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The Contribution of Matrix Metalloproteinase-1 Genotypes to Hepatocellular Carcinoma Susceptibility in Taiwan

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Abstract. *Background/Aim: Metalloproteinases (MMPs) are a family of proteases which have been shown to be overexpressed in various types of cancers. However, the contribution of MMP1 genotype to hepatocellular carcinoma (HCC) has not been well studied. This study aimed to evaluate the contribution of MMP1 promoter 1607 genotype to the risk of HCC in Taiwan, where HCC incidence is relatively high in the world. Materials and Methods: In this case–control study, MMP1 genotype and its interaction with consumption of cigarettes and alcohol in determining HCC risk was investigated among 298 HCC patients and 889 age- and gender-matched healthy controls. Results: The percentages of ever smokers and ever alcohol drinkers were much higher in the case group than in the control group. The percentages of 2G/2G, 1G/2G and 1G/1G for MMP1 promoter 1607 genotype were 37.2%, 38.3% and 24.5% in the HCC group and 34.8%, 44.0% and 21.2% in the control group, respectively (p for trend=0.2048). The allelic frequency distribution analysis showed the variant 1G allele of MMP1 promoter 1607 conferred similar HCC susceptibility as the wild-type 2G allele (odds ratio (OR)=1.01, 95% confidence interval (CI)=0.84-1.22, p=0.8735). As for the gene–lifestyle interaction, there was an obvious protective effect of MMP1 promoter 1607 1G allele on the risk of HCC among non-smokers, but not non-smokers, even alcohol drinkers or non-drinkers. Conclusion: The 1G allele of MMP1 promoter 1607 may have a protective effect on HCC risk for non-smokers in Taiwan and further validations are needed in other population groups.*

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Statistically, hepatocellular carcinoma (HCC) is diagnosed in more than 500,000 people worldwide annually and is one of the leading causes of cancer-related deaths (1). Geographically, HCC incidence is of relatively high density in Taiwan, China and other Asia-Pacific regions but has a low incidence in the United States and Europe (2), and has been reported to be closely associated with chronic infection with hepatitis B (HBV) or C virus (HCV), aflatoxin exposure, cigarette smoking, alcohol consumption, cirrhosis, male gender and family history of HCC (3, 4). In addition to these environmental factors, the genetic factors may contribute to the initiation and progression of HCC. In Taiwan, although specific biomarkers for HCC have been reported in recent years (5-9), the genomic susceptibility of HCC and the interactions among the genetic and environmental risk factors are largely unrevealed.

Extracellular matrix (ECM) components, which are composed of glycosaminoglycans and fibrous proteins, may contribute to the regulation and progression of morphogenesis, angiogenesis, inflammation, would healing and tumorigenesis (10). The matrix metalloproteinases (MMPs), also known as matrixins, are a family of calcium-dependent endopeptidases that play a key role in cell recruitment, migration (adhesion and dispersion), differentiation, angiogenesis, cell death

Table I. *Summary of selected characteristic data of the 298 patients with hepatocellular carcinoma and the 889 matched healthy controls.*

Characteristic	Controls (n=889)			Cases (n=298)			*p*-Value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			55.4 (4.9)			52.3 (4.5)	0.7418
Gender							
Male	636	71.5%		213	71.5%		0.9830
Female	253	28.5%		85	28.5%		
Personal habits							
Ever smokers	579	65.1%		224	75.2%		0.0017*
Ever drinkers	518	41.7%		206	69.1%		0.0011*

SD: Standard deviation; [a]abased on Student's *t*-test and Chi-square test. *Statistically significant, $p<0.05$.

(apoptosis and autophagy), and control degradation of the components of connective tissue matrices (10-14). Homeostasis statuses of MMPs is under the control of a complex network (15), and the imbalance of MMPs, include MMP-1, was a common feature for HCC etiology (16-19). MMP1, also known as collagenase-1, is the most abundant MMP in ECM and under the control of activator protein-1 (AP1) that binds to the promoter region of mitogen-activated kinase through polyomavirus-enhancing activity-3 (20, 21). A polymorphic site was found in the *MMP1* promoter region at upstream position of 1607 bp, which was reported to control the transcriptional activity of the *MMP1* gene and was also correlated with the incidence and progression of several cancer types (22).

The genomic contribution of *MMP1* to cancer has not been well elucidated and few scientific reports have investigated its role in HCC. In 2005, Okamoto and his colleagues reported that the 2G allele of *MMP1* promoter 1607 polymorphism was not associated with risk of HCC, compared with the 1G allele in a Japanese population with only 92 HCC cases and 83 controls (16). In 2010, the same group further proposed that *IL-1β* -31 T allele and *MMP-3* 5A allele, but not *MMP-1* allele, are cooperative risk factors for poor prognosis in HCC patients (23). Zhai and colleagues reported that *MMP1* promoter 1607 polymorphism was not associated with risk of HCC in 434 cases and 480 controls in south China (24). In the current study, we aimed to firstly reveal the contribution of *MMP1* genotype at the promoter 1607 site to the risk of HCC in Taiwanese.

Materials and Methods

Investigated population. Two hundred and ninety-eight patients diagnosed with HCC by Dr. Jeng were recruited at the Department of General Surgery at the China Medical University Hospital, Taiwan, in 2004-2010. Each patient and non-cancerous healthy person completed a self-administered questionnaire and provided their peripheral blood samples. Originally, three times as many non-cancer healthy volunteers as controls were selected by matching for age and gender after initial random sampling from the Health Examination Cohort of our hospital. The exclusion criteria of the controls included previous malignancy, metastasized cancer from other or unknown origin, and any genetic or familial diseases. The included control population was 898. Our study was approved by the Institutional Review Board of the China Medical University Hospital (DMR103-IRB-094), and written informed consent was obtained from all participants. The selected characteristic information extracted from personal questionales is summarized in Table I.

Genotyping conditions. The genomic DNA from the peripheral blood leucocytes of each investigated subject was prepared applying the QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and stored at −80˚C until processed as per our recent publications (25-28). The sequences of primers and the restriction enzymes for *MMP1* promoter 1607 genotyping are the same as our previous publication (29-31). Briefly, the sequences for forward and reverse primer pairs were 5'-TGACTTTTAAAACATAGTCTATGT-3' and 5'-GATTG ATTTGAGATAAGTCATAGC-3', respectively. The polymerase chain reaction (PCR) cycling conditions were set as: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 s, 58˚C for 30 s, and 72˚C for 30 s; and a final extension step at 72˚C for 10 min. After PCR amplification, the PCR products were subject to the digestion by *Alu*I restriction endonuclease for 2 h at 37˚C and separation *via* 3% agarose gel electrophoresis for 25 min. The genotypes were identified as homozygous 2G/2G with 269-bp product, heterozygous 1G/2G with 269-, 241- and 28-bp products, and homozygous 1G/1G with 241- and 28-bp products, respectively. All the genotypic processing was repeated by two researchers independently and blindly, and all the genotyping results were 100% concordant.

Statistical analyses. Student's *t*-test was applied for the comparison of ages between the HCC case and the control groups. Pearson's Chi-square test was applied to compare the distribution of the *MMP1* promoter 1607 genotypes among the subgroups. The associations between the *MMP1* promoter 1607 genotypes and HCC risk were estimated by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from logistic regression analysis. Statistically, any difference at $p<0.05$ was taken as significant between the two groups compared.

Table II. *Distribution of matrix metalloproteinase-1 (MMP1) promoter 1607 genotypes among the 298 patients with hepatocellular carcinoma and the 889 matched healthy controls.*

	Controls		Patients		OR (95% CI)	*p*-Value[a]
	n	%	n	%		
Genotype						
2G/2G	309	34.8%	111	37.2%	1.00 (Reference)	
1G/2G	391	44.0%	114	38.3%	0.82 (0.60-1.10)	0.1737
1G/1G	189	21.2%	73	24.5%	1.08 (0.76-1.52)	0.6815
P_{trend}						0.2048
Carrier comparison						
2G/2G +1G/2G	700	78.8%	225	75.5%	1.00 (Reference)	
1G/1G	189	21.2%	73	24.5%	1.20 (0.88-1.64)	0.2436
2G/2G	309	34.8%	111	37.2%	1.00 (Reference)	
1G/1G+1G/2G	580	65.2%	187	62.8%	0.90 (0.68-1.18)	0.4366

[a]Based on Chi-square test without Yate's correction.

Table III. *Distribution of allelic frequencies for matrix metalloproteinase-1 (MMP1) promoter 1607 among the 298 patients with hepatocellular carcinoma and the 889 matched healthy controls.*

	Controls, n	%	Patients, n	%	OR (95% CI)	*p*-Value[a]
Allele						
2G	1009	56.8%	769	56.4%	1.00 (Reference)	
1G	336	43.2%	260	43.6%	1.01 (0.84-1.22)	0.8735

[a]Based on Chi-square test without Yate's correction.

Results

The frequency distributions of selected characters including age, gender and personal lifestyles for the 298 HCC patients in the case group and 889 non-cancer healthy subjects in the control group are summarized and compared in Table I. Since we applied frequency matching to recruit the non-cancer healthy subjects as the controls, it is granted that there was no difference in the distributions of age and gender between the control and case groups (Table I). For these investigated individuals, it was found that the percentages of ever smokers and ever alcohol drinkers were much higher in the case group than in the control one (Table I). These findings fit the previous reports that smoking and alcohol drinking are risk factors for HCC in Taiwan.

The distributions of the MMP1 promoter 1607 genotype among the 889 non-cancer controls and the 298 HCC patients are presented and statistically analyzed in Table II. The results showed that the genotypes of *MMP1* promoter 1607 were not differently distributed between case and control groups (p for trend=0.2048) (Table II). In detail, the *MMP1* promoter 1607 heterozygous 1G/2G and homozygous 1G/1G were not associated with HCC risk, compared to wild-type 2G/2G genotype (OR=0.82 and 1.08, 95%CI=0.60-1.10 and 0.76-1.52, p=0.1737 and 0.6815, respectively; Table II). In the recessive and dominant models, there was still no association between the genotype of *MMP1* promoter 1607 and HCC risk (OR=1.20 and 0.90, 95%CI=0.88-1.64 and 0.68-1.18, p=0.2436 and 0.4366, respectively; Table II).

To confirm the results in Table II, the analysis of allelic frequency distribution for the *MMP1* promoter 1607 polymorphism was further conducted and the results are presented in Table III. Supporting the findings that neither heterozygous 1G/2G nor homozygous 1G/1G genotype of *MMP1* promoter 1607 was associated with HCC risk, the variant allele 1G was found at 43.6% in the case group, non-significantly different from that of 43.2% in the control group (OR=1.01, 95% CI=0.84-1.22, p=0.8735). To sum-up, there was no significant difference in the allelic frequencies of *MMP1* promoter 1607 between the case and control groups (Table III).

Since we found that smoking and alcohol drinking are risk factors for HCC in Taiwan, we were interested in investigating the interactions between the genotype of *MMP1*

Table IV. *Odds ratios for matrix metalloproteinase-1 (MMP1) promoter 1607 genotype and hepatocellular carcinoma after stratification by smoking status.*

Genotype	Non-smokers, n		OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value	Smokers, n		OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value
	Controls	Cases				Controls	Cases			
2G/2G	100	36	1.00 (ref)	1.00 (ref)		209	75	1.00 (ref)	1.00 (ref)	
1G/2G	139	26	0.52 (0.30-0.92)	0.54 (0.33-0.90)	0.0222*	252	88	0.97 (0.68-1.39)	0.99 (0.71-1.33)	0.8816
1G/1G	71	12	0.47 (0.23-0.97)	0.44 (0.25-0.95)	0.0371*	118	61	1.44 (0.96-2.16)	1.41 (0.94-2.11)	0.0777
Total	310	74				579	224			

[a]By multivariate logistic regression analysis. [b]By multivariate logistic regression analysis after adjusting of age, gender and alcohol drinking status; *Statistically significant.

Table V. *Odds ratios for matrix metalloproteinase-1 (MMP1) promoter 1607 genotype and hepatocellular carcinoma after stratification by alcohol drinking status.*

Genotype	Non-drinker, n		OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value	Drinkers, n		OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value
	Controls	Cases				Controls	Cases			
2G/2G	127	36	1.00 (ref)	1.00 (ref)		182	75	1.00 (ref)	1.00 (ref)	
1G/2G	163	37	0.80 (0.48-1.34)	0.82 (0.49-1.29)	0.3965	228	77	0.82 (0.56-1.19)	0.79 (0.57-1.21)	0.2952
1G/1G	81	19	0.83 (0.44-1.54)	0.84 (0.45-1.58)	0.5503	108	54	1.21 (0.79-1.85)	1.16 (0.82-1.93)	0.3701
Total	371	92				518	206			

[a]By multivariate logistic regression analysis. [b]By multivariate logistic regression analysis after adjusting of age, gender and smoking status; *Statistically significant.

promoter 1607 and personal cigarette smoking and alcohol drinking habits. Among the non-smokers, those with genotype of 1G/2G or 1G/1G at *MMP1* promoter 1607 were at 0.52- and 0.47-fold odds of having HCC (95% CI=0.30-0.92 and 0.23-0.97, p=0.0222 and 0.0371, respectively), seemingly conferring a protective effect, but this was not the case among those smokers (Table IV). After the adjusting of age, gender and alcohol drinking status, the significance were still existing at the similar level (OR=0.54 and 0.44, 95% CI=0.33-0.90 and 0.25-0.95, respectively, Table IV). There was no such close interaction of *MMP1* promoter 1607 genotype with personal alcohol drinking habits (Table V).

Discussion

In the literature, MMP-1, together with MMP-2, -9, -13 and their regulators TIMP-1 and -2 are involved in the liver fibrosis processions (32-36), but few has studied the genomic contribution of *MMP-1* to the carcinogenesis of HCC. In the current hospital–based case–control study, the contribution of *MMP1* promoter 1607 to HCC risk and its interaction with alcohol drinking and cigarette smoking were firstly examined among Taiwanese. The results showed that although neither the genotypic (Table II) nor the allelic frequencies (Table III) of *MMP1* promoter 1607 were differentially distributed among the HCC patients and non-cancer healthy controls, the 1G allele was a protective determinant for risk of HCC among the non-smokers (Table IV).

This is the first study to reveal an interaction between *MMP1* 1607 genotype and cigarette smoking on the susceptibility to HCC. Previously, long-term tobacco smoking has been shown to contribute to the etiology of HCC development (37-40) but little was known about the contributions of genomic factors to HCC development. Recently, others and our team have reported that specific genotypes may be combined with cigarette smoking habits and contribute to increased HCC risk, such as the polymorphisms on *CYP1A1* (41), *N-acetyltransferase* 2 (42) and *tumor necrosis factor-alpha* (9). Several genomic markers did not have joint effects with cigarette smoking habit on HCC risk (5, 8). However, the overall mechanisms are very complex and need more investigations. In Table I, it can be found that a higher proportion of individuals had consumed cigarettes and alcohol in the group of patients with HCC than the controls. However, the incomplete records of other factors, such as infection status with hepatitis B (HBV) or C virus (HCV), limited us to observe the interactions of genotypes of *MMP1* 1607 with these environmental factors of HCC in Taiwan.

MMP1 has been reported to be in charge of the degradation of the interstitial collagens, hence it is called collagenase-1. In normal conditions, MMP1 is under the suppression of TIMP1 (43, 44) and elevated MMP1 has been reported to play an important role in invasive and migration capacity of tumor cells (45). Mounting evidence indicates that elevated MMP1 expression was observed in the borders of solid tumors, such as breast and oral cancer (46-48). Mechanically, MMP1 is thought to promote invasion and metastasis through the degradation of the ECM as the main component of connective tissue, like to relieve the reins for the horses (49-51). In 2012, Liu and his colleagues performed a meta-analysis exploring the association between *MMP1* promoter 1607 1G/2G polymorphism and risk of several types of cancer, and the results showed that an elevated cancer risk was found regarding breast, colorectal, genitourinary neoplasm but not HCC (52). The dynamic balance between MMPs and TIMPs play a pivotal role in the maintenance of normal physiological conditions for cells, but it seems that the closely regulation of MMP1 by TIMP1 in HCC tissues is not as simple as a 'see-saw' relationship. In 2009, Altadill and his colleagues reported that overexpressed MMP-1 by fibroblast cells is correlated with poor prognosis (19), supported by the findings that overexpressed MMP-1 was associated with an elevated metastasis capacity of the HCC cells (53, 54). Altadill and his colleagues also reported that overexpressed TIMP1 by stromal cells is correlated with shortened overall survival period (19), and accordingly TIMP-1 overexpression was reported to be associated increased invasive and metastatic capacity of the HCC cells (55, 56). In the near future, further analysis of *TIMP1* genotype/phenotype may provide further evidence for evaluating the contribution of combined genotypes of *MMP-1* and *TIMP1* to the carcinogenesis of HCC. In addition, the promoter assay with the different genotypes of *MMP-1* 1607 and possible *TIMP1* promoter polymorphic site, may add more evidence for the functional differences between the different genotypes or haplotypes. Moreover, the involvement of smoking in etiology of HCC, especially the initial step of unbalanced DNA damage, was accessible with the treatment of cigarette components to the cells with different *MMP-1* and/or *TIMP1* genotypes. Of course, the genetic-environmental interactions could also be approached with the treatments of increasing doses of cigarette components to the cells with different *MMP-1* and/or *TIMP1* genotypes, and investigating their genomic instability. It is very possibly that the cells with 2G/2G genotypes at *MMP-1* 1607 with highest dose of BPDE, an ultimate carcinogenic metabolite of tobacco smoke carcinogen benzo[a]pyrene, was of the highest instable genomic integrity, which is mostly prone to carcinogenesis.

In conclusion, the study provides evidence that the 1G allele at *MMP1* promoter 1607 may interact with personal smoking status to determine the personal susceptibility to HCC, and more investigations should be conducted to reveal the detail alteration of ECM components with HCC risk and prognosis.

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