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Association of *Matrix Metalloproteinase-9* Genotypes With Nasopharyngeal Carcinoma Risk

CHAO-HSUAN CHEN[1,2,3*], LIANG-CHUN SHIH[3,4*], SHIH-WEI HSU[2,5,6*], HUI-CHI TIEN[7], YEN-FANG LIU[3], YUN-CHI WANG[2,3], CHIA-WEN TSAI[2,3], DA-TIAN BAU[2,3,8] and WEN-SHIN CHANG[2,3]

[1]*Department of Neurosurgery, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[2]*Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan, R.O.C.;*
[3]*Terry Fox Cancer Research Laboratory, Department of Medical Research, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[4]*Department of Otorhinolaryngology-Head and Neck Surgery, China Medical University Hospital, Taichung, Taiwan, R.O.C.;*
[5]*Division of Neurosurgery, Department of Surgery, Taichung Armed Forces General Hospital, Taichung, Taiwan, R.O.C.;*
[6]*National Defense Medical Center, Taipei, Taiwan, R.O.C.;*
[7]*Department of Audiology and Speech-Language Pathology, Asia University, Taichung, Taiwan, R.O.C.;*
[8]*Department of Bioinformatics and Medical Engineering, Asia University, Taichung, Taiwan, R.O.C.*

Abstract. *Background/Aim: The up-regulation of matrix metalloproteinase-9 (MMP-9) expression is a characteristic feature observed across various malignancies, including nasopharyngeal carcinoma (NPC). Nevertheless, the influence of MMP-9 genotype in the context of NPC remains underexplored. This study examined the implications of MMP-9 promoter rs3918242 genotypes on the susceptibility to NPC in Taiwan. Materials and Methods: In a cohort comprising 208 NPC cases and 416 healthy controls, genotyping of MMP-9 rs3918242 was conducted utilizing polymerase chain reaction-restriction fragment length polymorphism methodology. Results: Individuals harbouring the variant CT or TT genotype of MMP-9 rs3918242 did not demonstrate a discernible alteration in NPC risk when compared to wild-type CC carriers [odds ratio (OR)=0.83 and 0.79, with 95% confidence intervals (95%CI)=0.56-1.24 and 0.27-2.29; p=0.4205 and 0.8675, respectively]. Moreover, the presence of the variant T allele did not confer a modified risk of NPC (OR=0.84, 95%CI=0.60-1.19, p=0.3761). Intriguingly, a protective effect associated with the MMP-9 rs3918242 CT genotype against NPC risk was discerned among individuals abstaining from betel quid chewing behaviour (OR=0.51, 95%CI=0.30-0.87, p=0.0166). Notably, no significant association was established between the MMP-9 rs3918242 CT or TT genotype and NPC risk among individuals with or without smoking or alcohol consumption habits. Conclusion: Presence of the variant CT or TT genotype at MMP-9 rs3918242 did not appear to substantially contribute to an elevated risk of NPC. Notably, a protective effect against NPC risk was observed in individuals carrying the CT genotype, particularly in those abstaining from betel quid chewing.*

*These Authors contributed equally to this study.

Correspondence to: Wen-Shin Chang, Terry Fox Cancer Research Laboratory, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, R.O.C. Tel: +886 422053366 Ext. 5805, e-mail: halittlemelon@hotmail.com; Da-Tian Bau, Terry Fox Cancer Research Laboratory, China Medical University Hospital, 2 Yuh-Der Road, Taichung, 404 Taiwan, R.O.C. Tel: +886 422053366 Ext. 5805, e-mail: artbau2@gmail.com

Key Words: Genotype, matrix metalloproteinase-9, nasopharyngeal carcinoma, polymorphism, Taiwan.

Nasopharyngeal carcinoma (NPC) is a malignancy originating from the epithelial lining of the nasopharynx, characterized by a multifactorial etiology involving genetic, environmental, and viral elements (1, 2). Recent epidemiological investigations have enhanced our comprehension of the intricate etiology and distribution patterns of NPC. By pinpointing high-risk cohorts and modifiable risk determinants, such as lifestyle behaviors and viral exposures, these insights hold significant implications for targeted prevention and early detection endeavors. Despite the identification of several biomarkers as potential indicators

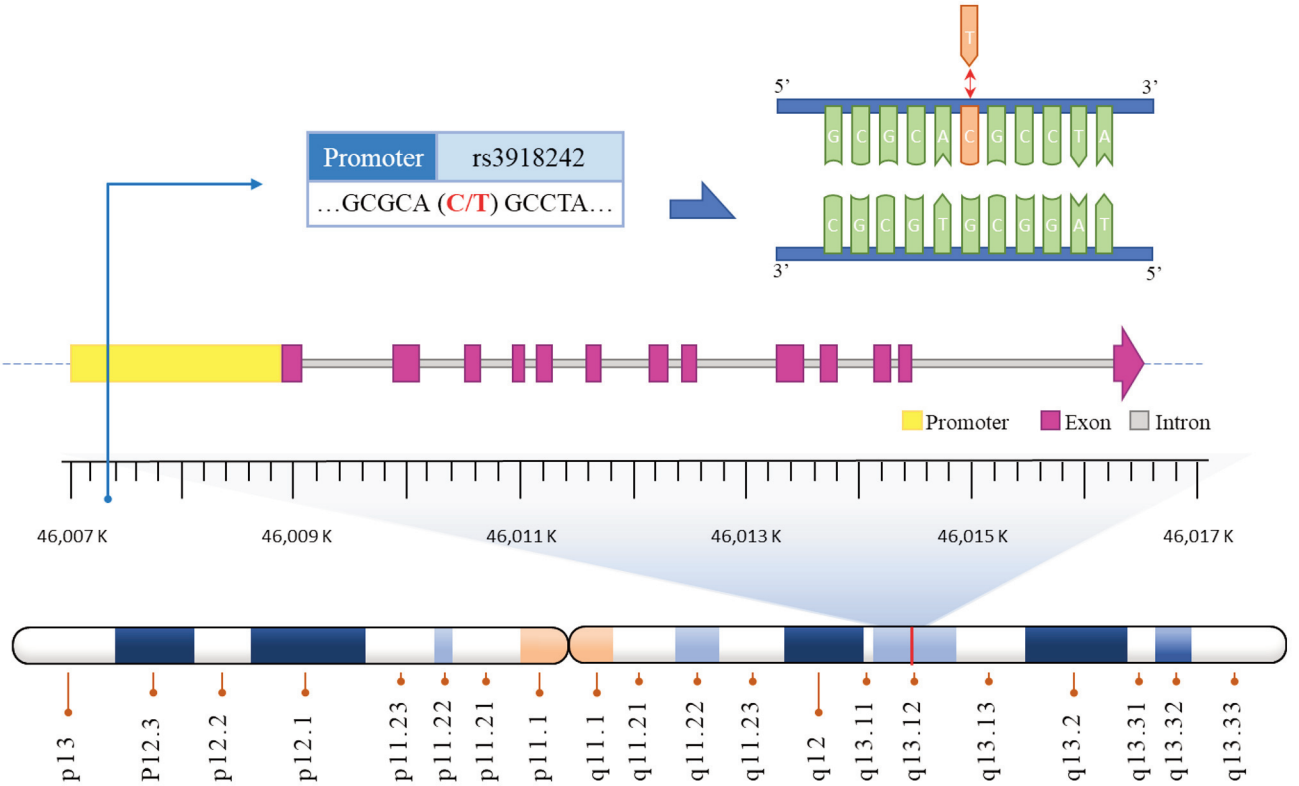

Figure 1. *Location of the MMP-9 rs3918242 polymorphic site along with the DNA sequences surrounding it.*

for NPC in recent research (3), there remains a pressing need to unearth clinically viable markers for NPC, particularly tailored to specific populations, such as the Taiwanese (4-8).

Matrix metalloproteinase-9 (MMP-9), a constituent of the matrix metalloproteinase family, participates in extracellular matrix remodeling and tumor advancement. Across the literature, MMP-9 has been underscored for its pivotal involvement in the initiation and progression of various human malignancies, encompassing cervical cancer (9, 10), ovarian cancer (11), osteosarcoma (12), giant-cell tumor of bone (13), breast cancer (14, 15), pancreatic cancer (16), hepatocellular carcinoma (17), and non-small cell lung cancer (18). MMP-9 orchestrates carcinogenesis through extracellular matrix remodeling and degradation of membrane proteins, modulating cancer cell behaviors encompassing proliferation, invasion, migration, and angiogenesis (19-21). Profound comprehension of the genomic aberrations linked with MMP-9 dysregulation in NPC is imperative for delineating its significance in disease pathogenesis.

Among the plethora of genetic variations linked with *MMP-9*, the most extensively scrutinized is the *MMP-9* rs3918242 polymorphism situated within the promoter region. The *MMP-9* rs3918242 polymorphisms have been scrutinized for their associations across a spectrum of cancer types and cancer-like diseases, encompassing salivary gland (22), esophageal (23), lung (24), breast (25, 26), gastric (27), colorectal (28, 29), bladder (30), prostate (31), cervical cancer (32), renal cell carcinoma (33, 34), and childhood leukemia (35, 36). However, concerning NPC, only two studies have delved into the involvement of *MMP-9* rs3918242 polymorphic genotypes (37, 38).

Based on the preceding information, the present investigation aimed to assess the correlation between *MMP-9* rs3918242 and the susceptibility to NPC in Taiwan. The physical location of *MMP-9* rs3918242 is illustrated in Figure 1. Furthermore, our objectives encompass exploring conceivable interactions between *MMP-9* rs3918242 genotypes and lifestyle factors, including smoking, alcohol consumption, and betel quid chewing. Additionally, this study undertook a comprehensive comparative analysis of existing literature concerning the involvement of *MMP-9* rs3918242 genotypes in NPC.

Materials and Methods

Recruitment of NPC cases and non-cancer controls. A cohort comprising 208 NPC patients was assembled from the Department of General Surgery at China Medical University Hospital in

Table I. *Demographic characteristics of the 416 control subjects and 208 nasopharyngeal carcinoma patients.*

Characteristics	Cases (n=208)			Controls (n=416)			*p*-Value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			50.6 (11.0)			49.9 (11.5)	0.4639[a]
Sex							
Male	153	73.6%		306	73.6%		1.0000
Female	55	26.4%		110	26.4%		
Personal behaviors							
Cigarette smoking	85	40.9%		158	38.0%		0.5422[b]
Alcohol drinking	95	45.9%		168	40.4%		0.2399[b]
Areca chewing	80	38.6%		156	37.5%		0.8840[b]
Classification							
KSCC (WHO type I)	8	3.8%					
NKC (WHO type II)	200	96.2%					
NKDC (WHO type IIa)	32	16.0%					
NKUC (WHO type IIb)	168	84.0%					

SD: Standard deviation; KSCC: keratinizing squamous cell carcinoma; NKC: non-keratinizing carcinoma; NKDC: non-keratinizing differentiated carcinoma; NKUC: non-keratinizing undifferentiated carcinoma; [a]Based on Student's *t*-test, [b]Based on Chi-square test with Yates' correction.

Taichung, Taiwan. These patients volunteered for participation, completed a self-administered questionnaire, and provided peripheral blood samples. Non-cancer controls were selected in a 2:1 ratio relative to cases, meticulously matched based on sex, age (±5 years), and behavioral habits (smoking, alcohol consumption, and betel quid chewing). Exclusion criteria for controls included prior malignancies, metastatic cancers of unknown or other origins, and any genetic or hereditary disorders. Information regarding the history and frequency of smoking, alcohol consumption, and betel quid chewing was obtained using identical self-reported questionnaires as employed for the cases. "Ever" usage was defined as more than twice a week for a minimum of one year. These behavioral patterns were quantitatively assessed and categorized as discrete variables. The study received approval and oversight from the Institutional Review Board of China Medical University Hospital (DMR101-IRB1-306). All clinical inquiries and documentation adhered strictly to the principles outlined in the Declaration of Helsinki. Selected demographic and clinical characteristics of both cases and controls are summarized in Table I.

Genotyping methodologies for MMP-9 in NPC cases and controls. DNA extraction was carried out on peripheral blood samples obtained from all participants within 24 h of collection using the QIAamp Blood Mini Kit (Qiagen, Valencia, CA, USA) (39, 40). The extracted DNA samples were stored at –80˚C for long-term preservation. Furthermore, the DNA samples were prepared for *MMP-9* rs3918242 genotyping determination by dilution, aliquoting, and establishment of a working stock collection, following established protocols (4, 41). The *MMP-9* rs3918242 genotype was determined according to previously described methodologies (35, 36). In brief, amplification of *MMP-9* rs3918242 was performed using forward and reverse primer sequences: 5'-TGGTCAACG TAGTGAAACCCCATCT-3' and 5'-TCCAGCCCCAATTATCACA CTTAT-3', respectively. Subsequently, restriction fragment length polymorphism analysis was conducted using *Sph* I restriction endonuclease (New England Biolabs, Taipei, Taiwan, ROC) on the PCR products. Upon enzyme digestion, DNA fragments corresponding to *MMP-9* rs3918242 CC, CT, and TT genotypes were distinguished as 386 bp, 386+320+66 bp, and 320+66 bp, respectively, through DNA adduct identification.

Statistical analysis. To ascertain the representativeness of controls within the general population, the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test to detect deviations in genotype frequencies within the control group. The unpaired Student's *t*-test was applied to compare mean ages between the case and control groups. Distribution of genotypes among subgroups was compared utilizing Pearson's Chi-square test with Yates' correction or Fisher's exact test (the latter when the expected count was less than 5). A significance threshold of $p<0.05$ was adopted for all analyses. Logistic regression was employed to estimate the odds ratios (ORs) and 95% confidence intervals (CIs) for NPC risk associated with specific genotypes.

Results

Analysis of basic characteristics between the NPC case and control groups. Table I displays the frequency distributions of selected characteristics for the 208 NPC cases and 416 cancer-free controls. Controls were selected through frequency-matching to achieve comparable distributions of sex and age with the cases ($p=0.4639$). Additionally, histological information is presented at the bottom of Table I. It is noteworthy that the elevated proportions of smokers (38.0%), alcohol drinkers (40.4%), and betel quid chewers (37.5%) in the control group are a result of the frequency-matching strategy and may not accurately reflect the broader Taiwanese population. Among the NPC cases, 8 (3.8%) were classified as keratinizing squamous cell carcinoma (WHO type I), and 200 (96.2%) as non-keratinizing carcinoma (WHO type II).

Table II. *Distribution of matrix metalloproteinase-9 rs3918242 variant genotypes among the controls and patients with nasopharyngeal carcinoma.*

Genotype	Frequency, n (%)		OR (95%CI)	*p*-Value[a]
	Cases (n=208)	Controls (n=416)		
rs3918242				
CC	159 (76.4)	303 (72.8)	1.00 (Reference)	
CT	44 (21.2)	101 (24.3)	0.83 (0.56-1.24)	0.4205
TT	5 (2.4)	12 (2.9)	0.79 (0.27-2.29)	0.8675
p_{trend}				0.6238
p_{HWE}				0.3162
Carrier comparison				
CC+CT	203 (97.6)	404 (97.1)	1.00 (Reference)	
TT	5 (2.4)	12 (2.9)	0.83 (0.29-2.39)	0.9307
CC	159 (76.4)	303 (72.8)	1.00 (Reference)	
CT+TT	49 (23.6)	113 (27.2)	0.83 (0.56-1.22)	0.3834

OR: Odds ratio; CI: confidence interval. [a]Based on chi-square test with Yates' correction. p_{trend}: *p*-Value for trend analysis; p_{HWE}: *p*-Value for Hardy-Weinberg Equilibrium.

Table III. *Allelic frequencies for matrix metalloproteinase-9 rs3918242 in the control and nasopharyngeal carcinoma patient groups.*

Allelic type	Frequency, n (%)		OR (95%CI)	*p*-Value[a]
	Cases (n=416)	Controls (n=832)		
C	362 (87.0)	707 (85.0)	1.00 (Reference)	
T	54 (13.0)	125 (15.0)	0.84 (0.60-1.19)	0.3761

CI: Confidence interval; OR: odds ratio. [a]Based on chi-square test with Yates' correction.

Furthermore, the type II NPC patients were subdivided into 32 (16.0%) cases of non-keratinizing differentiated carcinoma (WHO type IIa) and 168 (84.0%) cases of non-keratinizing undifferentiated carcinoma (WHO type IIb) (Table I).

Association of MMP-9 rs3918242 genotypes with NPC risk. Table II presents the genotypic distributions of *MMP-9* rs3918242 among the 416 non-cancer controls and the 208 NPC cases. Initially, the genotypic frequencies of *MMP-9* rs3918242 among control subjects were observed to conform well to the Hardy-Weinberg equilibrium (p=0.3162). Subsequently, the genotypic frequencies of *MMP-9* rs3918242 did not exhibit a significant disparity between the NPC case and non-cancer control groups (p for trend=0.6238). In detail, the heterozygous CT or the homozygous variant TT genotypes of *MMP-9* rs3918242 appeared to confer a protective effect against NPC risk; however, neither reached statistical significance (OR=0.83 and 0.79, 95%CI=0.56-1.24 and 0.27-2.29, p=0.4205 and 0.8675, respectively). Lastly, analysis under the recessive (TT *versus* CC+CT) or dominant model (CT+TT *versus* CC) revealed a non-significant association with the risk of NPC (OR=0.83 and 0.83, 95%CI=0.29-2.39 and 0.56-1.22, p=0.9307 and 0.3834, respectively; Table II).

Association of MMP-9 rs3918242 allelic frequencies with NPC risk. To further substantiate the pilot findings delineated in Table II, the allelic frequency analysis was conducted to scrutinize the involvement of *MMP-9* rs3918242 in NPC risk. In line with the observations in Table II, the data revealed that the presence of variant T alleles for *MMP-9* rs3918242 did not manifest a significant difference between the NPC case and control groups (p=0.3761). Individuals harboring the variant T allele exhibited a 0.84-fold OR (95%CI=0.60-1.19) for NPC susceptibility relative to those bearing the wild-type C allele (Table III). Consequently, the collective findings from Table II and Table III suggest that the variant CT or TT genotype of *MMP-9* rs3918242 may not substantially alter the susceptibility to NPC in Taiwan.

Stratified analysis of MMP-9 rs3918242 genotypes according to individual behaviors. We investigated the combined influence of *MMP-9* rs3918242 genotype with smoking, alcohol consumption, and betel quid chewing status on NPC risk (Table IV, Table V, and Table VI). No significant interaction was observed between *MMP-9* rs3918242 genotype and smoking status, whether among non-smokers or smokers (Table IV), nor between genotype and alcohol consumption status, among both non-drinkers and drinkers (Table V). Interestingly, our results revealed a protective effect of *MMP-9* rs3918242 genotype among non-betel quid chewers (p for trend=0.0150). Specifically, individuals carrying the heterozygous variant CT genotype of *MMP-9* rs3918242 exhibited a lower prevalence among non-betel quid chewers (OR=0.51, 95%CI=0.30-0.87, p=0.0166), even after adjustment for age, sex, smoking, and alcohol consumption status (adjusted OR=0.57, 95%CI=0.42-0.81). This contrasted with the findings for the homozygous variant TT genotype (Table VI, left section). Additionally, there was a potential risk elevation associated with the combination of *MMP-9* rs3918242 CT genotype and betel quid chewing behaviour (OR=1.94, 95%CI=1.00-3.76, p=0.0705, Table VI, right section).

Discussion

The over-expression of MMP-9 has been linked to cervical lymph node metastasis in NPC cases (42). However, conflicting evidence suggests that MMP-9 and pro-MMP-9

Table IV. *Distribution of matrix metalloproteinase-9 rs3918242 genotypes among 208 nasopharyngeal carcinoma cases and 416 controls 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			
CC	189	96	1.00 (ref)	1.00 (ref)		114	63	1.00 (ref)	1.00 (ref)	
CT	60	25	0.82 (0.48-1.39)	0.77 (0.54-1.26)	0.5450	41	19	0.84 (0.45-1.57)	0.93 (0.62-1.43)	0.6924
TT	9	2	0.44 (0.09-2.06)	0.36 (0.17-9.58)	0.3485	3	3	1.81 (0.35-9.23)	1.41 (0.52-7.80)	0.6688
Total	258	123				158	85			
p_{trend}					0.4545					0.6332

N: Number; OR: odds ratio; CI: Confidence interval; [a]Based on Chi-square with Yate's correction test; [b]Based on Chi-square with Yate's correction test (when every n≥5) or Fisher exact test (when any n<5) after adjustment of age, sex, alcoholism, and betel quid chewing status.

Table V. *Distribution of matrix metalloproteinase-9 rs3918242 genotypes among 208 nasopharyngeal carcinoma cases and 416 controls after stratification by alcoholism 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			
CC	176	88	1.00 (ref)	1.00 (ref)		127	71	1.00 (ref)	1.00 (ref)	
CT	65	24	0.74 (0.43-1.26)	0.77 (0.52-1.21)	0.3249	36	20	0.99 (0.54-1.85)	1.06 (0.73-1.94)	0.9841
TT	7	1	0.29 (0.03-2.36)	0.33 (0.17-13.56)	0.2799	5	4	1.43 (0.37-5.50)	1.31 (0.56-4.39)	0.7258
Total	248	123				168	95			
p_{trend}					0.2726					0.8693

N: Number; OR: odds ratio; CI: confidence interval; [a]Based on Chi-square with Yate's correction test; [b]Based on Chi-square with Yate's correction test (when every n≥5) or Fisher exact test (when any n<5) after adjustment of age, sex, smoking, and betel quid chewing status.

Table VI. *Distribution of matrix metalloproteinase-9 rs3918242 genotypes among 208 nasopharyngeal carcinoma cases and 416 controls after stratification by betel quid 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			
CC	176	104	1.00 (ref)	1.00 (ref)		127	55	1.00 (ref)	1.00 (ref)	
CT	76	23	0.51 (0.30-0.87)	0.57 (0.42-0.81)	**0.0166**	25	21	1.94 (1.00-3.76)	1.79 (1.05-3.44)	0.0705
TT	8	1	0.21 (0.02-1.72)	0.36 (0.14-7.25)	0.1627	4	4	2.31 (0.56-9.57)	2.03 (0.77-7.75)	0.2571
Total	260	128				156	80			
p_{trend}					**0.0150**					0.0880

N: Number; OR: odds ratio; CI: confidence interval; [a]Based on Chi-square with Yate's correction test; [b]Based on Chi-square with Yate's correction test (when every n≥5) or Fisher exact test (when any n<5) after adjustment of age, sex, smoking, and alcoholism status; the significant *p*-values are marked in bold.

may not serve as reliable markers for undifferentiated NPC (43). In a meta-analysis conducted by Liao *et al*. in 2016, pooling data from six studies, it was proposed that MMP-9 over-expression in both NPC tissue and blood samples could potentially predict poorer survival outcomes among NPC patients (44). Hence, it is hypothesized that the up-regulation

Table VII. *Existing literature regarding the genotypes of MMP-9 rs3918242 among nasopharyngeal carcinoma (NPC) populations worldwide.*

First author	Year	Ethnicity	CC, CT, TT genotype # of the controls	CC, CT, TT genotype # of the cases	Highlights of the study	Ref #
Chen	2024	Taiwan	303 : 101 : 12	159 : 44 : 5	No genotype significantly contributed to altered NPC risk. Specifically, CT genotypes decreased NPC risk for non-betel quid chewers, and increased NPC risk for betel quid chewers	Current
Nasr	2007	African	139 : 31 : 1	139 : 32 : 3	No genotype significantly contributed to altered NPC risk	37
Zhou	2007	Chinese	756 : 592 : 161	803 : 644 : 157	No genotype significantly contributed to altered NPC risk	38

of MMP-9 may be influenced by its inherited *MMP-9* rs3918242 polymorphism, located in the promoter region, which regulates MMP-9 expression itself (45, 46).

In the present study, we observed that the variant genotypes of *MMP-9* rs3918242 were not significantly associated with an altered risk of NPC (Table II). This finding aligns with previous investigations conducted in African and Chinese populations (37, 38). Both Nars's and Zhou's teams reported that the CT+TT genotypes of *MMP-9* rs3918242 were not the primary determinants of NPC susceptibility. We have compiled the population characteristics, case and control numbers, together with key findings of these studies in Table VII. It is noteworthy that the etiological factors contributing to NPC in Africa and East Asia (Taiwan and China) may differ, necessitating further investigations across diverse populations to validate these findings.

In addition to assessing the contribution of *MMP-9* rs3918242 genotypes to NPC risk, our investigation extended to exploring potential interactions between *MMP-9* rs3918242 genotypes and other factors related to NPC susceptibility. We found no significant associations among subgroups stratified by age or sex (data not shown). Moreover, there was no discernible difference in susceptibility among individuals with variant *MMP-9* rs3918242 genotypes with or without smoking or alcohol consumption habits for NPC risk (Table IV and Table V). However, our primary findings revealed higher proportions of NPC cases with the *MMP-9* rs3918242 CT genotype among betel quid users compared to betel quid users in the control group (Table VI, right part). Additionally, the CT genotype of *MMP-9* rs3918242 exhibited a protective effect for non-betel quid users (Table VI, left part). Currently, the underlying mechanisms by which *MMP-9* rs3918242 genotypes contribute to altered NPC risk in betel quid users or non-users remain unclear. However, evidence suggests that MMP-9 mRNA expression in human gingival epithelial progenitor cells can be induced by arecoline stimulation, a major component of betel quid (47). Nevertheless, our data support the critical role of *MMP-9* rs3918242 genotypes in determining individual NPC risk among betel quid users and non-users. Further investigations with larger sample sizes are urgently warranted to gain deeper insights into the involvement of *MMP-9* genotypes in NPC carcinogenesis.

Tumor growth, invasion, and metastasis necessitate the proliferation of tumor cells within the metastatic microenvironment, requiring the degradation of the extracellular matrix (ECM). This degradation is predominantly orchestrated by MMPs, among which MMP-9 exhibits specificity towards type IV collagen (48-50). MMP-9 levels are notably elevated in advanced stages, thereby heightening the propensity for bone metastasis (48, 51, 52). Although NPC can directly infiltrate the skull base, clinical occurrences of brain metastasis are exceedingly rare, with incidence rates ranging from 0.3% to 0.7% (53-55). While the regulatory mechanisms governing MMP-9 levels within brain regions remain elusive, recent reports suggest that lower MMP-9 levels may contribute to increased survival rates among glioblastoma cases, with MMP-9 demonstrating a positive correlation with the aggressiveness of brain tumors (56). Future investigations may unveil whether individuals harboring *MMP-9* rs3918242 CT or TT genotypes, associated with elevated MMP-9 expression levels compared to those with the CC genotype, exhibit differential susceptibility to NPC with brain metastasis *via* skull base invasion.

In conclusion, our findings suggest that the variant genotypes of *MMP-9* rs3918242 may exert a modest influence on NPC susceptibility. Specifically, the *MMP-9* rs3918242 CT genotype appears to confer a protective effect, particularly among individuals who do not chew betel quid as a habit. However, further investigations involving larger sample sizes and diverse populations are imperative to corroborate and expand upon our observations.

Conflicts of Interest

The Authors declare no conflicts of interest regarding this study.

Authors' Contributions

Research design: Chen CH, Bau DT, and Chang WS; patient and questionnaire summaries: Shih LC, Tien HC, and Liu YF; experimental work: Hsu SW, Wang YC, Chang WS, and Tsai CW; data clearance and identification: Chen CH, Shih LC, Hsu SW, and Tien HC; statistical analysis: Bau DT, Hsu SW, and Chang WS; literature review and manuscript writing: Chen CH, Shih LC, Hsu SW, Chang WS, and Bau DT; review and revision: Chang WS and Bau DT.

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