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# Association of Matrix Metalloproteinase-8 Genotypes with the Risk of Bladder Cancer

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**Abstract.** *Background/Aim: Matrix metalloproteinases (MMPs) control the homeostasis of the extracellular matrix and their genetic polymorphisms may contribute to cancer susceptibility. The aim of this study was to reveal the genotypes of MMP8 among the Taiwanese and examine the contribution of MMP8 C-799T, Val436Ala and Lys460Thr polymorphisms to bladder cancer. Materials and Methods: MMP8 C-799T, Val436Ala and Lys460Thr polymorphic genotypes were determined in 375 patients with bladder cancer and 375 healthy controls by polymerase chain reaction-restriction fragment length polymorphism methodology. Results: Regarding MMP8 C-799T, there was no significant differential distribution between patient and control groups [p for trend=0.6629]. The odds ratios (ORs) after adjusting for age, gender, smoking and alcohol drinking status for those carrying CT and TT genotypes at MMP8 C-799T were 1.13 (95%CI=0.89-1.44, p=0.3688) and 1.10 (95%CI=0.87-1.52, p=0.9030), respectively, compared to those carrying the wild-type CC genotype. Regarding MMP8 Val436Ala, there was no significant differential distribution between patient and control groups [p for trend=0.8166]. The adjusted OR for those carrying AC and CC genotypes at MMP8 Val436Ala were 0.71 (95%CI=0.31-2.28, p=0.6094) and 1.00 (95%CI=0.21-4.73, p=0.7247), respectively. The polymorphism Lys460Thr at MMP8 was not found among Taiwanese patients. Conclusion: MMP8 C-799T, Val436Ala and Lys460Thr may only play an indirect role in determining personal cancer susceptibility for bladder cancer in Taiwan.*

Bladder cancer is the 9th most common malignancy worldwide, contributing to about 5% of cancer death and is related to four million dollars of healthcare costs each year (1). According to the statistical data provided by the International Agency for Research on Cancer, there were an estimated 430,000 new cases of bladder cancer and 165,100 deaths in 2012 worldwide, while males are affected four times more than females (1, 2). In Taiwan, bladder cancer ranks seventh in incidence and mortality among the common types of cancer (3, 4). The major risk factors known for bladder cancer include a) tobacco smoking; b) industrial exposure to potential carcinogens such as aromatic amines and carbon black dust; c) long-term drinking of arsenic-contaminated or chlorinated water and d) family history of concordant cancers (5). Tumorigenesis of bladder cancer is a complex, multistep and multifactorial process being the consequence of interactions of personal lifestyle, environmental and genetic factors (3, 4, 6-10). Identification of genomic markers for

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bladder cancer may speed the development of personalized therapy and may allow early detection and prediction of susceptibility and responsiveness to therapy.

Matrix metalloproteinases (MMPs, also called matrixins) are a family of endopeptidases capable of degrading all kinds of extracellular matrix proteins and processing a number of extracellular matrix (ECM) components while playing important roles in inflammation, carcinogenesis and cancer cell migration (11, 12). Since early 1990's, several kinds of MMPs were found to be overexpressed in tumor and stromal cells of various types of cancer, and this was highly associated with invasion and progression of tumorigenesis (13, 14). In addition, MMPs released from distant organs, together with growth factors, can play a role in the initiation of metastasis (15, 16). As early as 1998, elevated levels of MMPs have been detected in the serum and/or urine of patients with various types of human cancer, including bladder, breast, lung, colon, head and neck and melanoma (17). During the following years, accumulating evidence indicated that functional polymorphisms of MMPs may contribute to inter-individual differences in susceptibility to several types of cancer (18-25). Among the MMPs, MMP8 is present in connective tissues acting as a collagen-cleaving enzyme and polymorphisms of *MMP8* may lead to dysfunctional MMP8, dysregulation of the microenvironment and potentially to carcinogenesis (26, 27).

There were several single nucleotide polymorphic sites on *MMP8*. The one in the promoter region of *MMP8* (-799C/T) and two nonsynonymous ones (Val436Ala and Lys460Thr) have been mostly examined (23, 28, 29). From the angle of genotype-phenotype correlation viewpoint, electrophoretic mobility shift assays revealed differences in nuclear protein binding to oligonucleotides associated with differences in *MMP8* C-799T genotype (30). In addition, promoter constructs containing the CT or TT genotype at *MMP8* C-799T had a 3-fold greater activity in chorion-like trophoblast cells compared to the constructs containing the C alleles (30). In 2014, it was reported that *MMP8* C-799T genotypes were not associated with bladder cancer risk in a Caucasian population (31). However, it is known that the genetic background is quite different among Caucasian and Eastern populations, for instance Taiwanese. In light of all the above, the aim of this study was to investigate the association of *MMP8* genotypes with the risk of bladder cancer in a representative (patient: control=375:375) Taiwan population.

## Materials and Methods

*Investigated population*. The current study was approved by the Institutional Review Board of the China Medical University Hospital (DMR104-IRB-158) and written-informed consents have been obtained from all the participants. All the clinical investigations and records were performed according to the principles expressed in the Declaration of Helsinki. Totally, three hundred and seventy-five patients diagnosed with bladder cancer were recruited at the China Medical University Hospital in central Taiwan. All the recruited patients voluntarily participated, completed a self-administered questionnaire and willingly provided 5 ml of their peripheral blood. The clinical characteristics of patients including histological details were graded and defined by expert surgeons. Equal number of non-cancer healthy controls were selected by matching for age and gender after initial random sampling from the Health Examination Cohort of the hospital. The exclusion criteria of the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. Both groups completed a short questionnaire which included personal habits. Smokers were defined as daily or almost daily smokers who had smoked at least five packs of cigarettes per year in their lifetime. Age of smoking initiation, whether they were currently smoking or had already quit, and if so, when they had quit, and on average, how many cigarettes they smoked or had smoked daily were recorded for smokers. The male *versus* female ratio was about 3:1 in each group. The mean age of the patients and the controls was 61.4 (SD=10.3) and 62.9 (SD=9.8) years, respectively. The selective demographic information for all the participants in this study is summarized in Table I.

*MMP8 Genotyping methodology*. Genomic DNA was extracted from peripheral blood leukocytes with a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan), stored long-term at −80˚C, diluted and aliquoted for genotyping as a working stock at −20˚C (32). The methodology for *MMP8* genotyping, such as the designed primers for *MMP8* C-799T, Val436Ala and Lys460Thr has been previously published by our group (23, 28, 29). Concisely, the polymerase chain reaction (PCR) cycling conditions were: one cycle at 94˚C for 5 min; 35 cycles of 94˚C for 30 sec, 57˚C for 30 sec and 72˚C for 30 sec, and a final extension at 72˚C for 10 min in My Cycler (Bio-Rad, Hercules, CA, USA). After amplification, the PCR products were subject to digestion and separation *via* 3% agarose gel electrophoresis. All the genotypic processing was repeated by at least two expert researchers listed in the acknowledgement independently and blindly, and their results were 100% concordant to each other. In addition, the success rate of PCR-restrictive fragment length polymorphism (RFLP) was also 100%, and the genotypes of 5% of the participants in both the control and patient groups were chosen randomly and analyzed by direct sequencing (Genomics BioSci & Tech Co). The concordance between direct sequencing and PCR-RFLP was perfectly 100%.

*Statistical analyses*. Those participants with complete genotypic and clinical data were subjects to final analysis. The descriptive statistics of patients and controls are presented as the mean and standard deviation (SD) or as percentages. In Table I, the Student's *t*-test was used for the comparison of ages between the patient and the control groups. The Pearson's chi-square test or Fisher's exact test (when any cell was less than five) was used to compare the distribution of the genotypes. Associations were evaluated and presented as odds ratios (ORs) with 95% confidence intervals (CIs). Data were deemed statistically significant when the *p*-value was less than 0.05.

## Results

*Basic characteristics of bladder cancer patients and healthy individuals in Taiwan*. The frequency distributions of age, gender, personal habits, tumor stage and grades for the 375

Table I. *Basic characteristics of the 375 patients with bladder cancer and 375 controls investigated in this study.*

| Characteristic | Controls (n=375) | | | Cases (n=375) | | | *p*-Value |
|---|---|---|---|---|---|---|---|
| | n | % | Mean (SD) | n | % | Mean (SD) | |
| Age (years) | | | 62.9 (9.8) | | | 61.4 (10.3) | 0.7315[a] |
| Age group (years) | | | | | | | 0.7108[b] |
| ≤55 | 152 | 40.5% | | 158 | 42.1% | | |
| >55 | 223 | 59.5% | | 217 | 57.9% | | |
| Gender | | | | | | | 0.5525[b] |
| Male | 287 | 76.5% | | 279 | 74.4% | | |
| Female | 88 | 23.5% | | 96 | 25.6% | | |
| Personal habits | | | | | | | |
| Cigarette smoking | 186 | 49.6% | | 201 | 53.6% | | 0.3063[b] |
| Alcohol drinking | 176 | 46.9% | | 189 | 50.4% | | 0.3807[b] |
| Stage | | | | | | | |
| Non-muscle-invasive | | | | 235 | 62.7% | | |
| Muscle-invasive | | | | 140 | 37.3% | | |
| Grade | | | | | | | |
| Low | | | | 151 | 40.3% | | |
| High | | | | 224 | 59.7% | | |

SD: Standard deviation; [a]based on Student's *t*-test; [b]based on Chi-square test.

bladder cancer patients and 375 non-cancer controls are summarized and compared in Table I. Since we had applied the frequency matching approach in selecting the recruited non-cancer healthy controls, as expected, there was no difference in the distribution of age and gender between bladder cancer patients and healthy control groups (Table I top penal). Among the investigated subjects, neither smokers nor alcohol drinkers were of higher percentages in bladder cancer patient group than in the control group (both $p>0.05$) (Table I, middle penal). The results indicated that smoking and alcohol drinking may not be the risk behavioral factors for bladder cancer in the investigated Taiwanese population. From the clinical viewpoint, the collected bladder cancer patients had more patients of non-muscle-invasive type (62.7%) and later stage (59.7%) than those of muscle-invasive type and early stage (Table I, bottom panel).

*Association of MMP8 genotypes and bladder cancer risk in Taiwanese*. The genotypes for the MMP8 C-799T, Lys460Thr and Val436Ala among the matched healthy controls and the bladder cancer patients are presented and compared in Table II. The results showed that there was no variant genotype among the 750 examined subjects at *MMP8* Val436Ala (Table II, bottom panel). The genotypic frequency distributions for *MMP8* C-799T or Lys460Thr were not different between the control and the bladder cancer patient groups ($p$ for trend=0.6629 and 0.8166) (Table II, top and middle panels). Regarding the *MMP8* C-799T variant, the heterozygous CT or homozygous TT genotypes seemed not to be associated with elevated risk of bladder cancer ($p$=0.3688 and 0.9030, adjusted OR=1.13 and 1.10, 95%CI=0.89-1.44 and 0.87-1.52, respectively; Table II, upper panel). After combination of the homozygotes and heterozygotes of the T allele (CT + TT), the analysis of the results still showed that the T allele at *MMP8* C-799T did not confer risk for bladder cancer ($p$=0.4217, adjusted OR=1.11, 95%CI=0.88-1.38) (Table II, upper panel). The non-differential distribution of genetic frequency between the two groups was also observed in the case of *MMP8* Lys460Thr (Table II, middle panel). In summary, the genotypes of the investigated three polymorphic sites of *MMP8* seemed to play an indirect role in determining personal susceptibility for bladder cancer in the Taiwanese population.

*Association of MMP8 allelic frequencies and bladder cancer risk in Taiwanese*. The distributions of allelic frequencies of the three *MMP8* polymorphisms among the bladder cancer patients and the healthy controls are presented and compared in Table III. Supporting the genotypic summary presented in Table II, the results of allelic analysis also showed that neither the T allele at *MMP8* C-399T nor the C allele at *MMP8* Lys460Thr were significantly associated with altered bladder cancer risk ($p$=0.5712 and 0.4607, adjusted OR=1.03 and 0.77, 95%CI=0.82-1.17 and 0.38-1.46, respectively) (Table III). In detail, the percentages of minor allele frequencies of *MMP8* C-799T in the bladder cancer patient and the healthy control groups were 30.1% and 28.8%, respectively (Table III, top panel). As for *MMP8* Lys460Thr, the percentages of minor allele frequencies of *MMP8* Lys460Thr in the bladder cancer patient and the healthy control groups were 1.7% and 2.3%,

Table II. *Distributions of matrix metalloproteinase-8 (MMP8) genotypic frequencies among the 375 patients with bladder cancer and the 375 healthy people.*

| *MMP8* | Cases (%) | Controls (%) | Adjusted OR (95%CI)[a] | *p*-Value[b] |
|---|---|---|---|---|
| C-799T | | | | |
| CC (wild-type) | 186 (49.6) | 197 (52.5) | 1.00 (Reference) | |
| CT | 152 (40.5) | 140 (37.3) | 1.13 (0.89-1.44) | 0.3688 |
| TT | 37 (9.9) | 38 (10.2) | 1.10 (0.87-1.52) | 0.9030 |
| CT+TT | 189 (50.4) | 178 (47.5) | 1.11 (0.88-1.38) | 0.4217 |
| $p_{trend}$ | | | | 0.6629 |
| Lys460Thr | | | | |
| AA (wild-type) | 365 (97.3) | 362 (96.5) | 1.00 (Reference) | |
| AC | 7 (1.9) | 9 (2.4) | 0.71 (0.31-2.28) | 0.6094 |
| CC | 3 (0.8) | 4 (1.1) | 1.00 (0.21-4.73) | 0.7247 |
| AC+CC | 10 (2.7) | 13 (3.5) | 0.78 (0.36-2.09) | 0.5252 |
| $p_{trend}$ | | | | 0.8166 |
| Val436Ala | | | | |
| TT (wildt-ype) | 375 (100.0) | 375 (100.0) | 1.00 (Reference) | |
| CT | 0 (0.0) | 0 (0.0) | -- | |
| CC | 0 (0.0) | 0 (0.0) | -- | |

OR: Odds ratio; CI: confidence interval; [a]adjusted for confounding factors including age, gender, cigarette smoking and alcohol drinking status; [b]based on Chi-square test without Yates' correction (when cell is larger than or equal to 5) and Fisher's exact test (when cell is less than 5).

Table III. *Allelic frequencies for matrix metalloproteinase-8 (MMP8) polymorphisms among the 375 patients with bladder cancer and the 375 healthy people.*

| Polymorphic allele | Cases (%) N=750 | Controls (%) N=750 | Adjusted OR (95%CI)[a] | *p*-Value[b] |
|---|---|---|---|---|
| C-799T | | | | |
| Allele C | 524 (69.9) | 534 (71.2) | 1.00 (reference) | 0.5712 |
| Allele T | 226 (30.1) | 216 (28.8) | 1.03 (0.82-1.17) | |
| Lys460Thr | | | | |
| Allele A | 737 (98.3) | 733 (97.7) | 1.00 (reference) | 0.4607 |
| Allele C | 13 (1.7) | 17 (2.3) | 0.77 (0.38-1.46) | |
| Val436Ala | | | | |
| Allele T | 750 (100.0) | 750 (100.0) | 1.00 (reference) | |
| Allele C | 0 (0.0) | 0 (0.0) | -- | |

OR: Odds ratio; CI: confidence interval; [a]adjusted for confounding factors including age, gender, cigarette smoking and alcohol drinking status; [b]based on Chi-square test without Yates' correction.

respectively (Table III, middle panel). We have also performed stratification analysis for the *MMP8* C-799T, Lys460Thr and Val436Ala genotypes according to age, gender, smoking and alcohol consumption status, however, no significant association was found in any of the stratification analyses among any subgroups (data not shown).

## Discussion

MMPs are a family of proteases that regulate the homeostasis of the tumor microenvironment, which provides the essential elements for tumor growth, such as cytokine release, loss of contact inhibition, angiogenesis, and invasion (33). Among them, the role of MMP8 and its genetic variants in carcinogenesis is largely unknown. Typically, MMP8 is produced by polymorphonuclear leukocytes, stored in the secretory granules, and released during pathological inflammatory conditions such as rheumatoid arthritis and osteoarthritis (34). In addition, the levels of MMP8 in the serum of patients with breast cancer were found to be higher than those of healthy individuals, indicating that MMP8 may play a role in the occurrence and development of breast cancer (35). Furthermore, plasma MMP8 levels were positively associated with lymph node involvement, but showed a negative correlation with the risk of distant metastasis among breast cancer patients, suggesting MMP8 has a protective effect against lymph node metastasis (36). Thus, it is reasonable to examine whether *MMP8* genetic variants can be used as markers for early detection of bladder cancer and prediction of prognosis.

In the present study, the genotypes of *MMP8* among a representative Taiwanese bladder cancer population were examined and was assessed whether there was an association between the genotypes of *MMP8* C-799T, Lys460Thr and Val436Ala and bladder cancer risk. The results showed that there was no significant association between the presence of the T allele at *MMP8* C-799T, the C allele at *MMP8* Lys460Thr or the C allele at *MMP8*C-799T with bladder cancer risk in this Taiwanese cohort (Tables II and III). Our findings suggest that these *MMP8* polymorphic genotypes may not play a determinant role in increasing susceptibility to bladder cancer. There are very few studies which investigated the association of *MMP8* genotype with bladder cancer. As far as we are aware of, the current study is the first to reveal that the genotypic variants of *MMP8* do not contribute significantly to bladder cancer in Taiwan. Our results are consisted with those obtained in a study that performed in a Caucasian population (37). Comparing the investigated population, it is noted that our population is more homogeneous (all Taiwanese *versus* multi-population with mainly Americans) and representative (375 bladder cancer patients and 375 healthy controls *versus* 200 cases and 200 controls). We investigated 3 polymorphic sites of *MMP8* while they investigated only one (37). In 2013, Srivastava and his colleagues found that *MMP8* C-799T was not associated with bladder cancer risk in Indian population (38). In 2014, Wieczorek and his colleagues found that the *MMP8* C-799T polymorphism was negatively associated with bladder cancer risk in a Polish population (with control/case=199/241).

In summary, the samples together with the clinical data collected in the study are currently most representative, genetically homogeneous and conservative, and the main findings are very consistent with other groups in other countries. Therefore, the results indicated that the polymorphic sites, *MMP8* C-799T, Lys460Thr and Val436Ala may not play a major role in the initiation of bladder cancer. However, we could not rule out the possibility that some other polymorphic sites in potentially more important members of MMP family play a role (38-40).

As for the contribution of *MMP8* genotypes to early prediction of prognosis, we have stratified the patients according to cancer invasiveness and stage. However, no positive association between specific MMP8 genotypes with cancer invasiveness or stage was found (data not shown). This is not consistent with the findings that plasma MMP8 levels were positively associated with lymph node involvement but showed negative correlation with the risk of distant metastasis among breast cancer patients (36). Our results indicated that MMP8 may have a protective effect against local (lymph node) or distant metastasis only for breast cancer but not for bladder cancer.

In conclusion, this study examined the genotypic patterns of *MMP8* C-799T, Lys460Thr and Val436Ala among Taiwanese. None of the genotypes in these *MMP8* polymorphic sites contributed to susceptibility for bladder cancer in Taiwan. These results may indicate that SNPs on other *MMP* genes should be examined in the near future to serve as early detective biomarkers for bladder cancer prediction in Taiwan.

## Conflicts of Interest

The Authors declare no conflicts of interest with any person or company.

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