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Association of functional polymorphisms in MMPs genes with gastric cardia adenocarcinoma and esophageal squamous cell carcinoma in high incidence region of North China

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Abstract The aim of the present study was to investigate the association of single nucleotide polymorphisms (SNPs) in matrix metalloproteinase (MMPs) with the risk of gastric cardia adenocarcinoma (GCA) and esophageal squamous cell carcinoma (ESCC). Genotypes were analyzed by polymerase chain reaction-restriction fragment-length polymorphism method in 592 patients and 624 healthy individuals. Significant differences in allele and genotype distributions of MMP-2 -1306C → T SNP were observed between ESCC and controls ($P = 0.02$ and 0.01, respectively). Compared with the C/T + T/T genotypes, C/C genotype significantly increased the risk of ESCC (OR = 1.57, 95% CI = 1.10–2.23), especially in individuals in smoker group and in the group with positive family history. The stratification analysis showed there were risk changes of GCA for -735C/C genotype carrier in non-smoker, for MMP-12 -82G allele and MMP-13 -77A/G genotype carrier in smoker. Our study indicated that these four functional polymorphisms might play roles in developing ESCC and GCA in high incidence region of North China.

Keywords Gastric cardia adenocarcinoma · Esophageal squamous cell carcinoma · Matrix metalloproteinase (MMPs) · Single nucleotide polymorphism

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Introduction

Esophageal squamous cell carcinoma (ESCC) is the common type of upper gastrointestinal cancer, with evident characteristics of geographical distribution about its development. China is a high incidence country for esophageal squamous cell carcinoma; the incidence and mortality are half of world level. Cixian County and Shexian County of Hebei province lie in the southern foot of Taihang Mountain, a border area of Hebei, Henan and Shanxi provinces, which is one of the high-risk areas for esophageal cancer in China. Gastric cardia adenocarcinoma (GCA) as another prevalent tumor is named as adenocarcinoma of the oesophago-gastric junction (OGJ) by World Health Organization (WHO) [1]. An increased incidence of GCA was observed in Europe [2]. Epidemiological studies have suggested that in China GCA shares very similar geographic distribution with ESCC, especially in a population of high incidence region. Studies have showed that the trend of the incidence and mortality of ESCC had decreased slightly, on the other hand, GCA showed a significant increase trend in Cixian County and Shexian County in Hebei province for the past few years [3]. The different trends of incidence between ESCC and GCA may have the indication that difference characteristic of molecular biology may exist. Thus, the comparative study on ESCC and GCA particularly in a local place is critical in order to understand the risk factor and pathogenesis of these diseases.

Matrix metalloproteinase (MMP), a zinc protease, decomposes the extracellular matrix (ECM) and basal membrane and plays a leading role in process of tumor invasion and metastasis. Recent studies have demonstrated that MMPs are involved in early tumorigenesis by modulating cell proliferation, apoptosis, and host immune surveillance

[4]. MMP-2, the major structural component of basement membrane, also known as 72 kDa gelatinase, primarily hydrolyzes type IV collagen [5]. In addition, active MMP-2 degrades insulin-like growth factor binding proteins and releases insulin-like growth factors, which are well known to have a strong effect on stimulating cell proliferation and inhibiting apoptosis [4]. MMP-12 (human macrophage metalloelastase) and MMP-13 (human collagenase-3) are located in the same chromosome region (11q22). MMP-12 promotes angiogenesis by cleaving structural components of the extracellular matrix, such as collagen type IV and fibrin [4]. MMP-13 cleaves native collagen but has a higher activity on type II collagen than MMP-1. It also acts to degrade various extracellular macromolecules including proteoglycans [6]. These activities of MMPs are believed to be linked to both cancer development and progression.

Somatic mutation of the MMPs gene in cancer has not been reported so far, suggesting that the overexpression of MMPs is probably due to the change of transcriptional and not gene amplification or an activating mutation. Several single nucleotide polymorphisms (SNPs) in the MMPs promoter region have been identified, functional analysis of these SNPs suggested that modulation the transcriptional activity of MMPs may increase the risk of individual tumor incidence [7–11]. Previously, we have investigated the association of the single nucleotide polymorphism in MMP-1, MMP-3 and MMP-7 genes promoter with the risk of ESCC and GCA, and the results indicate that the SNPs of MMPs may play different roles in developing ESCC and GCA [12–14]. MMP-2 -1306C → T, -735C → T; MMP-12 -82A → G and MMP-13 -77A → G, were functional polymorphisms have been described that seem to alter transcriptional levels [15–18]. Based on our previous finding, in this study, we further studied the association of the four polymorphisms with the risk of ESCC and GCA development.

Materials and methods

Study participants

This study included 592 patients (335 with ESCC and 257 with GCA) and 624 healthy individuals. The cases were outpatients for endoscopic biopsy or inpatients for tumor resection in the local tumor hospitals in Cixian County and Shexian County between 2003 and 2006. All patients were pathologically confirmed by the local county hospitals. Esophageal carcinomas were all squamous cell carcinomas. Gastric cardiac carcinomas were all adenocarcinomas with their epicenters at the gastroesophageal junction, i.e., from 1 cm above until 2 cm below the junction between the end of the tubular esophagus and the beginning of the saccular stomach [19]. Healthy subjects were recruited from Cixian County and Shexian County during the endoscopic screening campaign between 2003 and 2006. All the cancer patients and control subjects were unrelated Han nationals. Information of sex, age, smoking habit and family history was obtained from cancer patients and healthy controls by an interview following sampling. For smoking habit, the former and present smoking status, the number of cigarettes per day, and the time of starting and quitting were inquired. Individuals who formerly or currently smoked five cigarettes/day for at least. Individuals with at least one-first-degree relative or two-second-degree relatives having esophageal/cardiac/gastric cancer were defined as having a family history of upper gastrointestinal cancers (UGIC). Smoking status and family history were only available from a subset of cancer patients and healthy controls (Table 1). The study was approved by the Ethics Committee of Hebei Cancer Institute and informed consent was obtained from all recruited subjects.

DNA extraction

Venous blood (5 ml) was collected from each subject into Vacutainer tubes containing EDTA and stored at 4°C. After sampling, genomic DNA was extracted within 1 week by proteinase K (Merck, Darmstadt, Germany) digestion followed by a salting out procedure according to the previously described method [20].

MMP-2 -1306C → T, -735C → T; MMP-12 -82A → G and MMP-13 -77A → G genotyping

The MMP-2 -1306C → T, -735C → T; MMP-12 -82A → G and MMP-13 -77A → G genotypes were determined by polymerase chain reaction-restriction fragment-length polymorphism (PCR-RFLP) assay. The primers for amplifying the MMP-2, MMP-12, and MMP-13 promoter fragments are showed in Table 2. The PCR was performed in a 20 μl volume containing 100 ng of DNA template, 2.4 μl of 10× PCR buffer, 1 U of Taq DNA polymerase (Tiangen Biotech Co., Ltd, Beijing, China), 0.4 μl of 10 mmol/l dNTPs and 200 nM of each primer. The PCR cycling conditions were 5 min at 94°C followed by 35 cycles of 45 s at 94°C, 45 s at 58°C for -1306C → T, 65.5°C for -735C → T, 57°C for -82A → G and 53°C for -77A → G, and 45 s at 72°C, with a final step at 72°C for 10 min to allow for the complete extension of all PCR fragments. The 8 μl aliquot of every PCR product was subjected to digestion at 37°C overnight in a 10 μl reaction containing 10 U of respective restriction enzyme. After digestion, the products were separated on a 4% agarose gel that was stained with ethidium bromide. The length of PCR products, restriction enzymes, and fragments length are summarized in Table 1.

Table 1 PCR conditions for MMP-2, MMP-12 and MMP-13 restriction fragment length polymorphisms

Polymorphisms	Primers	Product length	Restriction enzyme	Fragment length
MMP-2				
-1306C–>T	5′-CTTCCTAGGCTG GTCCTTACTGA-3′ (F)	193 bp	*Xsp*I	188 + 5 bp(C)
	5′-CTGAGACCTGAAG AGCTAAAGAGCT-3′ (R)			162 + 26 + 5 bp(T)
-735C–>T	5′-GGATTCTTGGC TTGGCGCAGGA-3′ (F)	391 bp	*Hin*fI	391 bp(C)
	5′-GGGGGCTGGGTA AAATGAGGCTG-3′ (R)			338 + 53 bp(T)
MMP-12				
-82A–>G	5′-GAGATAGTCAAG GGATGATATCA-3′ (F)	199 bp	*Pvu*II	199 bp
	5′-AAGAGCTCCAG AAGCAGTGG-3′ (R)			175 bp + 24 bp
MMP-13				
-77A–>G	5′-GATACGTTCTTA CAGAAGGC-3′ (F)	445 bp	*Xsp*I	445 bp
	5′-GACAAATCATC TTCATCACC-3′ (R)			244 bp + 201 bp

Table 2 Demographic characteristics in ESCC, GCA patients and healthy controls

Group	Controls *n*(%)	ESCC		GCA	
		n (%)	*P* value[a]	*n* (%)	*P* value[a]
Gender					
Male	400(64.1)	225(67.2)		168(65.4)	
Female	224(35.9)	110(32.8)	0.343	89(34.6)	0.107
Mean age (SD)	60.4(8.42)	60.1(9.33)	0.55[b]	60.5(8.30)	0.95[b]
Smoking status					
Smokers	264(42.3)	133(39.7)		126(49.0)	
Non-smokers	360(57.7)	202(60.3)	0.44	131(51.0)	0.07
Family history of UGIC					
Positive	221(35.4)	162(48.4)		124(48.2)	
Negative	403(64.6)	173(51.6)	0.00[c]	133(51.8)	0.00[d]

ESCC esophageal squamous cell carcinoma; *GCA* gastric cardiac adenocarcinoma; *UGIC* upper gastrointestinal cancer

[a] *P* value for Chi-square test

[b] *P* value for *T* test

[c] Age, gender and smoking status adjusted odds ratio (OR) = 1.71, 95% confidence interval (CI) = 1.30–2.24

[d] Age, gender and smoking status adjusted odds ratio (OR) = 1.70, 95% confidence interval (CI) = 1.27–2.28

For a negative control, distilled water was used instead of DNA in the reaction system for each panel of PCR. The PCR reactions of 10% of the samples were run in duplicate for quality control.

Statistical analysis

Statistical analysis was performed using SPSS11.5 software package (SPSS Company, Chicago, Illinois, USA).

Hardy–Weinberg analysis was performed to compare the observed and expected genotype frequencies using the Chi-square test. Comparison of the MMP-2 -1306C → T, -735C → T; MMP-12 -82A → G and MMP-13 -77A → G genotype distributions in the study groups was performed by means of two-sided contingency tables using Chi-square test. The MMP-2 -1306C → T and -735C → T haplotype frequencies and linkage disequilibrium coefficient were estimated using the EH linkage software (version 1.2, Rockefeller University, New York) and 2LD program, respectively. The odds ratio (OR) and 95% confidence interval (CI) were calculated using an unconditional logistic regression model. A probability level of 5% was considered significant.

Results

Characteristic of subjects

The relevant characteristics of the study subjects are shown in Table 2. The mean age of ESCC cases, GCA cases, and controls was 60.1 ± 9.3 (range 34–85), 60.5 ± 8.3 (range 37–86), and 60.4 ± 8.4 years (range 31–78), respectively. The gender distribution in ESCC and GCA patients (67.2 and 65.4% men) was comparable to that in healthy controls (64.1% men) ($P = 0.34$ and 0.11, respectively). The proportion of smokers in ESCC, GCA patients (39.7 and 49.0%, respectively), and healthy controls (42.3%) were no significant different ($P = 0.44$ and $P = 0.07$, respectively). In addition, the frequency of positive family history of UGIC in ESCC (48.4%) and GCA (48.2%) patients was significantly higher than that in healthy controls (35.4%) ($P < 0.001$). Thus, family history of UGIC significantly increased the risk of developing ESCC (adjusted OR = 1.71, 95% CI = 1.30–2.24) and GCA (adjusted OR = 1.70, 95% CI = 1.27–2.28).

The allele frequencies and genotype distributions of MMPs in patients and controls are summarized in Table 3. The frequencies of MMP-2 -1306C → T, -735C → T; MMP-12 -82A → G and MMP-13 -77A → G genotypes in control groups did not significantly deviate from that expected for a Hardy–Weinberg equilibrium ($P > 0.05$).

Association of MMP-2 -1306C → T SNP with the risk of ESCC and GCA

The significant differences in allele frequencies and genotype distributions of the MMP-2 -1306C → T polymorphism were observed between ESCC and control ($P = 0.02$ and 0.01, respectively). Compared with the C/T + T/T genotypes, the

Table 3 Distributions of the MMPs SNP genotype/allele in ESCC,GCA patients and healthy controls

Polymorphisms genotype/allele	Controls *n*(%)	ESCC		GCA	
		n (%)	*P* value[a]	*n* (%)	*P* value[a]
MMP-2 -1306 C/T					
T/T	6(1.0)	39(0.9)	**0.01**	4(1.6)	0.41
C/T	137(21.0)	48(14.3)		46(17.9)	
C/C	487(78.0)	284(84.8)		207(80.5)	
T	143(11.5)	54(8.1)	**0.02**	54(10.5)	0.56
C	1,105(88.5)	616(91.9)		460(89.5)	
MMP-2 -735 C/T					
T/T	29(4.6)	13(3.9)	0.78	9(3.5)	0.06
C/T	187(30.0)	100(29.9)		63(24.5)	
C/C	408(65.4)	222(66.3)		185(72.0)	
T	245(19.6)	126(18.8)	0.66	81(15.8)	0.06
C	1,003(80.4)	544(81.2)		433(84.2)	
MMP-12 -82 A/G					
A/A	588(94.2)	322(96.1)	0.21	241(94.9)	0.79
A/G	36(5.8)	13(3.9)		16(5.1)	
A	1,176	657	0.18	498	0.87
G	36	13		16	
MMP-13 -77 A/G					
A/A	137(22.0)	76(22.7)	0.99	60(23.3)	0.54
A/G	324(51.9)	170(50.7)		123(47.9)	
G/G	163(26.1)	89(26.6)		74(28.8)	
A	598	322	0.95	243	0.81
G	650	348		271	

Bold values indicate positive significance

[a] *P* value for Chi-square test

C/C genotype significantly modified the risk of developing ESCC (OR = 1.57, 95% CI = 1.10–2.23) (Table 4). Furthermore, when stratified for smoking status and family history of UGIC, the C/C genotype significantly modified the risk of developing ESCC in the smoker or family history of UGIC groups (adjust OR = 1.96, 95% CI = 1.10–2.23 and OR = 2.06, 95% CI = 1.19–3.55, respectively) (Table 4).

Association of MMP-2 -735C → T SNP with the risk of ESCC and GCA

There was no significant difference in genotype and allelotype distributions of the MMP-2 -735C → T polymorphisms between patients (ESCC and GCA) and control ($P = 0.78$, 0.66 and $P = 0.06$, 0.06, respectively). Compared with the C/T + T/T genotypes, the C/C genotype increased the trend of risk of developing GCA (OR = 1.36, 95% CI = 0.99–1.87) (Table 4). In addition, compared with the C/T + T/T genotypes, the C/C genotype significantly modified the risk of developing GCA in nonsmoker (adjust OR = 1.70, 95% CI = 1.07–2.68) (Table 4).

Association of MMP-12 -82A → G SNP with the risk of ESCC and GCA

The allelotype and genotype distribution of the MMP-12 -82A → G SNP in the overall ESCC and GCA patients were not significantly different from that in healthy controls ($P > 0.05$). Compared with the A/A genotypes, the A/G genotype did not significantly modify the risk of developing ESCC and GCA (OR = 0.63, 95% CI = 0.32–1.20 and OR = 1.05, 95% CI = 0.56–1.95, respectively). When stratified by smoking status and family history of UGIC, the carriers with A/G genotype had a tendency of increasing susceptibility to GCA in smoker (adjust OR = 2.03, 95%CI = 0.90–4.60) (Table 5).

Association of MMP-13 -77A → G SNP with the risk of ESCC and GCA

The allelotype and genotype distribution of the MMP-13 -77A → G SNP in the overall ESCC and GCA patients was not significantly different from that in healthy controls

Table 4 Correlation between SNP of MMP-2 and susceptibility to ESCC and GCA

Groups	-1306 C/T genotype (cases,%)		*P* value[a]	OR(95% CI)[a]	-735 C/T genotype (cases, %)		*P* value[a]	OR(95% CI)[a]
	C/T + T/T	C/C			C/T + T/T	C/C		
Overall								
Control	137(22.0)	487(78.0)		1.00	216(34.6)	408(65.4)		1.00
ESCC	51(15.2)	284(84.8)	0.01	**1.57(1.10–2.23)**[b]	113(33.7)	222(66.3)	0.78	1.04(0.79–1.38)[b]
GCA	50(19.5)	207(80.5)	0.41	1.17(0.81–1.67)[b]	72(28.0)	185(72.0)	0.06	**1.36(0.99–1.87)**[b]
Non-smoker								
Control	78(21.7)	282(78.3)		1.00	124(34.4)	236(65.6)		1.00
ESCC	34(16.8)	168(83.2)	0.17	1.37(0.88–2.13)[c]	66(32.7)	136(67.3)	0.69	1.08(0.75–1.56)[c]
GCA	26(19.8)	105(80.2)	0.66	1.12(0.68–1.84)[c]	31(23.7)	100(76.3)	0.02	**1.70(1.07–2.68)**[c]
Smoker								
Control	59(22.3)	205(77.7)		1.00	92(34.8)	172(65.2)		1.00
ESCC	17(12.8)	116(87.2)	0.02	**1.96(1.09–3.53)**[c]	47(35.7)	86 (64.7)	0.92	0.98(0.63–1.51)[c]
GCA	24(19.0)	102(81.0)	0.46	1.22(0.72–2.08)[c]	41(32.5)	85(67.5)	0.65	1.11(0.71–1.74)[c]
Negative family history								
Control	83(20.6)	320(79.4)		1.00	129(32.0)	274(68.0)		1.00
ESCC	29(16.8)	144(83.2)	0.29	1.29(0.81–2.05)[d]	60(34.7)	113(65.3)	0.53	0.89(0.61–1.29)[d]
GCA	25(18.8)	108(81.2)	0.65	1.12(0.68–1.84)[d]	34(25.6)	99(74.4)	0.16	1.37(0.88–2.13)[d]
Positive family history								
Control	54(24.5)	167(75.6)		1.00	87(39.4)	134(60.6)		1.00
ESCC	22(13.6)	140(86.4)	0.01	**2.06(1.19–3.55)**[d]	53(32.7)	109(67.3)	0.18	1.34(0.87–2.04)[d]
GCA	25(20.2)	99(79.8)	0.37	1.28(0.75–2.19)[d]	38(30.6)	86(69.4)	0.11	1.47(0.92–2.35)[d]

Bold values indicate positive significance

[a] *P* value, ORs and 95% CIs were calculated by unconditional logistic regression with the *CT + TT* as the reference group

[b] Adjusted for age, gender, smoking status and UGIC family history

[c] Adjusted for age, gender and UGIC family history

[d] Adjusted for age, gender and smoking status

Table 5 Correlation between SNPs of MMP-12, MMP-13 and susceptibility to ESCC and GCA

Groups	MMP-12 genotype (cases, %)		*P* value[a]	OR(95% CI)[a]	MMP-13 genotype (cases, %)			*P* value[a]	OR(95% CI)[a]	*P* value[a]	OR(95% CI)[a]
	A/A	A/G			A/A	A/G	G/G				
Overall											
Control	588(94.2)	36(5.8)		1.00	137(22.0)	324(51.9)	163(26.1)		1.00		1.00
ESCC	322(96.1)	13(3.9)	0.21	0.66(0.35–1.26)[b]	76(22.7)	170(50.7)	89(26.6)	0.75	0.95(0.68–1.32)[b]	0.94	0.98(0.67–1.44)[b]
GCA	241(94.9)	16(5.1)	0.79	1.08(0.59–1.99)[b]	60(23.3)	123(47.9)	74(28.8)	0.45	0.87(0.60–1.25)[b]	0.86	1.04(0.69–1.56)[b]
Non-smoker											
Control	337(93.6)	23(6.4)		1.00	87(24.2)	166(46.1)	107(29.7)		1.00		1.00
ESCC	195(96.5)	7(3.5)	0.15	0.53(0.22–1.25)[c]	49(24.3)	94(46.5)	59(29.2)	0.98	1.01(0.65–1.55)[c]	0.93	0.98(0.61–1.57)[c]
GCA	126(96.2)	5(3.8)	0.28	0.58(0.22–1.56)[c]	24(18.3)	67(51.1)	40(30.5)	0.16	1.46(0.86–2.50)[c]	0.30	1.36(0.76–2.42)[c]
Smoker											
Control	251(94.4.)	13(5.6)		1.00	48(18.2)	158(59.8)	58(22.0)		1.00		1.00
ESCC	127(95.5)	6(4.5)	0.86	0.91(0.34–2.55)[c]	27(20.3)	76(57.1)	30(22.6)	0.57	0.86(0.50–1.48)[c]	0.80	0.92(0.48–1.75)[c]
GCA	114 (90.5)	12(9.5)	0.09	**2.03(0.90–4.60)**[c]	36(28.6)	56(44.4)	34(27.0)	**0.01**	**0.47(0.28–0.80)**[c]	0.43	0.78(0.43–1.43)[c]
Negative family history											
Control	379(91.8)	24(7.2)		1.00	85(21.1)	209(51.9)	109(27.0)		1.00		1.00
ESCC	169(97.7)	4(2.3)	0.07	0.37(0.13–1.10)[d]	44(25.4)	83(48.0)	46(26.6)	0.24	0.77(0.49–1.20)[d]	0.43	0.82(0.50–1.35)[d]
GCA	123(92.5)	10(7.5)		1.28(0.60–2.76)[d]	35(26.3)	59(44.4)	39(29.3)	0.13	0.69(0.42–1.12)[d]	0.61	0.87(0.51–1.49)[d]
Positive family history											
Control	209(94.6)	12(5.4)		1.00	52(23.5)	115(52.1)	54(24.4)		1.00		1.00
ESCC	153(94.4)	9(5.6)	0.96	1.03(0.42–2.49)[d]	32(19.8)	87(53.7)	43(26.5)	0.44	1.23(0.73–2.07)[d]	0.40	1.30(0.71–2.35)[d]
GCA	118(95.2)	6(4.8)	0.81	0.89(0.32–2.42)[d]	25(20.2)	64(51.6)	35(28.2)	0.61	1.16(0.66–2.04)[d]	0.36	1.35(0.71–2.55)[d]

Bold values indicate positive significance

[a] *P* value, ORs and 95% CIs were calculated by unconditional logistic regression with the A/A as the reference group

[b] Adjusted for age, gender, smoking status and UGIC family history

[c] Adjusted for age, gender and UGIC family history

[d] Adjusted for age, gender and smoking status

Table 6 Correlation between the MMPs haplotypes and susceptibility to ESCC and GCA

Haplotype	Control(*n* %)	ESCC(*n*%)	*P* value[a]	OR(95% CI)	GCA(*n* %)	*P* value[a]	OR(95% CI)
T_{1306}//T_{735}	28(2.2)	17(2.5)		1.00[a]	9(1.7)		1.00[b]
T_{1306}/C_{735}	116(9.3)	101(15.1)	0.28	1.43(0.74–2.77)	44(8.6)	0.70	1.18(0.52–2.70)
C_{1306}/T_{735}	211(16.9)	80(11.9)	0.16	0.62(0.32–1.20)	75(14.6)	0.80	1.11(0.50–2.45)
C_{1306}/C_{735}	893(71.6)	472(70.5)	0.66	0.87(0.47–1.60)	386(75.1)	0.45	1.35(0.63–2.88)
A_{12}/G_{13}	632(50.6)	342(51.0)		1.00[c]	264(51.4)		1.00[d]
A_{12}/A_{13}	579(46.4)	316(47.2)	0.93	1.01(0.83−−1.22)	233(45.3)	0.73	0.96(0.78–1.19)
G_{12}/G_{13}	20(1.6)	6(0.9)	0.21	0.55(0.22–1.59)	10(1.9)	0.65	1.20(0.55–2.59)
G_{12}/A_{13}	17(1.4)	6(0.9)	0.37	0.65(0.26–1.67)	7(1.4)	0.98	0.99(0.40–2.40)

[a] *P* value, ORs and 95% CIs were calculated by unconditional logistic regression with the T_{1306}//T_{735} as the reference group in ESCC

[b] ORs and 95% CIs were calculated by unconditional logistic regression with the T_{1306}//T_{735} as the reference group in GCA

[c] ORs and 95% CIs were calculated by unconditional logistic regression with the A_{12}/G_{13} as the reference group in ESCC

[d] ORs and 95% CIs were calculated by unconditional logistic regression with the A_{12}/G_{13} as the reference group in GCA

($P > 0.05$). When stratified by smoking status and family history of UGIC, the carriers with the A/G genotype in smoker had lower risk in developing GCA (adjust OR = 0.47, 95% CI = 0.28–0.80) (Table 5).

Haplotype of MMP-2 two SNPs with susceptibility to ESCC and GCA

The MMP-2 -1306C → T and -735C → T polymorphisms displayed linkage disequilibrium ($D' = 0.58$, $P = 0.00$) and the results are presented in Table 6. We did not observe a significant difference in haplotype frequencies between cases and controls. Compared with the haplotype of T_{-1306}–T_{-735}, the others did not significantly modify the risk of developing ESCC and GCA.

Haplotype of MMP-12 and MMP-13 SNPs with susceptibility to ESCC and GCA

The MMP-12 and MMP-13 polymorphisms displayed linkage disequilibrium ($D' = 0.51$, $P = 0.00$) and the results are presented in Table 6. We did not observe a significant difference in haplotype frequencies between cases and controls. Compared with the haplotype of MMP-12 A/MMP-13 A, the others did not significantly modify the risk of developing ESCC and GCA.

Discussion

This study showed that family history of UGIC significantly increased the risk of developing ESCC and GCA. The MMP-2 -1306C/C genotype significantly modified the risk of developing ESCC in smoker or positive family history of UGIC; on the other hand, the MMP-2 -735C → T C/C genotype significantly modified the risk of developing GCA in nonsmoker. The MMP-12 -82G allele significantly modified the risk of developing GCA, in contract, MMP-13 -77A → G A/G genotype significantly lowered the risk of developing GCA in smoker.

Previous studied have demonstrated that in the gene promoter region of MMP-2, there are sequence variations and several functional SNPs. Two transitions (-1306C → T and -735C → T), located at a core recognition sequence of Sp1 (CCACC box), lead to a strikingly low promoter activity because of abolishing the Sp1-binding site [15, 16]. Transient transfection experiments showed that reporter gene expression driven by the C allelic were greater than reporter gene expression driven by the T allelic, indicating the functional significance of these two polymorphisms [15]. Numerous studies have investigated if the -1306C → T and the -735C → T polymorphisms are associated with risk of some cancers, and the results obtained in different groups were inconsistent. Studies reported that C allele of -1306C → T SNP might be a potential risk factor for cancers, including lung, gastric cardia, oral, breast and cervical cancer [21–25]. In contrast, Rollin et al. and Grieu et al. [26, 27] found that no difference observed in MMP-2 -1306C → T genotypes between controls and patients for non-small cell lung cancer and breast cancer. About -735C → T polymorphism, the studies of lung cancer and ESCC had shown that -735C/C homozygote could increase the risk of cancers [21, 26]. In our study, we found that the -1306C/C increased the risk of developing ESCC. However, no significant differences were observed between GCA and control. Compared with the -735C → T C/T + T/T genotypes, the C/C genotype increased the trend of risk of developing GCA, but no significant differences were observed between ESCC and control. When stratified for smoking status and family history of UGIC, the -1306C → T C/C genotype could significantly modify the risk of developing ESCC in smoking groups and positive

family history of UGIC; and the MMP-2 -735C → T C/C genotype significantly modified the risk of developing GCA in nonsmoker.

The -82A → G SNP at position -82 in the promoter of the MMP-12 gene is located at a core recognition sequence of AP-1. In vitro experiments showed that the A allelic increased the binding ability of AP-1 to enhance the gene transcription [17]. Kader et al. [28] showed that the MMP-12 G allelic increased the metabasis of bladder cancer in smoking group. Su et al. [29] found that the MMP-12 G allelic significantly modified the risk of developing lung cancer in males group. Our study found that there was no significant difference in genotype and allelotype distributions of the MMP-12 -82A → G polymorphisms between patients (ESCC and GCA) and control of high-risk areas in Cixian County and Shexian County in Hebei province. When stratified for smoking status, genotypes carrying MMP-12 -82G allele significantly modified the risk of developing GCA in smoking group. It is worthy to mention that all smokers are males. These results indicate that the high transcription activity of MMP-12 G allelic may increase the risk of cancers in some group.

The MMP-13 -77A → G SNP at position -77 in the promoter of the MMP-13 gene is a binding site for the transcription factor, PEA3 (AGGAAG). In vitro experiments showed that the SNP altered the gene transcription activity [18]. Yoon et al. [18] found that the MMP-13 -77A → G SNP was associate with atherosclerosis in the abdominal aorta of black men. To the best of our knowledge, our study is the first molecular epidemiologic study with regard to the association of the MMP-13 polymorphism and the risk of cancer development. Our study demonstrated that the MMP-13 -77A → G genotypes were associated with a non-statistically significant higher risk of patients (ESCC and GCA) and control (all P values > 0.05), but when stratified for smoking status, the carriers of MMP-13 A/G genotype possibly lower the risk of developing GCA in smokers.

We also found the linkage disequilibrium between the MMP-2 -1306C → T and -735C → T polymorphisms ($D' = 0.58$). The haplotype analysis indicated that the C_{-1306}–C_{-735} was the most common haplotype in the control. Compared with the haplotype of T_{-1306}–T_{-735}, the other haplotypes did not significantly modify the risk of developing ESCC and GCA. Our result were not in good agreement with the previous finding [21] by Zhou et al. [21], in which that comparing with the T_{-1306}–T_{-735} haplotype, the subjects carrying C_{-1306}–C_{-735} were associated with increased risk of lung cancer significantly. The discrepancy could due to those participants in our study recruited from the high incidence regions of China, display the different heredity background. This needs further investigation.

MMP-12 and MMP-13, located at chromosome 11q22, are two neighboring genes. Our study have found the linkage disequilibrium between MMP-12 and MMP-13 ($D' = 0.51$). MMP-12 A/MMP-13 G (50.6%) and MMP-12 A/MMP-13 A (46.4%) were the common haplotype in the control of the high risk region. Comparing to the haplotype of MMP-12 A/MMP-13 A, the other haplotypes did not significantly modify the risk of developing ESCC and GCA.

In conclusion, our study indicated that the functional polymorphism in MMP-2, -12, -13 genes may be play a role in developing ESCC and GCA of high incidence region of North China. Also traditional epidemiological studies suggested that there may be common environmental and genetic factors to morbidity of ESCC and GCA, however, differences in molecular mechanism of ESCC and GCA development may exist. Thus, the exact etiologies mechanisms of the two malignancies need closer study and explore.

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