Bladder cancer risk associated with genotypic polymorphism of the matrix metalloproteinase-1 and 7 in North Indian population

Priyanka Srivastava, Ruchika Gangwar, Rakesh Kapoor and Rama D. Mittal*

Department of Urology and Renal Transplantation, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India

Abstract. Matrix metalloproteinases (MMPs) contribute to tumor invasion and microenvironment, hence are associated with bladder cancer risk. We therefore, tested whether polymorphisms in MMP genes modify the risk of bladder cancer (BC) and whether smoke exposure modifies this risk.

Genotyping was performed in 200 BC patients and 200 controls by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). MMP1-1607 2G/2G and MMP7-181 GG genotype were associated with increased risk of BC (p < 0.001; OR, 3.04; 95% CI- 1.71–5.39 and p, 0.005; OR, 2.38; 95% CI- 1.30–4.34) respectively. Smokers in BC patients showed significant increased risk for the same SNPs (p, 0.006; OR, 3.20; 95% CI- 1.40–7.31 and p, 0.009; OR, 2.85; 95% CI- 1.30–6.23 respectively). Haplotype analysis too revealed significant association with G/2G of MMP1-519-1607 (p < 0.001; OR, 2.62; 95% CI- 1.68–4.09). The 2G allele carrier (1G/2G + 2G/2G) of MMP1-1607 showed a protective effect and high recurrence free survival in Bacillus Calmette-Guérin (BCG) treated non muscle invasive BC (NMIBC) patients (log rank p, 0.030). Our data suggested that MMP1-1607 2G and MMP7-181 G allele were associated with high risk of BC, which was quite evident amongst smokers too. BCG treated NMIBC patients reflected protective effect for 2G allele carrier (1G/2G + 2G/2G) of MMP1-1607. This study provided new support for the association of MMP1-1607 and MMP7-181 in bladder cancer development, the tumorigenic effect of which was observed to be more enhanced in case of tobacco exposure.

Keywords: Bacillus Calmette-Guérin, bladder cancer, haplotypes, matrix metalloproteinase, polymorphism, recurrence free survival

1. Introduction

Transitional cell carcinoma (TCC) accounts for 90% of all cases of bladder cancer (BC) and remains as a significant cause of morbidity and death [1]. Most BC (70–80%) present as non-muscle-invasive papillary tumors which recur frequently (50–80%), but progress less often (5–30%) to invade bladder muscle wall. By contrast, the remaining 20–30% of BC is aggressive muscle-invasive tumors that have a much higher risk of metastasis, despite radical treatment [2].

Over the last three decades, intravesical immunotherapy with the biological response modifier Bacillus Calmette-Guérin (BCG) has been established as the most effective adjuvant treatment for preventing local recurrences and tumor progression following transurethral resection of non-muscle invasive BC [3], however, the response rate for BCG treatment is only 60% to 70%. A large number of clinical trials have
established a major role for BCG immunotherapy in urological oncology.

Although tobacco smoke and other environmental pollutants are responsible for more than 80–90% of the cases in men [4]. It is well established that less than 10–15% of smokers develop BC, indicating that other factors could also be responsible for the development of BC [5]. Polymorphisms represent natural variations of the genetic code in the population. The most common polymorphisms are single-nucleotide polymorphisms (SNPs). Polymorphisms in the promoter region of a gene, such as the 2G allele of MMP1 -1607, and MMP-7 -181A/G polymorphism has been shown to be associated with malignant diseases [6].

The matrix metalloproteinases MMPs are implicated in a number of pathological processes such as invasion and metastasis of tumor cells. Polymorphisms in the regulatory regions of MMPs have been associated with changes in the expression level of these genes in different human diseases [7,8]. In fact, the -1607 1G/2G polymorphism in the promoter region of MMP1 creates an Ets binding site which increases the promoter activity of this gene [9]. Thus, the 2G allele of MMP1 has significantly higher transcriptional activity than the 1G allele and has been associated with an increased risk of common cancers, including oral, colorectal, renal and head and neck [10,11]. Furthermore, in colorectal and ovarian cancer, the presence of the 2G allele in the MMP1 gene was significantly associated with poorer survival of patients with cancer [12,13].

Matrix metalloproteinase-7 (MMP-7) is a small secreted proteolytic enzyme with broad substrate specificity [14]. Its expression has been shown to be associated with tumor invasion, metastasis and survival for a variety of cancers. The gene encoding MMP-7 is localized on chromosome 11q21–q22. One of the MMP-7 -181A/G polymorphism has been shown to be associated with malignant diseases [15].

Given the genetic complexity of bladder cancer, individual polymorphisms are likely to have a modest effect on risk. However, examining multiple polymorphisms within biologically relevant pathways may reveal subgroups of individuals who are at significantly elevated risk for this disease. Besides the promoters of the MMP-1 and MMP-7 genes contain polymorphism and have allele-specific effects on the regulation of MMP gene transcription and are associated with development of some cancers.

In this study, we identified the possible association of MMP-1 -1607 1G/2G, MMP-1 -519 A/G and MMP-7 -181A/G genes in BC patients and healthy controls from North India with the risk of bladder cancer and to investigate the possible modulating effect of smoking on these associations.

2. Material and methods

2.1. Study subjects

The bladder cancer patients in this analysis were enrolled from an on-going case-control study of bladder cancer, which started patient recruitment in 2005. All enrolled patients were incident cases of histologically confirmed invasive or superficial bladder cancer and were recruited from the Urology department at Sanjay Gandhi Postgraduate Institute of Medical Sciences, a tertiary care center, from May 2005 to June 2009. A total of 200 patients with histologically confirmed transitional urothelial BC (mean age 58.5 years; 175 men and 25 women) were recruited for the study. Those with previous history of other cancer, cancer metastasized to the bladder from another origin, and previous radiotherapy was excluded. Healthy and genetically unrelated individuals visiting the hospital for a routine checkup or health awareness camps and hospital employees were recruited as the controls (n = 200). All the controls were age and sex matched with similar ethnicity and had no evidence of malignancy or chronic disease. The mean age of the controls was 56.8 years, and M: F ratio as 179:21. The disproportionate ratio between male and female bladder cancer in our population could be largely due to increased prevalence in case of males (3:1). Secondly due to social taboos females avoid visiting hospitals/clinics. The participation rate was 100%, and blood samples were available for all subjects. Ethnicity was based on self-report and categorized as North Indian. An epidemiologic questionnaire was designed for study participants to collect data on demographic characteristics, smoking history, occupation history, and other lifestyle factors were employed. At the end of the interview, a 5-ml blood sample was drawn into coded tubes.

Informed and written consent was taken from all subjects when interviewing for the demographic details and blood sample collection. The Ethical Review Board of the Institute approved the study.

2.2. Epidemiology data collection

The demographic details were obtained by interviewing each individual in cases and controls. The response
rate for the interview was 75% for the subjects. Individuals who smoked once a day for more than 5 years were defined as smokers. The individuals who had never smoked in their lifetime were regarded as non-smokers. At the conclusion of the interview, a 5 ml of blood sample was drawn into coded vials.

2.3. Clinical data collection

The demographic and clinical characteristics of the patients are presented in Table 1. The clinical information about tumor size, number, stage and tumor grade, intravesical therapy and dates of recurrence, chemotherapy, radical cystectomy and pathological findings at cystectomy were provided by the Uro-Oncologist in our department. The tumor stages were classified as per American Joint Committee on Cancer’s TNM staging system [16]. Of the 200 total patients enrolled in the study, 149 patients had non muscle invasive bladder cancer (NMIBC) while the rest 51 had muscle invasive bladder cancer (MIBC). Patients with NMIBC at high risk (high grade, multiple and large tumor) were treated with intravesical *Bacillus Calmette-Guerin* (BCG) (*n* = 78). The patients with NMI cancer of low risk (low grade and single small tumor) were kept on cystoscopic surveillance and considered as Non BCG patients. Subsequently, all the patients were examined by cystoscopy after every 3 months in first and second years and later at six monthly intervals as long as there was no tumor recurrence. BCG treatment consisted of 6 weekly instillation induction BCG (*n* = 78). Since the number of patients receiving maintenance BCG was too low, we did not categorize the patients according to BCG regime for statistical analysis. The end point of study included tumor recurrence, defined as a newly found bladder tumor following a previous negative follow-up cystoscopy, or end of study time (60 months). Patients with invasive BC (*n* = 51) were treated with radical cystectomy with or without adjuvant chemotherapy, which included cisplatin, gemcitabine followed by periodical cystoscopy. Blood sample was collected in EDTA from all subjects.

| Variable                  | Cases (200) | Controls (200) | Chi-square-value |
|---------------------------|-------------|----------------|------------------|
| Sex                       |             |                |                  |
| Female                    | 25(12.5)    | 21(10.5)       | 0.531            |
| Male                      | 175(87.5)   | 179(89.5)      |                  |
| Age (Years)               |             |                |                  |
| Mean age ± SD             | 58.5 ± 12.4 | 56.8 ± 10.8    | 0.117 $^S$       |
| Smoking*                  |             |                |                  |
| Never Smokers             | 78(50)      | 155(77.5)      | 0.001            |
| Smokers                   | 78(50)      | 45(22.5)       |                  |
| Tumor number*             |             |                |                  |
| Single                    | 115(60.8)   | –              | –                |
| Multiple                  | 74(39.2)    | –              | –                |
| Tumor Size (cm)*          |             |                |                  |
| < 1                       | 35(24.3)    | –              | –                |
| 1–3                       | 73(50.7)    | –              | –                |
| > 3                       | 36(25.0)    | –              | –                |
| Stage                     |             |                |                  |
| Ta                        | 64(32.0)    | –              | –                |
| T1                        | 85(42.5)    | –              | –                |
| T2                        | 51(25.5)    | –              | –                |
| Grade                     |             |                |                  |
| G1                        | 67(33.5)    | –              | –                |
| G2                        | 43(21.5)    | –              | –                |
| G3                        | 90(45.0)    | –              | –                |
| Intravesical Therapy      |             |                |                  |
| Non treated               | 71(47.7)    | –              | –                |
| BCG Induction (BCG i+m)   | 78(52.3)    | –              | –                |
| Event                     |             |                |                  |
| Recurrence                | 65(43.9)    | –              | –                |
| Non-Recurrence            | 83(56.1)    | –              | –                |

$^S$ Student t-test was used to determine the p-value

*The sum could not add up to the total due to some missing values.
Fig. 1. (a). Representative Gel picture of MMP1-1607 (1G/2G) polymorphism. Lane 1: 100bp ladder, Lane 2: wild (1G/1G), Lane 3: hetero (1G/2G), Lane 4: variant (2G/2G) (b). Representative Gel picture of MMP7-181 (A/G) polymorphism. Lane 1: wild (AA), Lane 2: hetero (AG), Lane 3: variant (GG), Lane 4: 50bp ladder.

for genotyping at the time of enrollment and stored at −70°C.

2.4. Genotyping

Genomic DNA was extracted from peripheral blood lymphocytes by salting out method [17]. Polymorphisms in MMP1 -1607 1G/2G, -519 A/G and MMP7 -181 A/G were analyzed using polymerase chain reaction-restriction fragment length polymorphism, as shown in Fig. 1(a), 1(b) respectively. Details of the primers and cycle conditions for MMP1 and MMP7 have been previously described [17,18]. Positive and negative controls were used in each genotyping assay, and 10% of the samples were randomly selected and run in duplicates with 100% concordance. The results were reproducible with no discrepancy in genotyping.

2.5. Statistical analysis

The power of the study was calculated using Quanto software, version 1.0 (available from: http://hydra.usc.edu/gxe) with input of following variables: case-control study design, significance level (alpha) > 0.05 (2 sided), model of inheritance was log additive, allele frequency was 0.28, and the genetic effect for odds ratio (OR) was 1.65 or greater. The present study achieved 80% of the statistical power for the minor allele of MMP1 519A/G, which exhibited the lowest allele frequency among the 3 polymorphisms. The goodness-of-fit chi square test was used to analyze any deviation from the Hardy-Weinberg equilibrium in controls. A binary logistic regression model was used to estimate the risk as the OR at the 95% confidence interval. Haplotypes of each individual consisting of 2 single nucleotide polymorphisms (SNP) in MMP1 was constructed, and the maximal likelihood haplotype frequencies were estimated using the expectation-maximization algorithm using the Arlequin program, version 2.000. Bonferroni’s correction was applied in case of multiple comparisons using the formula $p_c = p \times n$ ($P_c$ represents corrected value where $n$ is the number of comparisons performed). The statistical analysis was done using the Statistical Package for Social Sciences software, version 11.5 (SPSS, Chicago, IL), and $P < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of subjects

A total of 200 controls and 200 cases were recruited for this study. There was no significant age difference between the cases (58.5 ± 12.4 years) and the controls (56.8 ± 10.8 years) ($p > 0.117$). The cases had significantly higher percentage of smokers (50.0%) than the controls (22.5%) ($p = 0.001$). The demographic details of the study subjects and clinical characteristics of the patients are presented in Table 1.

3.2. MMP1-1607 1G>2G, -519A>G, MMP7-181A>G gene polymorphisms in bladder cancer

The genotype and allele frequencies of matrix metalloproteinase 1 and 7 gene polymorphism in healthy
controls and patients with BC are presented in Table 2. The genotype frequency in the controls was in Hardy Weinberg Equilibrium. The variant allele frequency (G) of MMP1-519A>G was higher in cases as compared to controls (32.3% vs 28.8%). Overall no statistically significant association was observed in MMP1-519A>G (p, 0.347; OR, 0.79). In MMP1-1607, significant association was observed with 2G/2G genotype (p < 0.001; OR, 3.04) which was also evident in case of alleles 2G (p < 0.001; OR, 2.24). Therefore, individuals carrying the 2G allele were at higher risk of developing BC. The variant allele (2G allele) frequency in MMP1-1607 was 51.8% in controls and 66.5% in cases. In MMP7-181A>G, the variant G allele was 47% prevalent in controls compared to 39.3% in controls. The homozygous variant genotype (GG) of MMP7-181 showed 2 folds increased risk with BC patients (p = 0.005; OR, 2.38) which was statistically significant.

Table 3
Association of MMP1-519A>G, -1609 1G>2G, MMP7-181A>G polymorphisms with tumor grade/stage categories of BC patients

| (a) | (b) | (c) | p-value (a-b) | OR, 95% CI* | p-value (a-c) | OR, 95% CI* |
|-----|-----|-----|--------------|------------|--------------|------------|
| MMP1-519 | AA | AG | GG | — Reference — | — Reference — | — Reference — |
| TaG1 | 15(40.5) | 18(46.8) | 4(10.9) | | | |
| TaG2-3,T1G1-3 | 52(46.8) | 45(40.5) | 14(12.7) | 0.263 | 0.61(0.26–1.44) | 0.994 | 1.00(0.27–3.78) |
| T2+ | 23(45.1) | 23(45.1) | 6(9.8) | 0.393 | 0.65(0.25–1.72) | 0.506 | 0.57(0.10–3.03) |
| MMP1-1607 | 1G/1G | 1G/2G | 2G/2G | — Reference — | — Reference — | — Reference — |
| TaG1 | 3(8.2) | 16(43.2) | 18(48.6) | | | |
| TaG2-3,T1G2-3 | 15(31.6) | 44(39.6) | 52(46.8) | 0.342 | 0.49(0.11–2.12) | 0.348 | 0.50(0.11–2.12) |
| T2+ | 10(19.6) | 16(29.4) | 26(51.0) | 0.065 | 0.23(0.05–1.09) | 0.154 | 0.34(0.08–1.50) |
| MMP7-181 | AA | AG | GG | — Reference — | — Reference — | — Reference — |
| TaG1 | 9(24.4) | 14(37.8) | 14(37.8) | | | |
| TaG2-3,T1G2-3 | 40(36.0) | 45(40.5) | 26(23.5) | 0.567 | 0.75(0.28–1.99) | 0.114 | 0.44(0.16–1.22) |
| T2+ | 15(29.4) | 24(47.1) | 13(23.5) | 0.933 | 0.95(0.30–2.97) | 0.244 | 0.49(0.14–1.63) |

*Age, gender, smoking adjusted odds ratio and 95% CI.

The patients with similar stage but with different grades respond to treatment differently [15]. Hence
we stratified the patients into three categories according to stage/grade [TaG1 (low risk NMIBC), TaG2,3+ T1G1−3 (High risk NMIBC) and T2+ (muscle invasive)] (Table 3). TaG1 was taken as a reference. No significant association was observed statistically in any of the three polymorphism with the tumor stages.

3.4. Association of MMP1 and MMP7 genotypes with smoking

The subjects were grouped as non smokers and smokers in both controls and patients respectively (Table 4). In case of MMP1-519, no significant association was observed. In MMP1-1607 the variant genotype 2G/2G was associated with high risk of BC among smokers ($p = 0.006; \text{OR}=3.20$) and in MMP7, the GG genotype was also significantly associated with high risk of 2 folds among smokers ($p = 0.009; \text{OR}=2.85$) in patients.

3.5. Association of MMP1 haplotypes with bladder cancer risk

Haplotype analysis was used to determine the association between the two promoter polymorphism of MMP1. A-1G was taken as reference. The haplotype results demonstrated that MMP1-519-1607 G-1G to be associated with 1.7 folds (OR= 1.7, 95%CI- 1.20–2.41, $P_{c} = 0.012$) increased risk in bladder cancer patients. MMP1-519-1607 G-2G (variant alleles of both sites) to be associated with 2.6 folds (OR= 2.62, 95%- 1.68–4.09, $P_{c} = 0.004$) risk for BC (Table 6).

3.6. Modulation of genotype variants and outcome after BCG immunotherapy

To analyze the association of MMP1 and MMP7 gene polymorphisms and risk of recurrence in NMIBC patients, further analysis was restricted to NMIBC patients only ($n = 148$). The median follow-up of NMIBC patients was 14 months (3–60 months). We analyzed the association of genotypes and risk of recurrence after BCG immunotherapy. We grouped patients into BCG treated ($n = 78$) and non-treated ($n = 70$) as these were patients of low grade tumors and did not require BCG immunotherapy. The patients of “BCG group” with heterozygous genotype of MMP1-1607 were observed to have reduced risk ($p, 0.017; \text{HR}, 0.28$) (Table 5). Similarly the variant allele carrier 1G+2G/2G+2G was also observed too be at statistical-
Table 5
Influence of MMP1 and MMP7 gene polymorphisms on the risk of recurrence in BCG treated NMIBC patients

|          | No Recurrence | BCG Recurrence | p value | HR (95%CI) |
|----------|---------------|----------------|---------|------------|
|          | n (%)         | n (%)          |         |            |
| **MMP1**-519(A/G) |       |               |         |            |
| AA       | 18(40.9)      | 12(35.3)       | Ref     |            |
| GA       | 23(52.3)      | 16(47.1)       | 0.801   | 0.91 (0.42–1.95) |
| GA + GG  | 3(6.8)        | 6(17.6)        | 0.158   | 2.03 (0.76–5.44) |
| **MMP1**-1607(1G/2G) |       |               |         |            |
| 1G/1G    | 3(6.8)        | 7(20.6)        | Ref     |            |
| 1G/2G    | 20(45.5)      | 10(29.4)       | 0.017   | 0.28 (0.10–0.80) |
| 2G/2G    | 21(47.7)      | 17(50.0)       | 0.129   | 0.480 (0.19–1.24) |
| 1G/2G+2G/2G | 41(93.2) | 27(79.4) | 0.039 | 0.39 (0.16–0.95) |
| **MMP7**-181(A/G) |       |               |         |            |
| AA       | 16(36.4)      | 10(29.4)       | Ref     |            |
| AG       | 15(34.1)      | 14(41.2)       | 0.393   | 1.45 (0.62–3.38) |
| GG       | 13(29.5)      | 10(29.4)       | 0.759   | 1.15 (0.47–2.84) |
| AG + GG  | 28(63.6)      | 24(70.6)       | 0.498   | 1.13 (0.61–2.81) |

HR, Age, gender and smoking adjusted Hazards Ratio; 95% CI, Confidence interval.

Table 6
Haplotype analysis of MMP1 gene polymorphisms in bladder cancer patients and healthy controls

|          | Controls n(%) | Patients n(%) | OR (95%CI) | p value |
|----------|---------------|---------------|------------|---------|
| **MMP1** haplotype (519 A/G-16071G/2G) |       |               |            |         |
| A-1G     | 127 (31.8)    | 86 (21.4)     | Ref        | Ref     |
| A-2G     | 66 (16.4)     | 45 (11.4)     | 1.01 (0.63–1.61) | 0.977 |
| G-1G     | 158 (39.4)    | 181 (45.2)    | 1.70 (1.20–2.41) | 0.003 |
| G-2G     | 49 (12.4)     | 88 (22.0)     | 2.62 (1.68–4.09) | < 0.001 |

(Bonferroni corrected P-value) Pc = 0.012, Pc = 0.004.

ly reduced risk of BC (p = 0.039; HR, 0.39). Subsequently, Kaplan–Meier analysis showed a higher median recurrence free survival of 54 months of 1G/2G + 2G/2G genotype as compared to 34 months for 1G/1G genotype carrying patients (log rank p = 0.030) (Fig. 2) in BCG treated patients. None of the other polymorphisms were associated with risk of recurrence free survival.

4. Discussion

MMPs are a class of proteases that contribute significantly and uniquely to the tumor microenvironment, which provides the elements needed for advanced tumor growth (i.e., cytokines, loss of contact inhibition, angiogenesis, and invasion [19]).

MMPs are essential for tumor cells to penetrate the basement membrane and colonize distant sites. Among MMPs, MMP-1 is the most ubiquitously expressed interstitial collagenase, thereby claiming a prominent role in collagen degradation [20]. Clinical research showed the presence of MMP-1 in cancer cells, and that MMP-1 expression is associated with a poor prognosis [21]. In the present study we have focused on two promoter polymorphism of MMP1. MMP1 -519 was not associated with BC risk or with any confounding factors. The other sequence polymorphism of MMP1 -1607 where, the insertion of a guanine nucleotide in the 2G allele of the MMP-1 -1607 1G/2G polymorphism creates a core binding site for transcription factor Ets, leading to a significantly higher promoter activity as reported by Ye et al [22]. MMP-1 SNPs have been correlated to the risk of BC [8], renal cell carcinoma [11], and colorectal cancer [12]. This study showed that the frequency of the 2G allele was significantly higher in BC patients compared to the controls (p = 0.002; OR 2.29), suggesting that variations in the MMP-1 promoter could facilitate bladder cancer formation and development. Our results were compatible to the study of Tasci et al. 2007 [23], who reported MMP1 2G allele to be significantly associated with the risk of BC. Hirata et al. [11] reported that the frequency of the 2G/2G genotype was significantly higher in patients with RCC than in controls. Zhu et al. [24] also showed that there was a significant association between the 2G/2G genotype and lung cancer risk.

Our results further showed that individuals with MMP-7 -181GG genotypes and -181G allele were at significant increased risk of BC (p = 0.005; OR 2.38). The underlying mechanism for this association may be related to the promoter activity variation of the 181G
alleles. Functional analysis revealed that MMP-7 -181G alleles can increase the gene transcription activity [14]. Previous studies have found that presence of -181G variant allele results in higher level of MMP-7 expression [25]. Presence of high expression MMP-7 -181G allele may alter cell surface signaling including cellular proliferation, invasion and apoptosis processes [26]. Therefore, individuals with excess MMP-7 activity by harboring the -181G allele may be predisposed to malignant transformation. In addition, enhanced expression of MMP-7 due to -181G allele may lead to increased activation of other members of the MMP family such as MMP-2. Association of MMP-7 -181G allele with gastric ulcer, colorectal carcinoma, and ovarian cancer has also been reported [15, 27, 28].

Furthermore, association of the genotypes with disease stages was carried out to explore the influence of variant genotypes on disease phenotype. Our results indicated that MMP1 and MMP7 gene polymorphism are not implicated with any of the stages in bladder tumor.

Interaction of MMP-1 and MMP-7 genotypes with tobacco exposure to investigate the modulation of risk was also conducted, since tobacco exposure is a significant risk factor in BC [4]. Association of MMP polymorphism with tumor development in smokers has also been reported by Yu et al [29] in which an additive interaction between the MMP-2 promoter polymorphism and smoking on the risk of developing lung cancer had been demonstrated. As MMPs expression can be influenced by smoking [30], we hypothesized that individual with MMP higher promoter activity alleles may react more strongly in smokers than those with lower activities. In the present study we observed MMP1-1607 2G/2G and MMP7 variant genotypes to be associated with high risk of BC among smokers. Given the small sample size in some strata and comparisons, these data need to be interpreted with caution, as it is possible that these results may have occurred due to chance.

Because individual polymorphisms are likely to confer modest effects to the risk of bladder cancer, we examined the effects of two MMP polymorphisms by performing haplotype analyses. Haplotype was constructed to analyze the association between the two polymorphic sites of MMP1. Patients with haplotype G-2G carrying variant allele of MMP1-519G and MMP1-1607 2G were at higher risk (Pc = 0.004). Additional research is required to investigate the functional effect of these haplotypes. Nevertheless, these results suggest that an appropriate combination of MMP-1 gene polymorphisms modifies the risk of overall bladder cancer.

We analyzed the association of MMP1-1607 polymorphism with risk of recurrence in NMIBC patients. The NMIBC patients were categorized on the basis of
BCG treatment in BCG group and no BCG group. On comparison of the patients receiving BCG immunotherapy vs the patients with low grade tumors not on BCG, we observed a reduced risk for recurrence in MMP1 1G/2G (HR = 0.28, p = 0.017) and 1G/2G+2G/2G genotype (HR = 0.39, p = 0.039). Since reduced recurrence risk for BC patients on BCG immunotherapy was observed, Kaplan-Meier recurrence free survival analysis was conducted. The Kaplan-Meier analysis revealed that 1G/1G genotype of MMP1-1607 had a lower recurrence free survival of 34 months in comparison to 54 months for 1G/2G + 2G/2G genotype (log rank p = 0.030). These results clearly suggest that mutant 2G allele at -1607 might be responsible for the delay in recurrence of bladder tumor in patients on BCG immunotherapy perhaps due to therapeutic effect of BCG.

Limitations and sources of bias should be considered when performing the study. Case control studies are subject to selection bias and recall bias. The biological role for many of the MMPs in bladder epithelium and the functional effects for many of the SNPs are still unknown. Therefore, these novel findings require further research in larger studies.

In summary, genetic variations in the MMP family of -1607 2G allele and MMP7-181G carrier genotype with risk of UBC particularly in smokers may contribute to the development of bladder cancer. Haplotype combination of G/2G projecting greater risk for BC and the variant allele of MMP1-1607 associated with delay in recurrence in BCG treated patients further project the significance of our study. These results, once validated, may help to identify high-risk populations as well as determine an individual’s risk of invasive bladder cancer. Further study of the biological ramifications of these polymorphisms may add to our understanding of the biology of the disease and provide potential foci for targeted therapies.

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