Contribution of Matrix Metallopeptidase-1 Genotypes, Smoking, Alcohol Drinking and Areca Chewing to Nasopharyngeal Carcinoma Susceptibility

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Abstract. *Background/Aim: The up-regulation of matrix metalloproteinase-1 (MMP-1) has been demonstrated to be correlated with lymph node metastasis of nasopharyngeal carcinoma (NPC); however, the genotypic role of MMP-1 is not well understand. The present study aimed to assess the contribution of MMP-1 promoter -1607 genotypes, combined with environmental carcinogens, on the predisposition to NPC tumorigenesis. Materials and Methods: The MMP-1 promoter -1607 genotypes were examined for 352 age- and gender-matched controls and 176 NPC patients by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methodology. Results: We found that the MMP-1 promoter -1607 heterozygous 1G/2G and homozygous 1G/1G genotypes, were more and more prone to be associated with NPC risk (odds ratio (OR)=0.64 and 0.63, 95% confidence interval (CI)=0.43-1.03 and 0.36-0.96, p=0.0659 and 0.0932, respectively). In the dominant models, there was a significant association between the genotype of MMP-1 promoter -1607 and NPC risk (OR=0.64, 95% CI=0.43-0.91, p=0.0359). In addition, individuals carrying the 1G allele at MMP-1 promoter -1607 were less susceptible to NPC (OR=0.73; 95%CI=0.59 to 0.96, p=0.0418) after adjustment for age, gender, cigarette, alcohol and areca consumption. Conclusion: The 1G/1G genotype of MMP-1 promoter -1607 may independently have a protective effect on NPC risk, without interaction with behavioral factors, including cigarette, alcohol and areca consumption.*

Nasopharyngeal carcinoma (NPC) is an Epstein-Barr virus (EBV) associated cancer that arises from epithelial cells at the nasopharynx (1). Geographically, NPC has remarkably high prevalence in Southeast Asia with an incidence rate of 20-30 per 100, 000, although it is a rare cancer in the Western countries and worldwide (2-4). Statistically, the 5-year overall survival rates of NPC patients were recently reported to exceed 80% (5-7). However, there are still 20% to 30% of patients developing distant metastasis and/or locoregional recurrence, which are the major causes of therapeutic failure (8). Thus, markers of NPC susceptibility, especially those for metastasis prediction, are urgently needed. Although several biomarkers for NPC susceptibility have been revealed recently (9-13), the genomic etiology of NPC and the interactions among the genetic and environmental factors are of great interest, but largely unknown.

The imbalance of extracellular matrix (ECM) components together with their percentages and relatively special distributions contribute to the micro-environmental remodeling during the processes of multiple phenomenon, including morphogenesis, angiogenesis, inflammation, would healing and

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Key Words: Genotype, MMP-1, nasopharyngeal carcinoma, polymorphism.

Table I. *Characteristics of 176 nasopharyngeal carcinoma patients and 352 non-cancer healthy controls.*

Characteristics	Controls (n=352)			Cases (n=176)			*p*-Value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			48.7 (10.8)			49.3 (9.4)	0.7138
Gender							1.0000
Male	256	72.7%		128	72.7%		
Female	96	27.3%		48	27.3%		
Behavioral habits							
Cigarette smoker	150	42.6%		73	41.4%		0.8519
Alcohol drinker	124	35.2%		72	40.9%		0.2150
Areca chewer	115	32.7%		54	30.7%		0.6926

SD, Standard deviation; [a]Based on Chi-square test; *means statistically significant in case of $p<0.05$.

tumorigenesis (1). Among the various contents of ECM component proteins, the matrix metalloproteinases (MMPs) are a family of endopeptidases that play a key role in ECM remodeling processes, which are found to be in charge of degradation of specific components of the connective tissue matrices (14, 15), with these alterations being reported to be related to the regulation of cancer invasion and metastasis (16). A polymorphic site has been found in the MMP-1 promoter region at 1607 bp upstream of *MMP-1* gene, reported to control the transcriptional activity of the *MMP-1* gene, and also correlated with the incidence and progression of several cancers (17). Mounting evidence suggested that there is a close relationship between the polymorphism in the *MMP-1* promoter region and an increase in tumor grade in astrocytoma, glioblastoma and pituitary adenoma cases (18-22). In an animal model using male Wistar rats, exposure to side stream cigarette smoke 5 days per week for one month induced an increase in MMP-1 *mRNA* levels in the lung tissue (23). Since smoking is the main personal behavioral factor for NPC, it is possible that abnormal expression of MMP-1 may play a role in the carcinogenesis of smoking-related NPC. Up to date, the genomic contribution of *MMP-1* to NPC has never been elucidated. In the current study, we firstly revealed that the *MMP-1* genotypes at promoter -1607 may serve as a predictive biomarker for the risk of NPC.

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 participants. One hundred and seventy-six patients diagnosed with NPC were recruited at the general surgery outpatient clinic of the study hospital in Taichung, Taiwan, between 2003 and 2009. All patients participated voluntarily, completed a self-administered questionnaire and provided peripheral blood samples. The questionnaire included questions on history and frequency of alcohol consumption, areca chewing and smoking habits; the reply 'ever' was defined as more than twice a week for years. Self-reported alcohol consumption, areca chewing and smoking habits were evaluated and classified as categorical variables.

For each case patient, two age- and gender-matched healthy controls, who had no NPC or any other type of cancer, were selected from those attending the hospital for a health examination (age matching was done within less than 5 years of the case patient's first diagnosis). These volunteers attended the hospital for regular health assessments by multidisciplinary team approach with registered health practitioners during the years 2002-2012; most of the volunteers underwent health examinations every 5-6 months. A total of 10,358 participants aged 1-104 years were recruited into this cohort and those who were cancer-free at diagnosis of the case patient, according to the International Classification of Diseases-Ninth Revision (ICD-9) codes, were chosen. Finally, 352 participants were included for analysis in the present study. For the convenience of the gene–environment interaction analysis, we preferentially selected those with alcoholism, areca chewing and smoking personal habits when selecting the controls for genotyping work and further analysis. Thus, the control group was not a general population control but, rather, an alcohol-, areca- and cigarette-consumption control group. The overall agreement rate in this study was more than 85% in collection.

Genotyping protocols. Genomic DNA from the peripheral blood leucocytes of each investigated subject was prepared using the QIAamp Blood Mini Kit (Qiagen, Valencia, CA, USA), further stored in -80˚C and processed as already described (24-26). The sequences of primers and the restriction enzymes for *MMP-1* promoter -1607 genotyping are modified from a previous published paper (27). The forward and reverse primers for *MMP-1* promoter -1607 genotyping were 5'-TGACTTTTAAAACATAGTCTATGT-3' and 5'-GATTGATTTGAGATAAGTCATAGC-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 digestion with *Alu I* restriction endonuclease for 2 h at 37˚C and separation of 3% agarose gel electrophoresis. The genotypes were identified as homozygous 2G/2G (269 bp), heterozygous 1G/2G (269, 241 and 28 bp) and homozygous 1G/1G (241 and 28 bp). All the genotypic procession was repeated by two researchers independently and blindly as previously performed (28, 29), with results being 100% concordant.

Table II. *Distribution of MMP-1 promoter -1607 genotypes among the nasopharyngeal carcinoma patients and control subjects.*

	Controls		Patients		aOR (95% CI)[a]	*p*-Value[b]
	n	%	n	%		
Genotype						
2G/2G	121	34.4%	77	43.8%	1.00 (Reference)	
1G/2G	158	44.9%	69	39.2%	0.64 (0.43-1.03)	0.0659
1G/1G	73	20.7%	30	17.0%	0.63 (0.36-0.96)*	0.0932
P_{trend}						0.1080
Carrier comparison						
2G/2G +1G/2G	279	79.3%	146	83.0%	1.00 (Reference)	
1G/1G	73	20.7%	30	17.0%	0.75 (0.51-1.22)	0.3127
2G/2G	121	34.4%	77	43.8%	1.00 (Reference)	
1G/1G+1G/2G	231	65.6%	99	56.2%	0.64 (0.43-0.91)*	0.0359*

[a]Adjusted with age, gender, smoking, alcohol drinking and areca chewing habits; [b]Statistically identified as significant based on Chi-square test without Yate's correction. CI, Confidence interval; OR, odds ratio.

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 *MMP-1* genotypes among the subgroups. The associations between the *MMP-1* genotypes and NPC risk were estimated by computing odds ratios (ORs) and their 95% confidence intervals (CIs) from logistic regression analysis. Any data with $p<0.05$ were considered statistically significant. All statistical tests were two-sided.

Results

The frequency distributions of age, gender, personal behavioral habits for the 176 NPC patients and 352 non-cancer controls are summarized and statistically compared in Table I. Since we have originally applied frequency matching to recruit the non-cancer healthy controls, there was no difference either in the distributions of age and gender between the two groups or in the distributions of personal behavioral habits (Table I).

The distributions of the *MMP-1* promoter -1607 genotype among the non-cancer controls and the NPC patients recruited in the study are presented and statistically analyzed in Table II. Overall, the genotypes of *MMP-1* promoter -1607 were at the borderline to be differently distributed between NPC and non-cancer control groups (p for trend=0.1080) (Table II). In detail, the *MMP-1* promoter -1607 heterozygous 1G/2G and homozygous 1G/1G were more and more prone to be associated with NPC risk (OR=0.64 and 0.63, 95% CI=0.43-1.03 and 0.36-0.96, p=0.0659 and 0.0932, respectively) (Table II). In the dominant models, there was significant association between the genotype of *MMP-1* promoter -1607 and NPC risk (OR=0.64, 95% CI=0.43-0.91, p=0.0359) (Table II).

To confirm the findings presented in Table II, analysis of allelic frequency distribution for the *MMP-1* promoter -1607 was further conducted and the results are summarized in Table III. Supporting these findings, the results showed that the variant allele 1G was 36.6% in the patient group, significantly lower than that of 43.2% in the non-cancer control group (OR=0.73, 95%CI=0.59-0.96, p=0.0418). To sum up, there was significant difference in the allelic frequencies of *MMP-1* promoter -1607 between the control and NPC patient groups (Table III).

Table III. *Allelic frequency analysis for matrix metalloproteinase-1 (MMP-1) promoter -1607 genotype and nasopharyngeal carcinoma.*

Allele	Controls n (%)	Patients N (%)	OR (95% CI)[a]	*p*-Value[b]
2G	400 (56.8%)	223 (63.4%)	1.00 (Reference)	
1G	304 (43.2%)	129 (36.6%)	0.73 (0.59-0.96)*	0.0418*

[a]The ORs were estimated with multivariate logistic regression analysis after age, gender, smoking, alcohol drinking and areca chewing habits adjustment. [b]Statistically identified as significant based on Chi-square test without Yate's correction. CI, Confidence interval; OR, odds ratio.

Next, we intended to examine the interactions among the genotype of *MMP-1* promoter -1607 and environmental factors, such as personal cigarette smoking, alcohol drinking and areca chewing habits. In Table IV, it is shown that the carriers with genotype of 1G/2G or 1G/1G at *MMP-1* promoter -1607 were of 0.64- and 0.70-fold ORs for NPC risk among non-smokers (95% CI=0.42-1.11 and 0.38-1.42, respectively) and of 0.71- and 0.53-fold ORs for NPC risk among smokers (95% CI=0.41-1.31 and 0.26-1.25, respectively). In summary, there was no interaction of *MMP-1* promoter -1607 genotypes with cigarette smoking (Table IV). Similarly, there was no interaction of *MMP-1* promoter -1607 genotypes with alcohol drinking and areca chewing habits (data not shown).

Table IV. *Odds ratios (OR) for matrix metalloproteinase-1 (MMP-1) promoter -1607 genotype and nasopharyngeal carcinoma after smoking status stratification.*

Genotypes	Non-smokers		OR (95% CI)[a]	Smokers		OR (95% CI)[a]
	Controls	Cases		Controls	Cases	
2G/2G	68	44	1.00 (Reference)	53	33	1.00 (Reference)
1G/2G	91	39	0.64 (0.42-1.11)	67	30	0.71 (0.41-1.31)
1G/1G	43	20	0.70 (0.38-1.42)	30	10	0.53 (0.26-1.25)
Total	202	103		150	73	

[a]The ORs were estimated with multivariate logistic regression analysis after age, gender, alcohol drinking and areca chewing habits adjustment. CI, Confidence interval.

Discussion

In the current study, the contribution of *MMP-1* promoter -1607 to NPC risk and its interaction with cigarette smoking, alcohol drinking and areca chewing was firstly evaluated among Taiwanese. The results showed that the 1G/1G genotypic frequencies of *MMP-1* promoter -1607 were 17.0% among the NPC patients, significantly less than 20.7% among the non-cancer healthy controls (Table II), while the 1G allelic frequencies of *MMP-1* promoter -1607 were associated with decreased risk of NPC in Taiwan (Table III).

This is the first study to analyze the interaction between *MMP-1* -1607 genotype and cigarette smoking on the susceptibility of carcinogens to NPC. Long-term tobacco smoking and EBV infection have been shown to contribute NPC development and affect clinical outcomes, such as survival rate (30-32). However, the mechanisms are very complex and need further research. In Taiwan, EBV infection (33), salted fish consumption during childhood (34), exposure to wood (34), areca consumption (34) and cigarette smoking (33, 35, 36) were reported to be risk factors for NPC, while alcohol consumption was not (36). In this study, we found that MMP-1 promoter -1607 genotypes were associated to NPC risk (Table II). However, the protective effects of *MMP-1* promoter -1607 genotype was observed on neither smokers, non-smokers (Table IV), alcohol drinkers, non-alcohol drinkers, areca chewers, nor non-areca chewers (data not shown). Thus, like EBV infection, *MMP-1* promoter -1607 genotype and cigarette smoking were independent risk factors for NPC susceptibility.

The MMP-1 protein, also called collagenase-1, is involved in the degradation of the native collagens in extracellular matrix (ECM). In normal conditions, MMP-1 is expressed at a relative low level under the suppressive regulation of TIMP-1 protein (37, 38), whereas abnormally up-regulated MMP-1 expression is observed in the borders of solid tumors, such as breast cancer (39, 40), oral cancer (41) and NPC (42). Elevated expression of MMP-1 has been reported to play an important role in tumor cells undergoing lymph node metastasis in NPC patients (43) and MMP-1 was thought to promote the migration behavior of the cells through the degradation of the ECM as the main component of connective tissue (44, 45). Recently, MMP-1 and TIMP-1 were shown to be up-regulated with cancer progression in a hamster cancer model (46). In 2012, a meta-analysis showed that *MMP-1* promoter -1607 1G/2G polymorphism was associated with the risk of breast, colorectal, genitourinary neoplasm but not oral cancer or NPC (47). In 2015, a meta-analysis reported that heterozygous 1G/2G at *MMP-1* promoter -1607 was associated with increased risk of NPC. However, in that study, there are no data for the Taiwanese and no significant finding concerning the homozygous variant 1G/1G genotype (48). Since the dynamic balance between MMP-1 and TIMP-1 play a main role for the maintenance of normal physiological conditions, further analysis of *MMP-1* and *TIMP-1* at DNA, RNA and protein angles simultaneously may provide an overall picture to evaluate the contribution of MMP-1 and TIMP-1 to NPC carcinogenesis.

In conclusion, our pilot study provided evidence that the homozygous 1G/1G genotype at *MMP-1* promoter -1607 may be a protective factor that independently interacts with personal cigarette, alcohol and areca nut consumption status to determine the personal susceptibility to NPC in Taiwan.

Acknowledgements

The Authors declare no conflicts of interest. We appreciate Tissue-bank of China Medical University Hospital, Chung-Hao Su, Min-Chun Chao and Kuo-Ting Sun for their excellent technical assistance. This study was supported mainly by Taiwan Ministry of Science and Technology to Professor Bau (MOST-104-2320-B-039-036) and partially by research grant from Taiwan Ministry of Health and Welfare Clinical Trial and Research Center of Excellence (MOHW105-TDU-B-212-133019).

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Received April 26, 2016
Revised May 31, 2016
Accepted June 1, 2016