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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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[8]. 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 motif of transcription factors[9]. The SNP on lymphatic endothelial cells and epithelial cells, and in various tumor cells[9]. 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 motif of transcription factors[9]. The SNP on lymphatic endothelial cells and epithelial cells, and in various tumor cells[9]. 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 motif of transcription factors[9]. The SNP on lymphatic endothelial cells and epithelial cells, and in various tumor cells[9]. 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 motif of transcription factors[9]. The SNP on lymphatic endothelial cells and epithelial cells, and in various tumor cells[9]. 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 motif of transcription factors[9]. The SNP on lymphatic endothelial cells and epithelial cells, and in various tumor cells[9]. 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 motif of transcription factors[9]. The SNP on lymphatic endothelial cells and epithelial cells, and in various tumor cells[9]. 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 motif of transcription factors[9]. The SNP on lymphatic endothelial cells and epithelial cells, and in various tumor cells[9]. 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 motif of transcription factors[9]. The SNP on lymphatic...
MMP1 promoter SNP genotyping

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only available from a subset of cancer patients and healthy

cancers (UGIC). The smoking status and family history were

defined as having a family history of upper gastrointestinal

relatives having esophageal/cardiac/gastric cancer were

at least one first-degree relative or at least two second-degree

five cigarettes per day for at least 2 years. Individuals with at

Smokers were defined as formerly or currently smoking

and healthy controls by interview following sampling.

pathological diagnosis. Information on sex, age, smoking

131 ESCC and 94 GCA patients from hospital records and

regions. Information on TNM staging was available from

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

areas. Information on TNM staging was available from

131 ESCC and 94 GCA patients from hospital records and

pathological diagnosis. Information on sex, age, smoking

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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 °C. Genomic DNA was extracted within 1 wk after
sampling by using proteinase K (Merk, Darmstadt, Germany)
digestion followed by a salting out procedure according to
the method published by Miller et al[10].

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’-TGACTTTTAAAAAC-
ATAGTCTATGTCA-3’ and reverse 5’-TCTTGGATTGATTTGAGATAAGTCATAGC-3’[9]. The reverse primer was specially designed to introduce a recognition site of restriction enzyme Alul (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 MgCl2, 1 unit of Taq-DNA-polymerase (BioDevTech., Beijing, China), 200 µMol of dNTPs and 200 mMol of sense and antisense primers. The PCR cycling conditions were
5 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at
58 °C, and 30 s at 72 °C, with a final step at 72 °C 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 °C in a 10-µL reaction containing 10 units of
Alul (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 χ² 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%) (χ²=2.79,
P=0.10). However, smokers in GCA patients (55.4%) were
more frequently seen than in healthy controls (χ²= 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%) (χ²= 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  | GCA n (%) | P  |
|----------------------|---------------|------------|----|-----------|----|
| 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             | 51.7 (10.7)   | 54.1 (10.2) | 0.06 | 55.0 (10.5) | 0.06 |
| Smoking status       |               |            |    |           |    |
| Ex-or current smoker | 120 (42.9)    | 96 (50.5)  | 0.10 | 92 (55.4)  | 0.01 |
| Non-smoker           | 160 (57.1)    | 94 (49.5)  | 74 (44.6) |
| Family history of UGIC |            |            |    |           |    |
| Positive             | 6 (4.7)       | 56 (30.4)  | <0.0001 | 62 (39.7) | <0.0001 |
| Negative             | 123 (95.3)    | 128 (69.6) | 96 (60.3) |
| MMP-1 SNP genotype   |               |            |    |           |    |
| 2G/2G                | 194 (55.4)    | 130 (56.6) | 0.61 | 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 alleletype |               |            |    |           |    |
| 2G                   | 493 (70.4)    | 336 (71.8) |        | 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. 'P value for χ² test; ²P value for t test; ³information of smoking status and family history was available from a subset of subjects; ⁴smoking significantly increased the risk for GCA development (the age and sex adjusted OR = 1.64, 95% CI = 1.12-2.38); ⁵positive 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 (χ² = 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 (χ² = 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

| Groups               | 1G/1G | 2G/2G | 2G/1G+2G/2G | aOR (95%CI)² | aOR (95%CI)³ |
|----------------------|------|------|-------------|--------------|--------------|
| Overall              |      |      |             |              |              |
| Normal               | 51   | 194  | 299 (85.4)  | 1.28 (0.78-2.09) | 1.23 (0.38-2.05) |
| ESCC                 | 28   | 130  | 206 (88.0)  | 1.39 (0.80-2.41) | 1.34 (0.74-2.40) |
| GCA                  | 20   | 112  | 163 (89.1)  | 1.12 (0.49-2.58) | 1.20 (0.38-3.82) |
| Non-smoker¹         |      |      |             |              |              |
| Normal               | 25   | 89   | 135 (84.4)  | 1.55 (0.65-3.46) | 1.54 (0.68-3.48) |
| ESCC                 | 10   | 58   | 84 (89.4)   | 1.19 (0.56-2.81) | 1.19 (0.56-2.81) |
| GCA                  | 10   | 40   | 64 (86.5)   | 1.12 (0.49-2.58) | 1.20 (0.38-3.82) |
| Smoker               |      |      |             |              |              |
| Normal               | 18   | 64   | 102 (85.0)  | 1.03 (0.48-2.19) | 0.93 (0.42-2.06) |
| ESCC                 | 14   | 46   | 82 (85.4)   | 1.80 (0.74-4.37) | 1.36 (0.46-0.45) |
| GCA                  | 8    | 59   | 84 (91.3)   | 1.19 (0.56-2.81) | 1.19 (0.56-2.81) |
| Negative family history² | 16    | 73    | 112 (87.5) | 1.19 (0.56-2.81) | 1.19 (0.56-2.81) |
| ESCC                 | 7    | 56   | 89 (92.7)   | 1.19 (0.56-2.81) | 1.19 (0.56-2.81) |
| GCA                  | 5    | 52   | 83 (93.9)   | 0.95 (0.59-1.53) | 0.72 (0.31-1.70) |

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

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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

| Groups | LM negative cases (%) | LM positive cases (%) | aOR(95%CI) |
|--------|-----------------------|-----------------------|------------|
| 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; 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; 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 aldihyde dehydrogenase-2 (ALDH2)[24], cytochrome P450 (CYP)[25], glutathione S-transferase (GST)[26], methyltetrahydrofolate reductase (MTHFR)[27], NAD(P)H: quinone oxidoreductase 1 (NQO1)[28], cyclin D1[29], X-ray repair cross-complementing group 1 (XRCC1) and xeroderma pigmentosum group D (XPD)[30], 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[31], 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[32], lung cancer[33] and colorectal cancer[34]. The 2G/2G genotype or the 2G allele has also been correlated to poorer prognosis of cutaneous malignant melanoma[35], ovarian cancer[36] and colorectal cancer[37].

Since MMP1 overexpression was an independent factor for tumor invasion and prognosis in ESCC[38], 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[39] and Japanese[40] 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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