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The Contribution of Matrix Metalloproteinase-1 Genotype to Oral Cancer Susceptibility in Taiwan

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Abstract. *Aim: Metalloproteinases (MMPs) are a family of multifunctional proteins which have been shown to be up-regulated in various types of cancer. However, the contribution of MMP1 genotype to oral cancer has not been elucidated. This study aimed to evaluate the contribution of MMP1 promoter 1607 genotype to the risk of oral cancer. Materials and Methods: In this case–control study, MMP1 genotype and its interaction with consumption of areca, cigarettes, and alcohol in determining oral cancer risk were investigated in 788 patients with oral cancer and 956 gender-matched healthy controls. Results: The distribution of 2G/2G, 1G/2G and 1G/1G for MMP1 promoter 1607 genotype was 36.8%, 40.2% and 23.0% in the oral cancer group and 34.3%, 44.9% and 20.8% in the non-cancer control group, respectively (p for trend=0.1454). We also analyzed the allelic frequency distributions and found that the variant 1G allele of MMP1 promoter 1607 conferred similar oral cancer susceptibility as the wild-type 2G allele (odds ratio=0.99, 95% confidence interval=0.87-1.14, p=0.9199). As for the gene–lifestyle interaction, there was an obvious protective effect of MMP1 promoter 1607 1G/2G genotype on the risk of oral cancer among smokers (odds ratio=0.71, 95% confidence interval=0.55-0.91, p=0.0076), but not non-smokers. There was no interaction between MMP1 promoter 1607 genotype and areca chewing or alcohol drinking habits. Conclusion: The 1G/2G genotype of MMP1 promoter 1607 may have a protective effect on oral cancer risk for smokers. The detailed mechanisms involved in this require further investigation.*

Statistically, oral cancer, the tenth most commonly diagnosed cancer worldwide, has the highest incidence density in Taiwan (1). According to the government annual report, oral cancer is the fourth cause of cancer-related death among males in Taiwan, and has been reported to be closely associated with tobacco, alcohol and betel nut consumption habits (2-5). Although several biomarkers for oral cancer in Taiwan have been revealed (6-12), the genomic etiology of oral cancer and the interactions among the genetic and lifestyle factors are of great interest but largely unknown.

Extracellular matrix (ECM) structures contribute to microenvironmental remodeling during the initial morphogenesis, angiogenesis, inflammation, would healing and tumorigenesis (13). The matrix metalloproteinases (MMPs) are a family of endopeptidases that play a key role in ECM remodeling, and control degradation of the components of connective tissue matrices (13, 14). They are also related to the regulation of oral cancer invasion and metastasis (15). The homeostasis of each MMP is also under the control of a complex network at several levels, including through their interactions with specific inhibitors, *e.g.*, the tissue inhibitors of metalloproteinases (TIMPs) (14). MMP1, also known as collagenase-1, is most abundant among the MMPs and under the control of activator protein-1 (AP1) which binds to the promoter region of mitogen-activated kinase through polyomavirus-enhancing activity-3 (16, 17). 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

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several cancer types (18). Studies of brain tumors suggest that there is a close relationship between the polymorphism of the *MMP1* promoter region and an increase in tumor grade in astrocytoma, glioblastoma and pituitary adenoma (17, 19-22). In an animal model, exposure to side-stream cigarette smoke 5 days per week for 1 month will induced an increase in *MMP1* mRNA levels in the lung tissue of male Wistar rats (23). Since smoking is a main lifestyle factor contributing to oral cancer, it is possible that abnormal expression of MMP1 may play a role in the carcinogenesis of smoking-related oral cancer.

The genomic contribution of *MMP1* to cancer has not been well elucidated. For oral cancer, Cao and colleagues reported that the 2G allele of *MMP1* promoter 1607 polymorphism was associated with higher risk of oral squamous cell carcinoma, compared with the 1G allele in a Chinese population (24). However, their investigation was limited to 96 cases and 120 controls. In the current study, we aimed to reveal the contribution of *MMP1* genotype at the promoter 1607 site to the risk of oral cancer in Taiwanese.

Materials and Methods

Investigated population. Our study was approved by the Institutional Review Board of the China Medical University Hospital (DMR101-IRB1-306) and written-informed consent was obtained from all the participants. Seven hundred and eighty-eight patients diagnosed with oral cancer were recruited at the China Medical University Hospital in central Taiwan. All patients voluntarily participated, completed a self-administered questionnaire and willingly provided 5 ml of their peripheral blood. The questionnaire administered to participants included questions on history and frequency of alcohol consumption, areca chewing and smoking habit. Self-reported alcohol consumption, areca chewing and smoking habits were evaluated and classified as categorical variables. Information on these factors was obtained as more than twice a week for years as ''ever''. A total of 956 non-cancer healthy individuals as controls were selected by matching for age and gender after initial random sampling from the Health Examination Cohort of the hospital. The male versus female ratio was 76% to 24% in each group. The mean age of the patients and the controls was 55.8 (SD=9.9) and 56.6 (SD=8.7) years, respectively. More detailed information is summarized in Table I.

Genotyping conditions. The genomic DNA from the peripheral blood leucocytes of each patient and control was prepared applying the QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and stored at −80˚C until processed as per our previous articles (6-8). The sequences of primers and the restriction enzymes for *MMP1* promoter 1607 genotyping are modified from a previous publication (25). The forward and reverse primers were 5'-TGACT TTTAAAACATAGTCTATGT-3' and 5'-GATTGATTTGAGATA AGTCATAGC-3', respectively. The polymerase chain reaction (PCR) cycling conditions were: 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 at 72˚C for 10 min. After amplification, the PCR products were subject to the digestion by *Alu*I restriction endonuclease for 2 h at 37˚C and separation using 3% agarose gel electrophoresis. 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. All the genotypic processing was repeated by two researchers independently, and blindly, and the results were 100% concordant.

Statistical analyses. Student's *t*-test was used for the comparison of ages between the case and the control groups. Pearson's Chi-square test was used to compare the distribution of the *MMP1* promoter 1607 genotypes among the subgroups. The associations between the *MMP1* promoter 1607 genotypes and oral cancer risk were estimated by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from logistic regression analysis. Any difference at $p<0.05$ was considered statistically significant, and all statistical tests were two-sided.

Results

The frequency distributions of selected characters including age, gender, personal habits and primary tumor sites for the 788 patients with oral cancer and 956 non-cancer controls are summarized and compared in Table I. Since we applied frequency matching to recruit the non-cancer healthy controls, there was no difference in the distributions of age and gender between the control and case groups (Table I). For these investigated individuals, betel quid chewers and smokers were more freqent in patients with oral cancer than in the controls (Table I). Most primary tumors occurred in the tongue and buccal mucosa.

The distributions of the *MMP1* promoter 1607 genotype among the non-cancer controls and the oral cancer patients are presented and statistically analyzed in Table II. The genotypes of *MMP1* promoter 1607 were not differently distributed between oral cancer and non-cancer control groups (p for trend=0.1454) (Table II). In detail, the *MMP1* promoter 1607 heterozygous 1G/2G and homozygous 1G/1G were not associated with oral cancer risk (p=0.1011 and 0.8283, respectively; Table II). In the recessive and dominant models, there was still no association between the genotype of *MMP1* promoter 1607 and oral cancer risk (p=0.2782 and 0.2788, respectively; Table II).

To confirm the findings in Table II, the analysis of allelic frequency distribution for the *MMP1* promoter 1607 was also conducted and the results are summarized in Table III. Supporting the findings that neither heterozygous 1G/2G nor homozygous 1G/1G genotype of *MMP1* promoter 1607 was associated with oral cancer risk, the variant allele 1G was found at 43.1% in the patient group, non-significantly different from that of 43.3% in the control group (p=0.9199). To sum up, there was no significant difference in the allelic frequencies of *MMP1* promoter 1607 between the control and oral cancer groups (Table III).

Next, we examined the interactions among the genotype of *MMP1* promoter 1607 and lifestyle factors, such as

Table I. *Characteristics of the 788 patients with oral cancer and 956 controls investigated.*

Characteristic	Controls (n=956)			Cases (n=788)			*p*-Value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			56.6 (8.7)			55.8 (9.9)	0.7951
Gender							1.0000
Male	727	76.0%		599	76.0%		
Female	229	24.0%		189	24.0%		
Personal habits							
Areca chewing	506	52.9%		661	83.9%		<0.0001*
Cigarette smoking	667	69.8%		595	75.5%		0.0084*
Alcohol drinking	641	67.1%		560	71.1%		0.0773
Primary tumor site							
Tongue				325	41.2%		
Buccal mucosa				294	37.3%		
Mouth floor				30	3.8%		
Retromolar trigone				26	3.3%		
Alveolar ridge				18	2.3%		
Palate				18	2.3%		
Lip				39	4.9%		
Others				38	4.9%		

SD: Standard deviation; [a]based on Chi-square test. *Statistically significant, $p<0.05$.

Table II. *Distribution of matrix metalloproteinase-1 (MMP1) promoter 1607 genotypes among the patients with oral cancer and controls.*

	Controls		Patients		OR (95% CI)	*p*-Value[a]
	n	%	n	%		
Genotype						
2G/2G	328	34.3%	290	36.8%	1.00 (Reference)	
1G/2G	429	44.9%	317	40.2%	0.84 (0.67-1.04)	0.1011
1G/1G	199	20.8%	181	23.0%	1.03 (0.80-1.33)	0.8283
*P*trend						0.1454
Carrier comparison						
2G/2G +1G/2G	757	79.2%	607	77.0%	1.00 (Reference)	
1G/1G	199	20.8%	181	23.0%	1.13 (0.90-1.42)	0.2782
2G/2G	328	34.3%	290	36.8%	1.00 (Reference)	
1G/1G+1G/2G	628	65.7%	498	63.2%	1.10 (0.91-1.33)	0.2788

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

personal cigarette smoking, betel quid chewing, and alcohol drinking habits. Among smokers, those with genotype of 1G/2G at *MMP1* promoter 1607 were at 0.71-fold odds of having oral cancer (95% CI=0.55-0.91, $p=0.0076$), seemingly conferring a protective effect, but this was not the case among non-smokers (Table IV). There was no interaction of *MMP1* promoter 1607 genotype with areca chewing and alcohol drinking habits (Table V and VI).

Table III. *Distribution of allelic frequencies for matrix metalloproteinase-1 (MMP1) promoter 1607 among patients with oral cancer and controls.*

	Controls, n	%	Patients, n	%	OR (95% CI)	*p*-Value[a]
Allele						
2G	1085	56.7%	897	56.9%	1.00 (Reference)	
1G	827	43.3%	679	43.1%	0.99 (0.87-1.14)	0.9199

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

Table IV. *Odds ratios (OR) for matrix metalloproteinase-1 (MMP1) promoter 1607 genotype and oral cancer 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	108	59	1.00 (ref)	1.00 (ref)		220	231	1.00 (ref)	1.00 (ref)	
1G/2G	123	89	1.32 (0.87-2.01)	1.38 (0.82-1.83)	0.1876	306	228	0.71 (0.55-0.91)*	0.77 (0.63-0.89)*	0.0076*
1G/1G	58	45	0.94 (0.58-1.52)	0.98 (0.64-1.39)	0.4939	141	136	0.92 (0.68-1.24)	0.81 (0.73-1.08)	0.5562
Total	289	193				667	595			

aOR: Adjusted OR. [a]By multivariate logistic regression analysis. [b]By multivariate logistic regression analysis after adjusting for age, gender, alcohol drinking and areca chewing status. [‡]For OR. *Statistically significant.

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

Genotype	Non-drinkers, 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	105	86	1.00 (ref)	1.00 (ref)		223	204	1.00 (ref)	1.00 (ref)	
1G/2G	146	90	0.75 (0.51-1.11)	0.78 (0.45-1.15)	0.1503	283	227	0.88 (0.68-1.13)	0.90 (0.67-1.21)	0.3179
1G/1G	63	52	1.01 (0.63-1.60)	1.05 (0.58-1.56)	0.5348	136	129	1.04 (0.76-1.41)	1.01 (0.75-1.35)	0.5371
Total	314	228				642	560			

aOR: Adjusted OR. [a]By multivariate logistic regression analysis. [b]By multivariate logistic regression analysis after adjusting for age, gender, smoking and areca chewing status. [‡]For OR. *Statistically significant.

Table VI. *Odds ratios for matrix metalloproteinase-1 (MMP1) promoter 1607 genotype and oral cancer after stratification by areca chewing status.*

Genotype	Non-chewers, n		OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value[‡]	Chewers, n		OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value[‡]
	Controls	Cases				Controls	Cases			
2G/2G	148	45	1.00 (ref)	1.00 (ref)		180	245	1.00 (ref)	1.00 (ref)	
1G/2G	206	52	0.83 (0.53-1.30)	0.81 (0.55-1.34)	0.4189	223	265	0.87 (0.67-1.13)	0.89 (0.71-1.15)	0.3101
1G/1G	96	30	1.03 (0.61-1.74)	1.05 (0.66-1.81)	0.6604	103	151	1.08 (0.79-1.48)	1.11 (0.75-1.60)	0.4125
Total	450	127				506	661			

aOR: Adjusted OR. [a]By multivariate logistic regression analysis. [b]By multivariate logistic regression analysis after adjusting for age, gender, smoking and alcohol drinking status. [‡]For OR. *Statistically significant.

Discussion

In the current case–control association study, the contribution of *MMP1* promoter 1607 to oral cancer risk and its interaction with alcohol drinking, cigarette smoking and areca chewing was firstly evaluated among Taiwanese. The results showed that although neither the genotypic nor the allelic frequencies of *MMP1* promoter 1607 were differentially distributed among the patients and non-cancer healthy controls (Table II), the heterogeneous 1G/2G genotype was associated with increased risk of oral cancer for the smokers (Table IV).

This is the first study to reveal an interaction between *MMP1* 1607 genotype and cigarette smoking on the susceptibility to oral cancer. Long-term tobacco smoking and areca chewing have been shown to contribute to etiology of oral cancer development (6, 7, 26-33). However, the mechanisms are very complex and need more investigations. In Table I, it can be seen that a higher proportion of individuals had consumed areca,

cigarettes and alcohol in the group of patients with oral cancer than the controls.

The MMP1 protein is involved in the degradation of the native fibrillar collagen, hence it is called collagenase-1. In normal conditions, MMP1 is expressed at a relatively low level under the suppressive regulation of TIMP1 protein (34, 35). MMP1 has been reported to play an important role in tumor cells undergoing invasion and migration (36, 37). Mounting evidence indicates that activated MMP1 is observed in the borders of solid tumors, such as breast and oral cancer (38-40). MMP1 is thought to promote invasion and metastasis through the degradation of the ECM as the main component of connective tissue, especially in the tumorigenesis of oral mucosal cancer (41-44). In 2016, MMP1 and TIMP1, together with MMP1 and TIMP2, were shown to gradually increase with progression of tongue cancer in a hamster model of tongue cancer (45). In 2012, Liu and 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 oral cancer (46). The dynamic balance between MMP1 and TIMP1 play a pivotal role in the maintenance of normal physiological conditions for cells, but it seems that the balance between *MMP1* and *TIMP1* in oral tissues is not as simple as a 'see-saw' relationship. In the near future, an overall analysis of MMP1 and TIMP1 genotype/phenotype may provide further evidence for evaluating the contribution of these genotypes to oral carcinogenesis.

In conclusion, our pilot study provides evidence that the heterozygous 1G/2G genotype at *MMP1* promoter 1607 may interact with personal smoking status to determine the susceptibility to oral cancer, and this should be confirmed in multicenter and multi-population studies.

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