.56	0.002	115/68	96/21	2.63	0.002	115/68	96/21	2.63	0.002
Sex												
Male	130/137	122/74	1.84	0.006	136/139	116/73	1.72	0.014	135/139	117/72	1.76	0.010
Female	38/86	34/59	1.34	0.339	41/90	30/55	1.25	0.476	43/90	29/54	1.16	0.636
Tumor site												
EC	25/223	23/133	2.61	0.013	24/229	23/128	3.01	0.005	25/229	23/126	1.40	0.022
CC	11/223	24/133	5.99	<0.001	12/229	23/128	5.31	<0.001	12/229	23/126	5.31	<0.001
NCC	142/223	114/133	1.47	0.036	152/229	104/128	1.36	0.098	152/229	104/126	1.36	0.096
Pathology												
Adenocarcinoma	172/223	141/133	1.45	0.041	181/229	132/128	1.36	0.092	180/229	133/126	1.38	0.078
Squamous carcinoma	21/223	20/133	2.23	0.037	20/229	21/128	2.71	0.010	20/229	21/126	2.72	0.009
Differentiation												
Poor	85/223	59/133	1.30	0.222	86/229	58/128	1.33	0.182	86/229	58/126	1.40	0.022
Moderate to well	92/223	83/133	1.72	0.026	97/229	78/128	1.61	0.050	96/229	79/126	1.67	0.034
Tumor size												
≤ 5 cm	104/223	100/133	1.81	0.005	114/229	90/128	1.59	0.027	115/229	89/126	1.54	0.039
> 5 cm	58/223	43/133	1.44	0.148	60/229	41/128	1.39	0.192	60/229	41/126	1.40	0.183
Tumor stage												
I-II	129/223	115/133	1.60	0.016	136/229	108/128	1.50	0.036	137/229	107/126	1.47	0.047
III-IV	45/223	32/133	1.38	0.262	47/229	30/128	1.35	0.297	45/229	32/126	1.53	0.134

Notes: The adjusted OR is derived from the correction for age and gender. The results were in bold, if the P value < 0.05.

EC, esophageal cancer; GC, gastric cancer; CC, cardia cancer; NCC, noncardia cancer.

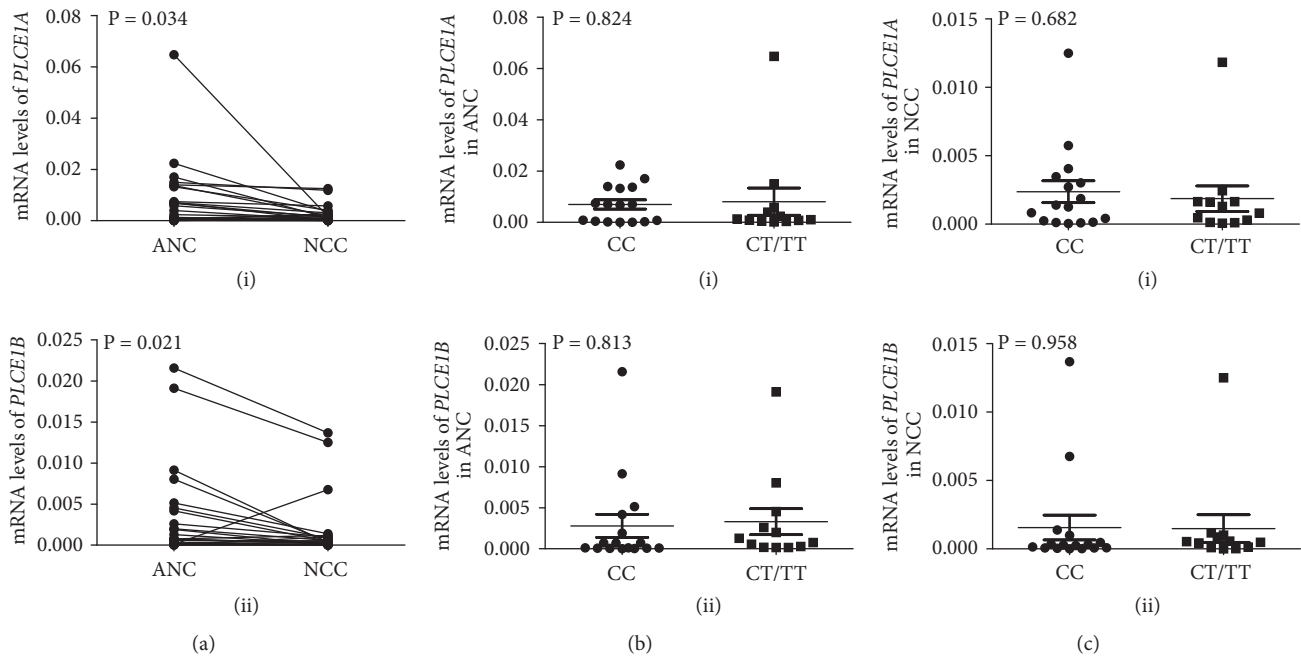


FIGURE 3: *PLCE1* mRNA expression in adenocarcinoma of noncardia cancer (NCC) and adjacent noncancerous (ANC) tissues by qRT-PCR. (a) *PLCE1A* (i) and *PLCE1B* (ii) mRNA expression in ANC and NCC tissues, respectively. (b) *PLCE1A* (i) and *PLCE1B* (ii) mRNA expression in ANC tissues with different genotypes of rs3765524. (c) *PLCE1A* (i) and *PLCE1B* (ii) mRNA expression in NCC tissues with different genotypes of rs3765524. Horizontal lines indicate mean \pm SE (n = 28).

rs3765524 CC and rs3765524 CT/TT both in ANC (Figures 3(b)(i) and 3(b)(ii)) and in NCC (Figures 3(c)(i) and 3(c)(ii)).

For protein translation, immunohistochemistry (IHC) staining revealed that PLCE1 protein expression was generally downregulated in NCC than in their ANC tissues regardless of rs3765524 genotype (6 of 13, 46.15%, $P = 0.018$, see in Figures 4(a), 4(b), and 4(c)(i)). By genotyping, the PLCE1 protein expression was found higher in group of rs3765524 CT/TT than in group of rs3765524 CC both in ANC (Figure 4(c)(ii). $P = 0.031$) and NCC tissues (Figure 4(c)(iii). $P = 0.045$).

4. Discussion

SNPs are the most common type of genetic variation, which makes them excellent biological markers [30]. On the other hand, SNPs, including those that fall within the coding or noncoding regions of genes, may affect the gene transcription and translation, as well as the structure and function of protein, contributing to changing the host susceptibility to diseases [31].

GWAS study found that some SNPs in *PLCE1* corresponding to Y, C2, and RA domain were associated with the risk of EC and GC [8–10]. These are very important domains to PLCE1. The Y domain folds to form the catalytic core of the phospholipase and the C2 domain can bind to phospholipid [32]. RA domain is in the C terminal of PLCE1 protein, which interacts directly with upstream regulators of Ras, Rap, and others [33]. The genomic region for Y, C2, and RA domains spans from exon 24 to exon 33. By referring to the frequencies of SNPs in Chinese Han population in HapMap database,

after removing the SNPs with minimum allele frequency (MAF) less than 0.05, seven candidate SNPs in the region were selected in our study, where rs3765524 was in exon 24 and in Y domain, rs3818432 was in intron 24, rs2274223 was in exon 26 and in C2 domain, rs10509670 was in intron 26, rs11187852 and rs3781264 were in intron 27, and rs11187866 was in intron 32.

By genotyping and logistic regression, we not only confirmed the two previous reported SNPs of rs3765524 and rs2274223 [8–10] but also revealed that another SNP of rs10509670 in *PLCE1* was associated with the risk of EC and GC susceptibility. rs3765524 C>T causes an amino acid change from Thr to Ile (ACC177ATC), and rs2274223 A>G can also cause a missense mutation of His to Arg (CAC192CGC). These two SNPs are corresponding to the Y and C2 domain of PLCE1 protein, respectively. We noticed that Thr, His, and Arg are frequently modified amino acid residues in human proteins. Different posttranslational modification may alter the structure, stability, and function of PLCE1 protein [34]. In the case of rs3765524, we found that although there was no difference in mRNA transcription between wild type and mutant type (Figure 3), there was a difference in protein expression (Figure 4). Among them, the expression of CT/TT genotype was higher than that of CC genotype in both NCC and ANC groups, implying that the amino acid change by the polymorphism of rs3765524 might lead to different protein modifications or structural changes, ultimately affecting *PLCE1* expression or stability.

The third loci of rs10509670 located in the intron of *PLCE1* gene has also shown to be associated with risk of EC and GC

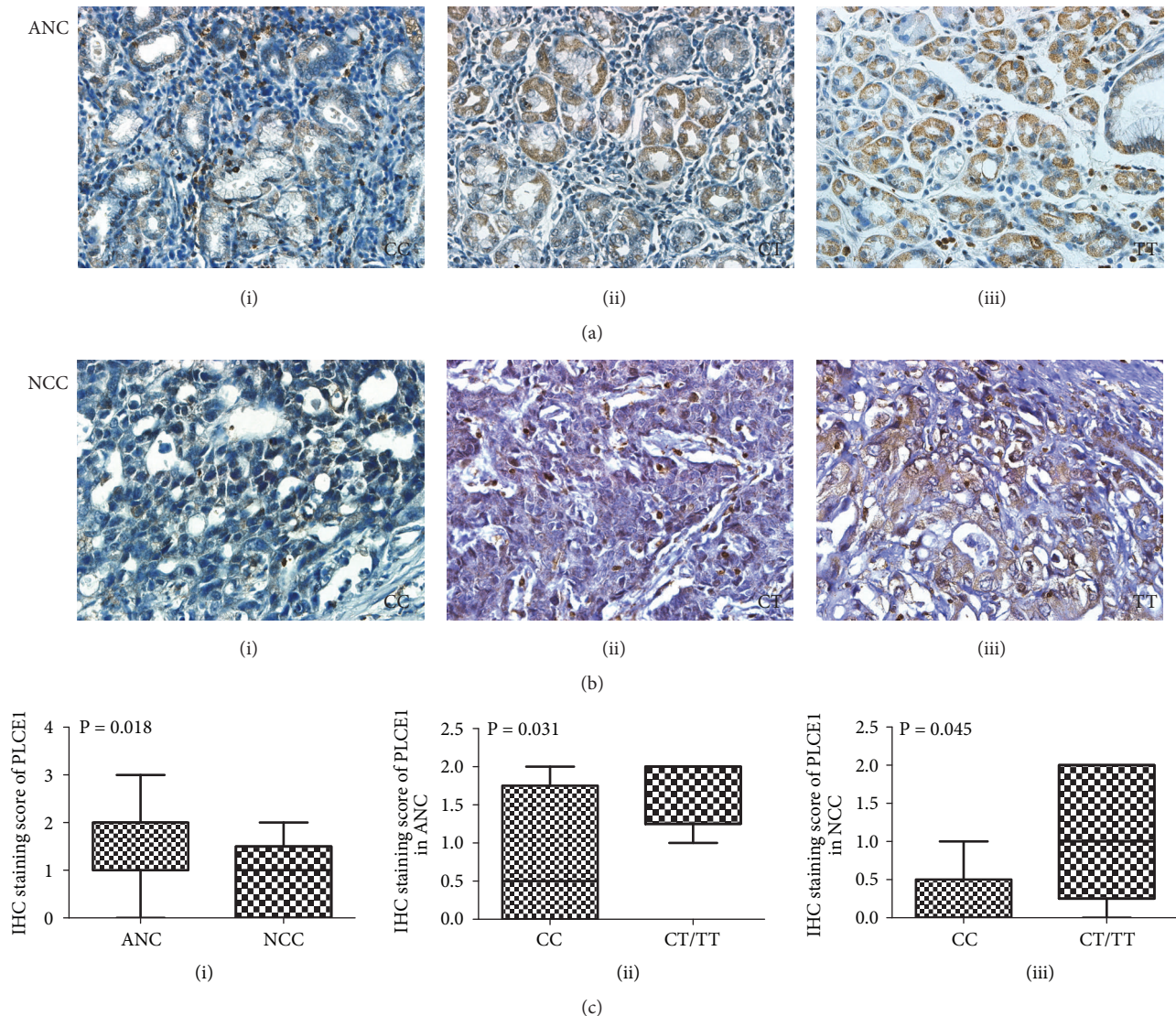


FIGURE 4: *PLCE1* protein expression in adenocarcinoma of noncardia cancer (NCC) and adjacent noncancerous (ANC) tissues by immunohistochemistry (IHC) staining. (a) Representative images of *PLCE1* expression in ANC tissues with different genotypes of rs3765524 (i) CC, (ii) CT, and (iii) TT (400 \times). (b) Representative images of *PLCE1* expression in NCC tissues with different genotypes of rs3765524 (i) CC, (ii) CT, and (iii) TT (400 \times). (c) IHC staining score comparison of *PLCE1* (i) between ANC and NCC tissues regardless of rs3765524 genotyping, (ii) in ANC tissues with different genotypes of rs3765524, (iii) in NCC tissues with different genotypes of rs3765524. The data was represented as mean \pm SE (n = 13).

in the experiment. We hypothesize that rs10509670 A>G may affect *PLCE1* gene structure or expression by regulating gene splicing or transcription [31]. In the study, the seven tSNPs have been proved to be in LD. Moreover, we identified two haplotypes associated with EC and GC risk. The haplotype of “CCAAGTC” (corresponding to Y, C2, and RA domains) and the haplotype in subblock 1 of “CCAA” (corresponding to Y and C2 domains) have decreased risk of EC and GC of 33% and 28%, respectively.

Previous studies have exhibited different associations between *PLCE1* polymorphisms and the risk of EC and GC, especially for different tumor subsites of GC in several candidate-gene studies [11–24]. The latest large meta-analyses

confirmed the G allele of *PLCE1* rs2274223 to be associated with an increased risk of cardia cancer (CC) rather than noncardia cancer (NCC) [35]. In our stratification analysis, we not only confirmed the T allele of rs3765524 and G allele of rs2274223 but also identified that the G allele of rs10509670 was associated with increased risk of EC and CC susceptibility. Furthermore, we revealed a significant association of rs3765524 C>T with the increased risk of NCC and adenocarcinoma. As we know, NCC has predominant incidence among digestive tract tumors in China [36, 37].

So far, the literature reports about *PLCE1* expression and distribution were still unclear and conflicting. Previously, we conducted a comprehensive analysis of *PLCE1* expression

in atrophic gastritis and GC tissues, which revealed that differential expression of *PLCE1* may distinguish GC from inflammation lesions [28]. In terms of tumorous-normal comparison, upregulation and downregulation of *PLCE1* were both found in EC and CC at mRNA and/or protein levels [9, 15, 25, 26], while there was only one study that identified downregulation of *PLCE1* at mRNA level in NCC [26]. In terms of the comparison of minor-major alleles of rs2274223 with *PLCE1* expression, the results for EC were also inconsistent [14, 15, 38], and there is no report about CC and NCC until now. Another two studies reported the expression of *PLCE1* in GC but without specific tumor subsites information (CC or NCC), which presented opposite conclusions for tumorous to normal comparison [23, 28].

By tissue microarray, we identified that *PLCE1* protein is expressed not only in cardia but also in gastric fundus glands both in GC and ANC tissues. This result, together with the association of rs3765524 C>T with NCC risk, suggests that *PLCE1* protein may be involved in carcinogenesis of NCC. Therefore, we used qRT-PCR and IHC to study genetic variation effects on *PLCE1* expression in NCC and their ANC tissues. Results showed that the expression of *PLCE1* at both mRNA and protein levels was lower in NCC tissues than in their ANC tissues, which supports the hypothesis that *PLCE1* may function as a tumor suppressor. We also found that rs3765524 genotype may affect *PLCE1* expression, where *PLCE1* expression was higher in group of rs3765524 CT/TT than in group of CC. This strongly suggests, as one of the contributors, the reference allele C of rs3765524 loss of expression in tumor, but the mutated T allele, on the other hand, produces a “dominant negative” phenotype, which is related to the increased risk of NCC. Of course, the exact mechanism needs to be studied further. To our knowledge, this is the first report about *PLCE1* expression distribution in NCC by genotypes.

PLCE1A and *PLCE1B* arise from alternative splicing at the amino terminus of *PLCE1* protein. *PLCE1A* is composed of 2303aa. *PLCE1B* is composed of 1994aa which is truncated at the amino terminal of the peptide [39]. The different distribution and function of the two subunits in gastric carcinogenesis have not been studied yet. We demonstrated, through qRT-PCR, that both *PLCE1A* and *PLCE1B* were downregulated in NCC than their ANC tissues. This suggests that *PLCE1A* and *PLCE1B* may be involved in NCC carcinogenesis.

5. Conclusion

Our study reveals that *PLCE1* polymorphisms may affect gene expression and function and are associated with the risk of not only EC and CC, but also, to some extent, NCC in northwestern Chinese population. The tSNPs of *PLCE1* may have a potential possibility to be biomarkers for prewarning and diagnosis against these diseases.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this article.

Authors' Contributions

All authors contributed to the design, experiment of the study, and the preparation and critical revision of the manuscript and agreed to be accountable for all aspects of the study. The manuscript is approved by all authors for publication. Ping Liang and Wentao Zhang contributed equally to this work.

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