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No association of the *matrix metalloproteinase 1* promoter polymorphism with susceptibility to esophageal squamous cell carcinoma and gastric cardiac adenocarcinoma in northern China

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Abstract

AIM: To investigate association of the *2G* or *1G* single nucleotide polymorphism (SNP) in *matrix metalloproteinase 1* (*MMP1*) promoter with susceptibility to esophageal squam-ous cell carcinoma (ESCC) and gastric cardiac adenocarcinoma (GCA) in a population of North China.

METHODS: *MMP1* promoter SNP was genotyped by polymerase-chain reaction (PCR)-restriction fragment length polymorphism (RFLP) analysis in 417 cancer patients (234 ESCC and 183 GCA) and 350 healthy controls.

RESULTS: The genotype frequencies of the *MMP1* promoter SNP in healthy controls were 55.4% (*2G/2G*), 30% (*1G/2G*) and 14.6% (*1G/1G*), respectively. The genotype and allelotype distribution in ESCC and GCA patients was not significantly different from that in healthy controls (all *P* values were above 0.05). Compared with the *1G/1G* genotype, neither the *2G/2G* nor in combination with the *1G/2G* genotype significantly modified the risk of developing ESCC and GCA, the adjusted odds ratio was 1.28 (95%CI = 0.78-2.09), 1.23 (95%CI = 0.38-2.05) in ESCC and 1.39 (95%CI = 0.80-2.41), 1.34 (95%CI = 0.74-2.40) in GCA, respectively. When stratified by smoking status and family history of upper gastrointestinal cancer, the *2G/2G* genotype alone or in combination with the *1G/2G* genotype also did not show any significant influence on the risk of ESCC and GCA development. In addition, influence of the *MMP1* SNP on lymphatic metastasis in ESCC and GCA was also not obs-erved.

CONCLUSION: The *2G* or *1G* SNP in the *MMP1* promoter might not modify the risk of ESCC and GCA development and might not be used as a stratification marker to predict the potential of lymphatic metastasis in these two tumor types.

Key words: SNP; ESCC; GCA

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INTRODUCTION

Matrix metalloproteinases (MMPs) are a family of enzymes that degrade the extracellular matrix and have been implicated in invasion and metastasis of tumor cells. Twenty-six human MMPs have been identified currently and these enzymes are classified according to their substrate specificity and structural similarities[1]. MMP1 belongs to interstitial collagenase, a subfamily of MMPs that cleaves stromal collagens. *MMP1* gene is localized on 11q22 and expressed in a wide variety of normal cells, e.g., stromal fibroblasts, macrophages, endothelial cells and epithelial cells, and in various tumor cells[1]. The level of MMP1 expression, and its potential to mediate connective tissue degradation and tumor progression, can be influenced by a genetic variation in *MMP1* promoter[2]. This variation is a single nucleotide polymorphism (SNP) located at-1 607 bp, where an insertion of a guanine base (G) creates the sequence of 5'-GGAT-3', the core binding site for members of the EST family of transcription factors[2]. The *MMP1* SNP has been correlated to the risk of renal cell carcinoma[3], lung cancer[4] and colorectal cancer[5]. This polymorphism has also been associated with the invasiveness of cutaneous malignant melanoma[6], ovarian cancer[7] and colorectal cancer[8].

China is a country with high incidence regions of esophageal squamous cell carcinoma (ESCC) and gastric cardiac adeno-carcinoma (GCA). Although the expression of MMP1 was associated with local invasion and poor prognosis in ESCC[9] and gastric cancer[10], the role of the *MMP1* promoter SNP in the development and progression of ESCC and GCA is

still unknown. Since *in vitro* study suggested that the *MMP1* SNP might modify MMP1 expression by increasing its binding with transcription factors[2,11], we hypothesize that the genotype which leads to higher MMP1 expression (i.e., *2G/2G*) might increase the susceptibility to ESCC and GCA and enhance the potential of lymphatic metastasis of these two tumor types. Therefore, in the present study, we conducted a hospital-based case control study to explore the role of the *MMP1* promoter SNP in the development and lymphatic metastasis of ESCC and GCA in a population of north China.

MATERIALS AND METHODS

Subjects

This study included 417 patients (234 with ESCC and 183 with CAC) and 350 healthy individuals without overt cancer. The cases were outpatients for endoscopic biopsy or inpatients for tumor resection in the Fourth Affiliated Hospital, Hebei Medical University between 2001 and 2003. Histological tumor typing was carried out on basis of the biopsy or resection specimens in the Department of Pathology of the same hospital. The esophageal carcinomas were all squamous cell carcinomas. The 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 tubular esophagus and the beginning of the saccular stomach[12]. The healthy subjects, who had no history of cancer and genetic diseases, visited the same hospital for physical examination between 2001 and 2003. All of the cancer patients and control subjects were unrelated Han nationality and from Shijiazhuang city or its surrounding regions. Information on TNM staging was available from 131 ESCC and 94 GCA patients from hospital records and pathological diagnosis. Information on sex, age, smoking habit and family history was obtained from cancer patients and healthy controls by interview following sampling. Smokers were defined as formerly or currently smoking five cigarettes per day for at least 2 years. Individuals with at least one first-degree relative or at least two second-degree relatives having esophageal/cardiac/gastric cancer were defined as having a family history of upper gastrointestinal cancers (UGIC). The 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

Five milliliters of venous blood from each subject was drawn in Vacutainer tubes containing EDTA and stored at 4 ℃. Genomic DNA was extracted within 1 wk after sampling by using proteinase K (Merck, Darmstadt, Germany) digestion followed by a salting out procedure according to the method published by Miller *et al*[13].

MMP1 promoter SNP genotyping

The *MMP1* genotyping was determined by PCR-RFLP assay. The PCR primers used for amplifying the *MMP-1* polymorphism were: forward 5'-TGACTTTTAAAACATAGTCTATGTTCA-3' and reverse 5'-TCTTGGATTGATTTGAGATAAGTCATAGC-3'[4]. The reverse primer was specially designed to introduce a recognition site of restriction enzyme *Alu*I (AGCT) by replacing a T with a G at the second position close to the 3' end of the primer. The *1G* allele has this recognition site, whereas the *2G* allele destroys the recognition site by inserting a guanine. The PCR was performed in a 20-μL volume containing 100 ng of DNA template, 2.0 μL of 10× PCR buffer, 1.5 mmoL of $MgCl_2$, 1 unit of *Taq*-DNA-polymerase (BioDev-Tech., Beijing, China), 200 μmoL of dNTPs and 200 nmoL of sense and antisense primers. The PCR cycling conditions were 5 min at 94 ℃ followed by 35 cycles of 30 s at 94 ℃, 30 s at 58 ℃, and 30 s at 72 ℃, with a final step at 72 ℃ for 5 min to allow for the complete extension of all PCR fragments. An 8 μL aliquot of PCR product was digested overnight at 37 ℃ in a 10-μL reaction containing 10 units of *Alu*I (TakaRa Biotechnology Co., Ltd, Dalian, China) and 1× reaction buffer. After overnight digestion, the products were resolved and separated on a 4% agarose gel stained with ethidium bromide. After electrophoresis, homozygous *2G* alleles were represented by a DNA band with a size of 269 bp, homozygous *1G* alleles were represented by DNA bands with sizes of 241 and 28 bp, whereas heterozygotes displayed a combination of both alleles (269, 241, and 28 bp). For a negative control, each PCR reaction used distilled water instead of DNA in the reaction system. For 10% of the samples, the reaction was repeated once for *MMP1* genotyping and all of the genotypes matched with the original results.

Statistical analysis

Statistical analysis was performed using SPSS10.0 software package (SPSS Company, Chicago, IL, USA). Comparison of the *MMP1* genotype distribution in the study groups was performed by means of two-sided contingency tables using χ^2 test. A probability level of 5% was considered significant. The odds ratio (OR) and 95%CI were calculated using an unconditional logistic regression model and adjusted according to age and sex.

RESULTS

The mean age of all ESCC cases was 54.1±10.2 years (range 34-76 years), of all GCA cases was 55.0±10.5 (range 37-76 years) and of controls was 51.7±10.7 years (range 30-68 years). The gender distribution in ESCC and GCA patients (72.2% and 73.2% men) was comparable to that in healthy controls (65.4% men) ($P = 0.08$ and 0.07, respectively). The proportion of smokers in ESCC patients (50.5%) was not significantly different from that in healthy controls (42.9%) ($\chi^2 = 2.79$, $P = 0.10$). However, smokers in GCA patients (55.4%) were more frequently seen than in healthy controls ($\chi^2 = 6.78$, $P = 0.01$). Therefore, smoking significantly increased the risk for GCA development (the age and sex adjusted OR = 1.64, 95%CI = 1.12-2.38). In addition, the frequency of a positive family history of UGIC in ESCC (30.4%) and GCA (39.7%) patients was significantly higher than that in healthy controls (4.7%) ($\chi^2 = 31.74$ and 47.87, respectively, $P<0.0001$). Thus, a family history of UGIC significantly increased the risk to develop ESCC (the age and sex adjusted OR = 7.89, 95%CI = 3.25-15.49) and GCA (the age and

sex adjusted OR = 13.24, 95%CI = 5.98-26.40). Among 131 ESCC patients with tumor resection, lymphatic metastasis was reported in 59 cases and the rest (72 cases) were diagnosed as lymph node negativity, whereas among 94 GCA patients with operation, positive and negative lymphatic metastases were reported in 46 and 48 cases, respectively. The demographic distribution of ESCC and GCA patients as well as healthy controls is shown in Table 1.

Table 1 Characteristics of ESCC, GCA patients, and healthy individuals

Groups	Control *n* (%)	ESCC*n* (%)	*P*[1]	GCA *n* (%)	*P*[1]
Sex					
Male	229 (65.4)	169 (72.2)	0.08	134 (73.2)	0.07
Female	121 (34.6)	65 (27.8)		49 (26.8)	
Mean age in yr (SD)	51.7 (10.7)	54.1 (10.2)	0.06[1]	55.0 (10.5)	0.06[2]
Smoking status[3]					
Ex-or current smoker	120 (42.9)	96 (50.5)	0.10	92 (55.4)	0.01[5]
Non-smoker	160 (57.1)	94 (49.5)		74 (44.6)	
Family history of UGIC[4]					
Positive	6 (4.7)	56 (30.4)	<0.0001[6]	62 (39.7)	<0.0001[6]
Negative	123 (95.3)	128 (69.6)		96 (60.3)	
MMP-1 SNP genotype					
2G/2G	194 (55.4)	130 (55.6)	0.611	12 (61.2)	0.35
1G/2G	105 (30.0)	76 (32.5)		51 (27.3)	
1G/1G	51 (14.6)	28 (11.9)		20 (10.9)	
MMP-1 SNP allelotype					
2G	493 (70.4)	336 (71.8)	0.61	275(75.1)	0.10
1G	207 (29.6)	132 (28.2)		91 (24.9)	

ESCC: esophageal squamous cell carcinoma; GCA: gastric cardiac adenocarcinoma; UGIC: upper gastrointestinal cancer. [1]*P* value for χ^2 test; [2]*P* value for *t* test; [3,4]information of smoking status and family history was available from a subset of subjects; [5]smoking significantly increased the risk for GCA development (the age and sex adjusted OR = 1.64, 95%CI = 1.12-2.38); [6]positive family history of UGIC significantly increased the risk of developing ESCC (the age and sex adjusted OR = 7.89, 95%CI = 3.25–15.49) and GCA (the age and sex adjusted OR = 13.24, 95%CI = 5.98-26.40).

MMP1 SNP genotyping was successfully performed in all study subjects. The SNP genotype distribution was not correlated with gender, age and smoking status both in healthy controls and in ESCC and GCA patients (data not shown). In healthy controls, the frequencies of the *2G/2G, 1G/2G* and *1G/1G* genotypes were 55.4%, 30.0% and 14.6% while the distribution of the *2G* and *1G* allele was 70.4% and 29.6%, respectively. The genotype distribution in healthy controls was not in Hardy-Weinberg equilibrium ($P = 0.002$). In contrast, the genotype frequencies in ESCC and GCA patients were consistent with Hardy-Weinberg equilibrium ($P = 0.10$ and 0.09, respectively).

As shown in Table 1, there was no statistic difference in allele distribution between ESCC, GCA patients and healthy controls ($\chi^2 = 0.25$ and 2.65, $P = 0.61$ and 0.10, respectively). The overall *MMP1* genotype distribution in ESCC and GCA patients was also not significantly different from that in healthy controls ($\chi^2 = 0.98$ and 2.08, $P = 0.61$ and 0.35, respectively). By using *1G/1G*, the genotype with a lower MMP1 expression as reference, neither the *2G/2G* genotype alone nor in combination with the *1G/2G* significantly modified the risk of ESCC and GCA, the adjusted OR for ESCC was 1.28 (95% CI = 0.78-2.09) and 1.23 (95% CI = 0.38-2.05), for GCA it was 1.39 (95% CI = 0.80-2.41) and 1.34 (95% CI = 0.74-2.40), respectively. When stratified by smoking status and family history of upper gastrointestinal cancer, the frequencies of the *MMP1* genotypes in ESCC and GCA patients were also not significantly different from that in healthy controls. Consistently, the *2G/2G* genotype, alone or in combination with the *1G/2G*, did not show any significant influence on the risk of ESCC and GCA in the stratification groups (Table 2), when compared with the *1G/1G* genotype.

Furthermore, we tried to identify that if *MMP1* genotyping played a role in predicting lymphatic metastasis in ESCC and GCA in the study subjects. As shown in Table 3, in both ESCC and GCA groups, the distribution of the *MMP1* genotypes was not significantly different between patients with

Table 2 Association analysis of the *MMP1* SNP with the risk of ESCC and GCA development

	1G/1G	2G/2G	2G/1G+2G/2G	aOR (95%CI)[3]	aOR (95%CI)[4]
Overall					
Normal	51 (14.9)	194 (55.4)	299 (85.4)		
ESCC	28 (12.0)	130 (55.6)	206 (88.0)	1.28 (0.78-2.09)	1.23 (0.38-2.05)
GCA	20 (10.9)	112 (61.2)	163 (89.1)	1.39 (0.80-2.41)	1.34 (0.74-2.40)
Non-smoker[1]					
Normal	25 (15.6)	89 (55.6)	135 (84.4)		
ESCC	10 (10.6)	58 (61.7)	84 (89.4)	1.55 (0.65-3.46)	1.54 (0.68-3.48)
GCA	10 (13.5)	40 (54.1)	64 (86.5)	1.12 (0.49-2.58)	1.20 (0.38-3.82)
Smoker					
Normal	18 (15.0)	64 (53.3)	102 (85.0)		
ESCC	14 (14.6)	46 (47.9)	82 (85.4)	1.03 (0.48-2.19)	0.93 (0.42-2.06)
GCA	8 (8.7)	59 (64.1)	84 (91.3)	1.80 (0.74-4.37)	1.36 (0.46-4.05)
Negative family history[2]					
ESCC	16 (12.5)	73 (57.0)	112 (87.5)	1.19 (0.65-2.18)	1.18 (0.63-2.20)
GCA	7 (7.3)	54 (56.2)	89 (92.7)	1.19 (0.56-2.51)	0.42 (0.16-1.04)
Positive family history[3]					
ESCC	8 (14.3)	29 (51.8)	48 (85.7)	1.02 (0.46-2.29)	0.95 (0.41-2.21)
GCA	10 (16.1)	38 (61.3)	52 (83.9)	0.95 (0.59-1.53)	0.72 (0.31-1.70)

ESCC: esophageal squamous cell carcinoma; GCA: gastric cardiac adenocarcinoma; [1,2]information of smoking status and family history was available from a subset of subjects; [3,4]the age and sex adjusted odds ratio of the *2G/2G* (c) and *1G/2G+2G/2G* genotype (d) against the *1G/1G* genotype.

or without lymphatic metastasis. Compared to the *1G/1G* genotype, neither the *2G/2G* nor the *1G/2G+2G/2G* genotype showed modification in the potential of lymphatic metastasis, with age and sex adjusted OR of 1.72 and 1.73 (95%CI = 0.58-5.33 and 0.60-4.97) in ESCC, and of 3.80 and 3.66 (95%CI = 0.71-20.41 and 0.71-18.87) in GCA, respectively.

Table 3 Influence of the *MMP-1* SNP on lymphatic metastasis in ESCC and GCA[1]

Groups	LM negative cases (%)	LM positive cases (%)	aOR (95%CI)[2]
ESCC			
1G/1G	12 (16.7)	6 (10.2)	1.0 (ref.)
2G/2G	31 (43.0)	29 (49.2)	1.72 (0.58-5.33)
1G/2G+2G/2G	60 (83.3)	53 (89.8)	1.73 (0.60-4.97)
GCA			
1G/1G	7 (14.6)	2 (4.4)	1.0 (ref.)
2G/2G	25 (52.1)	28 (60.9)	3.80 (0.71-20.41)
1G/2G+2G/2G	41 (85.4)	44 (95.6)	3.66 (0.71-18.87)

ESCC: esophageal squamous cell carcinoma; GCA: gastric cardiac adenocarcinoma; LM: lymphatic metastasis, [1]all of the 131 ESCC (53 LM positive and 60 negative) and 96 GCA patients (46 LM positive and 48 negative) with available related data were considered; [2]the age and sex adjusted odds ratio of the *2G/2G* and *2G/1G+1G/1G* genotype against the *1G/1G* genotype.

DISCUSSION

Several exogenous factors were correlated to the development of ESCC and GCA in China[14-18]. However, genetic background has been suggested to play important roles in cancer occurrence, as displayed in this study which showed that a family history of UGIC significantly increased the risk of ESCC and GCA. In addition, some polymorphic genes encoding metabolic enzymes, cell cycle regulators and mismatch repair enzymes, such as aldehyde dehydrogenase-2 (ALDH2)[19], cytochrome P450(CYP)[20], glutathione S-transferase (GST)[20], methylenetetrahydrofolate reductase (MTHFR)[21], NAD(P)H: quinone oxidoreductase 1 (NQO1)[22] , cyclin D1[23], X-ray repair cross-complementing group 1(XRCC1) and xeroderma pigmentosum group D (XPD)[24], have been found to be able to modify the susceptibility to chemically induced cancers including esophageal and gastric cardiac cancer. Therefore, these polymorphic genes, alone or in combination with each other or through interaction with exogenous risk factors, may be used as predicative parameters for screening individuals at a high risk of ESCC and GCA.

Carcinogenesis is a multicellular and multistage process in which destruction of the microenvironment is required for the conversion of normal tissue to tumor. Molecular analysis of the microenvironment and its deregulation during neoplasia is an essential step to understand the mechanism of malignant conversion process. Given the fact that MMPs, produced by both tumor and normal cells, influence the microenvironment by degrading extracellular matrix and altering cellular signals[25], they may be also involved in the initial stages of tumor development. MMP1 is the most highly expressed interstitial collagenase degrading fibrillar collagens, the most abundant protein in human body. Expression of MMP1 is partially regulated by the upstream promoter sequence in which the *2G* or *1G* SNP site is located. The *MMP1 2G/2G* genotype, which leads to higher expression of MMP1, has been reported to increase the susceptibility to renal cell carcinoma[3], lung cancer[4] and colorectal cancer[5]. The *2G/2G* genotype or the *2G* allele has also been correlated to poorer prognosis of cutaneous malignant melanoma[6], ovarian cancer[7] and colorectal cancer[8].

Since MMP1 overexpression was an independent factor for tumor invasion and prognosis in ESCC[9], we presently conducted a case control study to explore the role of the *MMP1* SNP in the development and lymphatic metastasis of ESCC as well as of GCA, another common carcinoma with similar geographic epidemic regions to ESCC. In line with the results from Caucasian[4] and Japanese[18] populations, the genotype distribution of the *MMP1* promoter SNP in our healthy controls was not in Hardy-Weinberg equilibrium. Although the underlying reason is unknown, the random recruitment of healthy controls and reproducible genotyping method used in this study should not influence the feasibility of control group.

In contrary to our expectation, the *MMP1* genotype distribution difference was not found between the two cancer groups and healthy controls, as well as in the stratification comparisons according to smoking status (never smoking or currently and previously smoking) and family history of UGIC. The result suggests that although the *MMP1* promoter SNP is correlated with some cancer types, this genetic alteration may not be associated with the susceptibility to ESCC and GCA in a population of north China. In addition, lymphatic metastasis, which is one of the main factors to influence prognosis and survival of upper gastrointestinal tumors, is also not correlated with this *MMP1* promoter polymorphism, suggesting that MMP1 expression might influence ESCC progress via mechanisms other than regulation by the promoter SNP. Our result is consistent with a recent study on gastric cancer in Japan, which showed that the genotype distribution of the *MMP1* promoter SNP in cancer patients was similar to that in healthy controls, and the SNP showed no influence on tumor invasion, lymph node metastasis and clinical stage of gastric cancer[26].

In summary, the result from gastric cancer[26], together with the finding in this study, indicate that the *MMP1* promoter SNP might not be used as a stratification marker to predict the susceptibility to upper gastrointestinal carcinoma and the potential of lymphatic metastasis in these tumor types, at least in Asian population.

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