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Genetic Polymorphisms in *Matrix Metalloproteinases -1* and *-7* and Susceptibility to Gastric Cancer: an Association Study and Meta-Analysis

Wen-Liang Fang[1,2], Wei-Bo Liang[2], Lin-Bo Gao[3], Bin Zhou[3], Feng-Li Xiao[1], and Lin Zhang[2,3]

[1] *Central Laboratory of Clinical College, Anhui Medical University, Hefei 230601, P. R. China*
[2] *Department of Forensic Biology, West China School of Preclinical and Forensic Medicine, Sichuan University, Chengdu 610041, P. R. China*
[3] *Laboratory of Molecular Translational Medicine, West China Second University Hospital, Sichuan University, Chengdu 610041, P. R. China*

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ABSTRACT

Matrix Metalloproteinases (*MMPs*) play an important role in gastric cancer (GC). Accumulated evidence suggests that functional *MMP-1* and *MMP-7* gene polymorphisms are associated with several tumors. The aim of this study was to investigate two single nucleotide polymorphisms, *MMP-1* -1607 1G/2G and *MMP-7* -181 A/G, and their potential relationship with GC.

We examined 246 GC patients and 252 age-and sex-matched controls from Sichuan province in China. Genotypes were determined using a polymerase chain reaction-restriction fragment length polymorphism strategy and DNA sequencing. We also performed a meta-analysis of relevant studies, involving 1084 cases and 1721 controls, to place our findings in a broader context.

No significant relationship was observed between the MMP-1 -1607 1G/2G alleles and genotypes and the risk of GC. There were significant differences in the genotypes and allele distributions of the -181 A/G polymorphism of the MMP-7 gene between cases and controls. The -181 A allele carriers had a significantly increased risk of GC compared with -181 G allele carriers (OR=3.051, 95% CI, 1.475-6.310, *P*=0.002), and the AA genotype of -181 A/G was associated with an increased risk of GC compared with the AG genotype (OR=3.189, 95% CI, 1.523-6.676, *P*=0.001).

A meta-analysis of six studies also showed a significant risk of GC associated with *MMP-7* polymorphism.

Keywords: Gastric cancer; Matrix metalloproteinases; Meta-analysis; Polymorphism, Single Nucleotide

Corresponding Author: Lin Zhang, MD, PhD;
Department of Forensic Biology, West China School of Preclinical and Forensic Medicine, Sichuan University, NO.17 Renmin South Road (Section III), Chengdu 610041, Sichuan, China. Tel: (+86 28) 8546 9033; Fax: (+86 28) 8540 5541, E-mail: zhanglin@scu.edu.cn

INTRODUCTION

Gastric cancer (GC) is the fourth most common cancer and second leading cause of cancer-related

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deaths worldwide.[1] Previous studies have shown that China is one of areas with the highest risk of GC and that there has been a continuous increase in GC incidence over the past 30 years.[2,3] Environmental and genetic factors possibly play a role in the etiology of the disease.[4,5] Inherited susceptibility is an important factor in upper gastrointestinal (UGI) carcinogenesis; for example, Gao et al found an increased risk of UGI carcinogenesis among individuals with a family history of malignant tumors.[6]

The *matrix metalloproteinase* (*MMP*) family is a class of zinc-dependent proteolytic enzymes and includes at least 28 distinct human gene products.[7] *MMP-2*, *MMP-9* and *VEGF* expression were positively correlated with tumor size, depth of invasion, lymphatic and venous invasion and lymph node metastasis in gastric carcinomas.[8] The *MMP-1* gene is located on chromosome 11q22.2. *MMP-1* acts as a molecular ratchet tethered to the cell surface, which suggests a novel mechanism for its role in tissue remodeling and the cell-matrix interaction.[9] *MMP-7* was mapped to 11q22.3, and *MMP-7* expression was confirmed to be independently associated with tumor metastasis.[10]
Single-nucleotide polymorphisms (SNPs) within *MMP* genes are thought to influence gene expression, and some are thought to be associated with cancer susceptibility.[8,10] To investigate the association between *MMP-1* and *MMP-7* and GC, we conducted a case-control study of two polymorphisms in *MMP-1* and *MMP-7* in Chinese GC patients and controls and further performed a meta-analysis of the published studies and our results.[11-15]

MATERIALS AND METHODS

Study Population

Blood samples were taken from 246 patients with gastric cancer and 252 healthy control subjects. All of the cases and healthy controls were unrelated Chinese Han individuals who were selected from the West China Hospital, Sichuan University between July 2006 and September 2009. The case group comprised endoscopic biopsy outpatients or tumor resection inpatients who were histopathologically confirmed to have gastric cancer. The patients (163 males; 83 females) had an average age of 59.7±11.9 years. The healthy control group comprised 252 healthy volunteers who visited the general health check-up division at West China Hospital, Sichuan University. The average age of the healthy control group (167 males and 85 females) was 58.8±11.2 years. There was no significant difference in the gender or age distribution between the patient and healthy control groups. Written informed consent was obtained from all the subjects, and the study was approved by the ethics committee of the Chinese Human Genome.

Genotyping

Genomic DNA was extracted from peripheral blood with an extraction kit (Bioteke Corporation: Beijing, China) according to the manufacturer's instructions. *MMPs* were identified using polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) analysis (PCR instrument: Bio-Rad, USA). The primer sequences and reaction conditions were described previously.[13,16] To confirm the genotyping results, the PCR-amplified DNA samples were sequenced by ABIPRISM 3730XL automated sequencer (Applied Biosystems, USA).

Statistical Analysis

The genotype and allele frequencies of the *MMP* genes were compared in the two groups using the $\chi 2$ test and Fisher's exact test as appropriate. Odds ratios (OR) with 95% confidence intervals (CI) were calculated to assess the relative risk conferred by a particular allele or genotype. Demographic and clinical data between groups were compared by $\chi 2$ and Student's t-tests. Hardy-Weinberg equilibrium was tested with a goodness of fit $\chi 2$-test with one degree of freedom to compare the observed genotype frequencies with the expected genotype frequencies. Statistical significance was assumed at the $p<0.05$ level in a two-tailed test. The SPSS statistical software package version 11.5 was used for all statistical analyses.

For the meta-analysis, we used allele frequency data extracted from each eligible study to examine the association of GC with *MMP-1* -1607 1G/2G and *MMP-7* -181 A/G. We sharply contrasted the *MMP-1* -1607 1G/2G allelic effect, comparing 2G versus 1G, 2G/2G+2G/1G versus 1G/1G and 2G/2G versus 2G/1G +1G/1G genotypes, and the *MMP-7* -181 A/G allelic effect, comparing A versus G, AA+AG versus GG and AA versus AG+GG genotypes. OR was used as the summary statistic. The point estimate of the pooled OR was considered statistically significant at two-sided *p*-value levels less than 0.05, and its significance was determined by the *z*-test.[17] Random- and fixed-effects models were used to calculate pooled effect estimates in

the presence ($p \leq 0.1$) and absence ($p > 0.1$) of heterogeneity, respectively. The heterogeneity of the OR was assessed using a χ^2 test of goodness of fit. I^2 was used to describe the percentage of the total variation between studies that was due to heterogeneity rather than to chance.[18] The influence of each study on the pooled OR was determined by sequentially removing each study and recalculating the pooled OR and its 95% confidence interval (95% CI), i.e., sensitivity analysis. Stratification analysis was implemented according to the populations studied. Publication bias was examined visually in a funnel plot of log OR against standard error, and the degree of asymmetry was tested using Egger's unweighted regression asymmetry test.[19] This test detects funnel plot asymmetry by determining whether the intercept deviates significantly from zero in a regression of the standardized effect estimates against their precision. All of the above statistical analyses were performed using the software package Stata Version 8.0 (Stata Corporation, College Station, TX).

RESULTS

The *MMP-1*-1607 1G/2G and *MMP-7*-181 A/G polymorphism genotype and allele distributions in cases and controls are shown in Table 1. Both polymorphisms were in Hardy-Weinberg equilibrium in both cases and controls.

The frequency of *MMP-1* -1607 2G>1G alleles did not differ significantly between cases and controls (OR=0.944; 95% CI=0.693-1.287; p=0.718), and the same result was observed for genotype frequencies (p=0.232).

The genotypes of *MMP-7*-181 A/G were differentially distributed in gastric cancer patients and control subjects: AA 95.94%, AG 4.06% in patients versus AA 88.10%, AG 11.90% in controls (OR=3.189 95% CI=1.523-6.676; p=0.001). A similar result was observed for allelic polymorphisms: A 97.97% and G 2.03% in patients versus. A 94.05% and G 5.95% in controls (OR=3.051 95% CI=1.475-6.310; p=0.002).

The meta-analysis of 6 case-control association studies included 1084 cases and 1721 controls (Table 2). These results demonstrated that carrying the 2G allele of *MMP-1* -1607 were not at a significantly increased risk of GC compared to individuals with the1G allele (OR=1.03, 95% CI=0.94–1.12, p=0.118) and1G/1G genotype (OR=1.00, 95% CI=0.89–1.12, p=0.221) (Table3-4, Figure1-3). However, there was significant

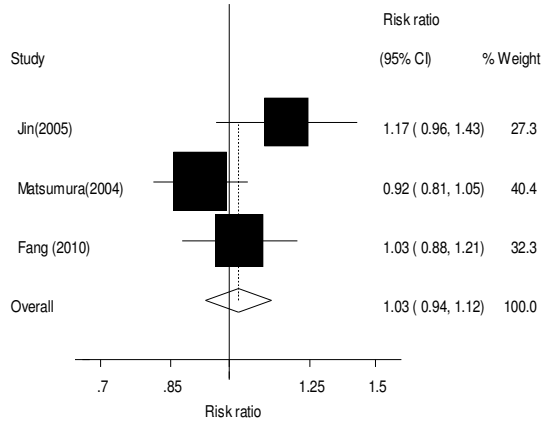

Figure 1. Meta-analysis of the association between GC and the *MMP-1*-1607 2G/1G allele among all populations in a fixed model.

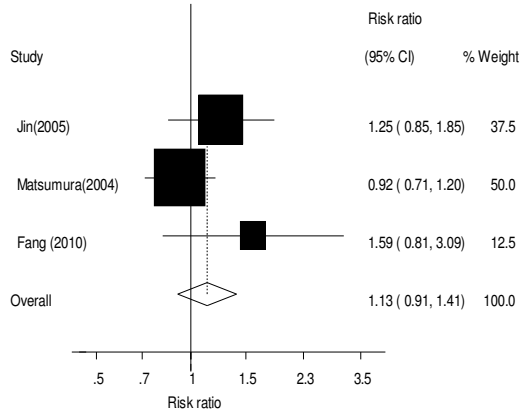

Figure 2. Meta-analysis of the association between GC and the *MMP-1*-1607 2G/2G versus 2G /1G+1G/1G allele among all populations in a fixed model.

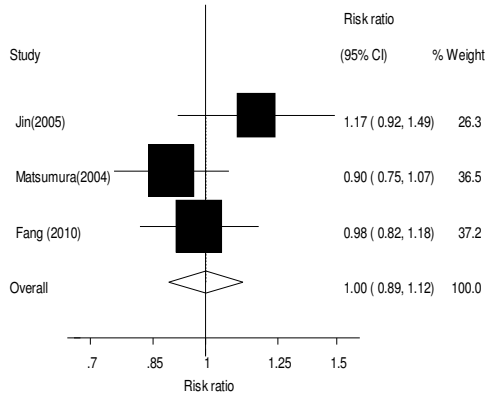

Figure 3. Meta-analysis of the association between GC and the *MMP-1*-1607 2G/2G+2G/1G versus 1G/1G allele among all populations in a fixed model.

difference between the *MMP-7* -181 A and G alleles in patients with GC and controls. Individuals carrying the A allele or AA genotype of *MMP-7*-181A/G had significantly increased risk of GC compared to individuals with the G allele (OR=0.91, 95% CI=0.78–1.06, p=0.006) or GG genotype (OR =1.01, 95% CI=0.85–1.19, p<0.001) (Table 3-4, Figure 4-6).

Table 1. The genotype and allele frequencies of *MMP-1* -1607 1G/ 2G and *MMP-7* -181 A/G in GC patients and controls.

Polymorphisms	Patients n=246 (%)	Controls n=252 (%)	OR (95% CI)	*P value*	
MMP-1-1607 1G/2G					
Genotypes					
1G/1G	6 (2.44%)	13 (5.16%)			
1G/2G	85 (34.55%)	78 (30.95%)	0.424 (0.153-1.169)	0.090	
2G/2G	155 (63.01%)	161 (63.89%)	0.479 (0.178-1.293)	0.139	0.232
Alleles					
1G	97 (19.72%)	104 (20.63)			
2G	395 (80.28%)	400 (79.37)	0.944 (0.693-1.287)	0.718	
MMP-7 -181 A/G					
Genotypes					
AA	236 (95.94%)	222 (88.10%)			
AG	10 (4.06%)	30 (11.90%)	3.189 (1.523-6.676)	0.001	
Alleles					
A	482 (97.97%)	474 (94.05%)			
G	10 (2.03%)	30 (5.95%)	3.051 (1.475-6.310)	0.002	

P: compared in alleles; P: compared in genotypes

Table 2. Characteristics of individual studies included in this meta-analysis.

First author (year)	Country	Selection/characteristics of cases	Selection/characteristics of controls
Matsumura (2004)	Japan	215 gastric cancer patients, 153 males and 62 females (median age 67.7±11.4 years), including 122 patients with an intestinal type of gastric cancer and 93 patients with a diffuse type.	166 healthy control subjects, 95 males and 71 females.
Jin (2005)	China	183 patients with gastric cardiac adenocarcinoma, 134 males and 49 females, average age 55.0±10.5.	350 healthy individuals, 229 males and 121 females. Average age 51.7±10.7.
Zhang (2005)	China	201 gastric cardiac carcinoma patients, 146 males and 55 females, average age 55.6±10.2 years.	350 healthy controls, 229 males and 121 females, average age 51.7±10.7 years.
Kubben (2006)	Netherlands	79 primary gastric adenocarcinoma patients (21 females and 58 males, average age 66 years, range 35–91 years).	169 healthy volunteers (64 male and 105 males, median age 33 years (range 18–73 years), > 95% Caucasian).
Sugimoto (2008)	Japan	160 gastric cancer patients, 122 males and 38 females, average age 68.6 ±0.8 years.	434 non-cancer patients, including 156 gastritis patients, 157 gastric ulcer patients, and 121 duodenal ulcer patients, with average ages of 52.6±0.8, 54.2 ±1.1 and 50.7±1.2 and male/female ratios of 106/50, 131/26 and 99/22, respectively.
Fang (2010)	China	246 gastric cancer patients, 163 males and 83 females, average ages 57.9 ±11.8 years.	252 normal controls, 167 males and 85 females, average ages 58.8±11.2 years.

Table 3. Sample sizes and *MMP-1* -1607 1G/2G, *MMP-7* -181A/G allele and genotype distributions in the studies considered in this meta-analysis.

Gene	First author (year)	Cases						Controls					
		N	2G/2G	1G/2G	1G/1G	G	C	N	2G/2G	1G/2G	1G/1G	G	C
MMP-1-1607 1G/2G	Matsumura (2004)	215	101	88	26	290	140	166	88	61	17	237	95
MMP-1-1607 1G/2G	Jin (2005)	183	112	51	20	275	91	350	194	105	51	493	207
MMP-1-1607 1G/2G	Fang (2010)	246	155	85	6	395	97	252	161	78	13	400	104
MMP-7 -181A/G	Zhang (2005)	201	167	34	0	368	34	350	316	33	1	665	35
MMP-7 -181A/G	Kubben (2006)	79	34	37	8	105	53	169	46	106	17	198	140
MMP-7 -181A/G	Sugimoto (2008)	160	133	27	0	293	27	434	393	40	1	826	42
MMP-7 -181A/G	Fang (2010)	246	236	10	0	482	10	252	222	30	0	482	10

Table 4. Meta-analysis of the association between the studied *MMP-1, 7* alleles and GC in different populations.

Gene	Genotypes	Group	A fixed-effects model			Heterogeneity		
			OR (95%CI)	Z	*P*	X^2	I^2 (%)	$P_{Q\text{-test}}$
MMP-1 -1607 1G/2G	2G vs. 1G	total (Asian)	1.03 (0.94-1.12)	0.55	0.585	4.28	53.2%	0.118
	2G/2G+2G/1G vs. 1G/1G	total	1.13 (0.91-1.41)	1.10	0.270	3.55	43.7%	0.169
	2G/2G vs. 2G/1G +1G/1G	total	1.00 (0.89-1.12)	0.02	0.983	3.02	33.8%	0.221
MMP-7 -181 A/G	A vs. G	total	0.91 (0.78-1.06)	1.19	0.235	12.52	76.0%	0.006
		Asian	0.74 (0.62-0.89)	3.25	0.001	2.21	9.5%	0.331
		Caucasian	1.26 (0.96-1.66)	1.65	0.099	0	0	-
	AA+AG vs. GG	total	1.03 (0.58-1.82)	0.10	0.923	0.10	0	0.953
		Asian	1.27 (0.23-6.96)	0.28	0.782	0.03	0	0.861
		Caucasian	0.99 (0.54-1.82)	0.02	0.987	0	0	-
	AA vs. AG+ GG	total	1.01 (0.85-1.19)	0.06	0.953	27.94	89.3%	0.000
		Asian	0.87 (0.71-1.06)	1.37	0.171	16.00	87.5%	0.000
		Caucasian	1.59 (1.11-2.27)	2.53	0.011	0	-	-

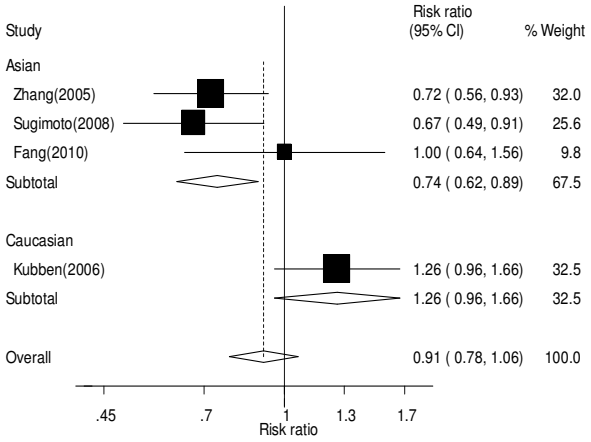

Figure 4. Meta-analysis of the association between GC and *MMP-7* -181 A/G allele among all populations in a fixed model.

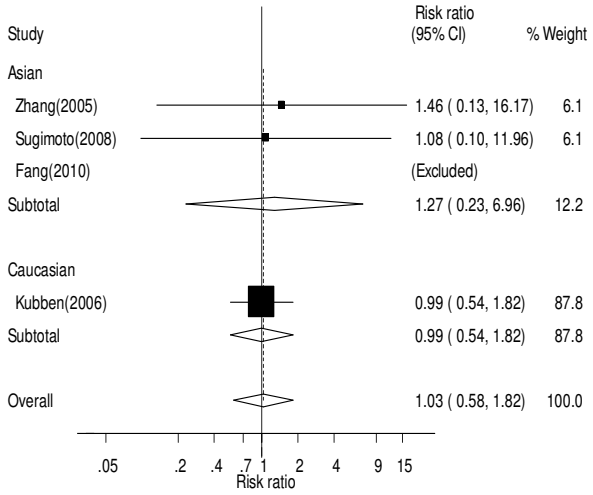

Figure 5. Meta-analysis of the association between GC and *MMP-7* -181 AA+AG/GG allele among all populations in a fixed model.

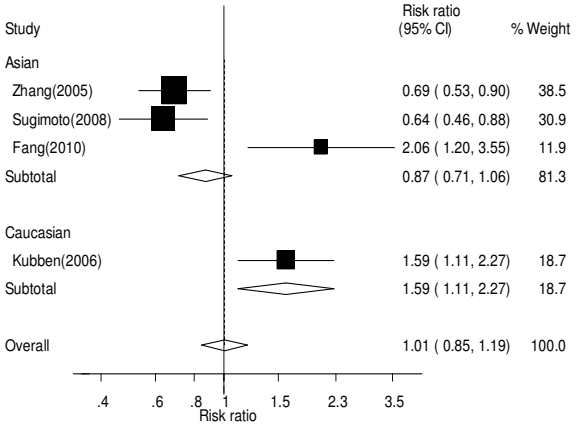

Figure 6. Meta-analysis of the association between GC and *MMP-7* -181 AA /GG+AG allele among all populations in a fixed model.

DISCUSSION

MMPs are able to degrade the extracellular matrix and basement membrane, which is an important event in many physiological and pathological processes, including tumor invasion and metastasis. Metalloproteinase expression has been found in a variety of human tumors and has a positive correlation with tumor invasion, metastasis and therapeutic response.[20,21]

We detected the MMP-1-1607 1G/2G and MMP-7-181 A/G allele and genotype distributions using the PCR-RFLP analysis. No significant change of the MMP-1-1607 1G/2G polymorphism was found between the GC patients and the healthy controls. It was consistent with the results of two other studies[11,12]. Our results showed an association between the MMP-7-181 A/G allele and genotype and GC in Chinese Hans. Interestingly, the MMP-7-181 A/G polymorphism was found to be associated with GC in Asian and Caucasian people in previous studies[13-15].

We added our numbers to those of similar previously published studies for a meta-analysis of 1804 cases and 1721 controls. These data confirm that there were significant differences in the genotype and allele distributions of the -181 A/G polymorphism of the *MMP-7* gene among cases and controls. The -181 A allele was associated with a significantly increased risk

of GC compared with the -181 G allele. However, no significant relationship was observed between the *MMP-1* -1607 1G/2G alleles and genotypes and the risk of GC. The frequency of *MMP-1* -1607 1G/2G alleles was similar in both groups in our study.

To date, the SNP screening of 23 *MMP* genes has included only SNPs in the gene promoter regions, and few polymorphisms in the coding region of the gene have been examined. However, with the recent developments in protein structure research and the role of *MMPs* in tumor metastasis, the effects of structural polymorphisms on *MMP* function and enzyme activity are gradually becoming clear. It is hoped that the further study of more SNPs in *MMPs* will reveal more information about the association between *MMPs* and tumor biology and provide a basis for cancer diagnosis, prognosis assessment, gene diagnosis and gene therapy.

In summary, a meta-analysis of our data and data from previous studies indicates that the -181A/G polymorphism of *MMP-7* is likely to be associated with GC. These findings should be further validated by larger, preferably prospective studies with more diverse ethnic groups and more detailed environmental exposure data.

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