

Associations of Cyclooxygenase 2 Polymorphic Genotypes with Bladder Cancer Risk in Taiwan

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Abstract. Aim: Bladder cancer is the sixth most common cancer worldwide and its incidence is particularly high in southwestern Taiwan. However, the genetic contribution to its etiology is not well-understood. The aim of this study is to evaluate the association of cyclooxygenase 2 (Cox-2) polymorphic genotypes with Taiwan bladder cancer patients. Materials and Methods: Six polymorphic variants of Cox-2 were analyzed regarding their association with bladder cancer risk, and three hundred and seventy-five patients with bladder cancer and same amount of age- and gender-matched healthy controls recruited were genotyped by the PCR-RFLP method. Results: Among the six polymorphic sites examined, only the Cox-2 promoter G-765C (rs20417) genotypes were positively associated with bladder cancer risk ($p=0.0102$). Individuals with the Cox-2 -765GC genotypes were associated with higher prostate cancer risk than those with -765GG. Conclusion: Our findings provide evidence that the C allele of Cox-2 promoter G-765C may be associated with the overexpression of COX-2 during bladder cancer development and may be a useful marker for the early detection of bladder cancer.

Bladder cancer is the most serious urinary neoplasm worldwide. In the western countries, bladder cancer is the fourth most common cancer among males, accounting for

7% of the total malignancies (1). In Taiwan, bladder cancer ranks seventh in incidence and mortality among common carcinomas (2). Carcinogenesis of bladder cancer is a complex, multi-step and multi-factorial process resulting following interactions of both environmental and genetic factors. In literature, the environmental risk factors for bladder carcinogenesis may include cigarette smoking, carcinogenic aromatic amine exposure and harmful drug consumption, such as phenacetine, chlornaphrazine and cyclophosphamide (3, 4).

Cyclooxygenases (also known as prostaglandin endoperoxide synthases, PTGSs) are key enzymes that convert arachidonic acids to prostaglandin H₂ (5). There are two forms of cyclooxygenases, Cox-1 and Cox-2. Cox-1 is a housekeeping enzyme involved in intracellular signaling, whereas Cox-2 is absent from many cell types unless induced by tumor promoters, growth factors, or cytokines (6-8). Accumulating evidence has shown that up-regulation of Cox-2 is closely-associated with malignant progression (9-12). Evidence collected from mRNA and protein levels of Cox-2 showed that levels may vary dramatically among the investigated subjects, and the variation may be partially determined by genetic variations, such as single nucleotide polymorphisms (SNPs) of Cox-2 itself (13, 14).

In literature, the association between SNPs of Cox-2 and bladder cancer susceptibility has been examined in Korean (15), New England (16), and India (17) populations, however, never among the Taiwanese. The present work is focused in two purposes, one is to perform the large case-control genotyping study of bladder cancer in Taiwan, a highly genetically-conserved population; the other is to examine the biological plausibility that genetic variation of Cox-2 could alter its coded enzyme expression levels or biochemical function and consequently have an impact on modifying the individual risk for bladder cancer. To examine the above

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Table I. Primer sequences, PCR and restriction fragment length polymorphism (RFLP) conditions for the Cox-2 genotyping.

Polymorphism (location)	Primers sequences (5' to 3')	Restriction enzyme	SNP sequence	DNA product size (bp)
G-1195A (rs689466)	F: CCCTGAGCACTACCCATGAT R: GCCCTTCATAGGAGATACTGG	<i>Hha I</i>	A	273
G-765C (rs20417)	F: TATTATGAGGAGAATTTACCTTTTCGC R: GCTAAGTTGCTTTCAACAGAAGAAT	<i>PvuII</i>	C	100
T+8473C (rs5275)	F: GTTTGAAATTTTAAAGTACTTTTGAT R: TTTCAAATTATTGTTTCATTGC	<i>Bcl I</i>	T	147
intron 1 (rs2745557)	F: GAGGTGAGAGTGTCTCAGAT R: CTCTCGGTTAGCGACCAATT	<i>Taq I</i>	G	439
intron 5 (rs16825748)	F: GCGGCATAATCATGGTACAA R: CAGCACTTCACGCATCAGTT	<i>BsrG I</i>	A	353+76
intron 6 (rs2066826)	F: ACTCTGGCTAGACAGCGTAA R: GCCAGATTGTGGCATAATC	<i>Aci I</i>	T	417
			A	314+103
			A	327
			G	233+94

*F and R indicate forward and reverse primers, respectively.

Table II. Characteristics of bladder cancer patients and non-cancer healthy controls.

Characteristics	Controls (n=375)			Patients (n=375)			p-Value ^a
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			62.3 (9.7)			61.4 (10.3)	0.73
Age group (years)							0.71
≤55	152	40.5%		158	42.1%		
>55	223	59.5%		217	57.9%		
Gender							0.55
Male	287	76.5%		279	74.4%		
Female	88	23.5%		96	25.6%		
Habits							
Cigarette smokers	186	49.6%		201	53.6%		0.31
Alcohol drinkers	176	46.9%		189	50.4%		0.38

^ap-Values based on Chi-square test.

hypothesis that the SNP variants of *Cox-2* are associated with the risk of bladder cancer, the genetic polymorphisms of six *Cox-2* SNPs, including G-1195A (rs689466), G-765C (rs20417), T+8473C (rs5275), intron 1 (rs2745557), intron 5 (rs16825748), and intron 6 (rs2066826), were analyzed in a Taiwanese population (control/case; 375/375).

Materials and Methods

Study population and sample collection. Three hundred and seventy-five patients diagnosed with bladder cancer were recruited at the outpatient clinics of general surgery between 2003-2009 at the China Medical University Hospital, Taichung, Taiwan, Republic of China. All patients who voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. As many non-bladder cancer healthy controls were selected by matching for age and gender after initial random sampling from the Health Examination Cohort of the hospital. Exclusion criteria for

the control group included previous malignancy, metastasized cancer from other or unknown origin, and any familial or genetic diseases. In addition, all members in the two groups completed a short questionnaire.

Genotyping assays. Genomic DNA was prepared from peripheral blood leukocytes of each subject using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed as previous genotyping studies (18-21). The polymerase chain reaction (PCR) cycling conditions for all the six genotyping work were: one cycle at 94°C for 5 min; 35 cycles of 94°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec, and a final extension at 72°C for 10 min. The PCR primer sequences and restriction enzyme for each DNA product are listed in Table I.

Statistical analyses. To ensure that the controls used were representative of the general population, and to exclude the possibility of genotyping error, the deviation of the genotype frequencies of *Cox-2* SNPs in the controls from those expected under the Hardy-

Table III. Distribution of Cox-2 genotypes among bladder cancer patient and control subjects.

Genotype	Controls	%	Patients	%	p-Value ^a	OR (95%CI) ^b
A-1195G (rs689466)					0.7236	
AA	107	28.5%	105	28.0%		1.00 (reference) ^c
AG	171	45.6%	181	48.3%		1.08 (0.77-1.52)
GG	97	25.9%	89	23.7%		0.94 (0.63-1.39)
G-765C (rs20417)					0.0102*	
GG	315	84.0%	286	76.3%		1.00 (reference)
GC	60	16.0%	89	23.7%		1.63 (1.13-2.35)*
CC	0	0%	0	0%		ND
T+8473C (rs5275)					0.4831	
TT	258	68.8%	248	66.1%		1.00 (reference)
TC	117	31.2%	127	33.9%		1.13 (0.83-1.53)
CC	0	0%	0	0%		ND
Intron 1 (rs2745557)					0.6727	
GG	284	75.7%	274	73.1%		1.00 (reference)
AG	84	22.4%	92	24.5%		1.14 (0.81-1.59)
AA	7	1.9%	9	2.4%		1.33 (0.49-3.63)
Intron 5 (rs16825748)					1.0000	
TT	371	98.9%	372	99.2%		1.00 (reference)
AT	4	1.1%	3	0.8%		0.75 (0.17-3.37)
AA	0	0%	0	0%		ND
Intron 6 (rs2066826)					0.4871	
GG	329	87.7%	339	90.4%		1.00 (reference)
AG	40	10.7%	32	8.5%		0.78 (0.48-1.27)
AA	6	1.6%	4	1.1%		0.65 (0.18-2.31)

ND, Not determined for the observed counts in case or control are zero; Significant ^ap-values and ^bORs, 95% CIs are bold and followed with a star; ^cset as control for comparison.

Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's chi-square test or Fisher's exact test (when the number in any cell was less than five) was used to compare the distribution of the genotypes between cases and controls. Data were deemed significant when $p < 0.05$. Cancer risk associated with the genotypes was estimated as odds ratios (ORs) and 95% confidence intervals (95% CIs) using unconditioned logistic regression.

Results

The frequency distributions for age, gender, cigarette smoking and alcohol drinking habits of the 375 bladder cancer patients and 375 controls are shown in Table II. The characteristics of the patients and controls were all well-matched. None of the differences in these characteristics between both groups were statistically significant ($p > 0.05$) (Table II).

The distribution of genotypic frequencies for the Cox-2 SNPs in controls and bladder cancer patients are shown in Table III. The genotypic frequencies of Cox-2 promoter G-765C polymorphism were differentially distributed between bladder cancer and control groups ($p = 0.0102$), while those for other five polymorphisms were not significant, ($p > 0.05$) (Table III). In detail, compared to those with GG, patients with the GC genotype may have 1.63-fold OR of bladder cancer susceptibility (95%CI=1.13-2.35).

The frequencies of the alleles for Cox-2 SNPs in controls and bladder cancer patients are shown in Table IV. The C allele of the Cox-2 promoter G-765C polymorphism was found to be associated with bladder cancer ($p = 0.0123$). Compared to those with the G allele, genotypes with C allele at Cox-2 promoter G-765C may have 1.55-fold higher OR for bladder cancer susceptibility (95%CI=1.09-2.18). Thus, from the data in Tables III and IV it can be concluded that the Cox-2 promoter -765G allele appears to be associated with higher risk for bladder cancer in Taiwan, while the other Cox-2 genotypes investigated in this study do not.

Discussion

In recent literature, several studies have demonstrated that variant genotypes in Cox-2 were associated with the risk of prostate cancer (22-27). However, there is no article investigating its association with bladder cancer.

In the present study one variant, GC genotype of G-765C (rs20417) was associated with an increased risk of bladder cancer among Taiwanese, while no other variant did (Table III). In the allelic frequency analysis, the C allele was found to be associated with a higher risk of bladder cancer (Table IV). We have summarized findings of the present study along

Table IV. *Cox-2* Allelic frequencies among bladder cancer patient and control subjects.

Allele	Controls	%	Patients	%	p-Value	OR (95%CI)
A-1195G (rs689466)					0.7565	
Allele A	385	51.3%	391	52.1%		1.00 (reference)
Allele G	365	48.7%	359	47.9%		0.97 (0.79-1.19)
G-765C (rs20417)					0.0123*	
Allele G	690	92.0%	661	88.1%		1.00 (reference)
Allele C	60	8.0%	89	11.9%		1.55 (1.09-2.18)*
T+8473C (rs5275)					0.4842	
Allele T	633	84.4%	623	83.1%		1.00 (reference)
Allele C	117	15.6%	127	16.9%		1.10 (0.84-1.45)
Intron 1 (rs2745557)					0.3700	
Allele G	652	86.9%	640	85.3%		1.00 (reference)
Allele A	98	13.1%	110	14.7%		1.14 (0.85-1.53)
Intron 5 (rs16825748)					0.7048	
Allele T	746	99.5%	747	99.6%		1.00 (reference)
Allele A	4	0.5%	3	0.4%		0.75 (0.17-3.36)
Intron 6 (rs2066826)					0.1966	
Allele G	698	93.1%	710	94.7%		1.00 (reference)
Allele A	52	6.9%	40	5.3%		0.76 (0.49-1.16)

Significant ORs, 95%CI and p-values are bold-marked and followed with a star.

Table V. Summary of current and previous studies on the association of *Cox-2* G-765C (rs20417) polymorphism and bladder/prostate cancer risk.

First author, year	Country	Race	Cases	Controls	Association with cancer
Chang WS, 2013	Taiwan	Chinese	375	375	Bladder cancer, associated
Panguluri RC, 2004	USA	European/African	90/124	90/164	Prostate cancer, associated
Cheng I, 2007	USA	European/African	416/89	417/88	Prostate cancer, not associated
Fernandez P, 2008	South Africa	African	151	134	Prostate cancer, associated
Wu HC, 2011	Taiwan	Chinese	218	436	Prostate cancer, associated
Mandal RK, 2011	India	Indian	195	250	Prostate cancer, associated

with those of other reports regarding prostate cancer in Table V. These findings are of pioneer significance for other groups to investigate its contribution in other populations. As for ourselves, further studies with larger population groups and the detail mechanisms are warranted, and should be compared with updated multi-ethnic studies to elucidate the role of *Cox-2* in bladder cancer. Furthermore, sophisticated gene-gene and gene-environment interactions together with the contribution of *Cox-2* genotype to prognosis of bladder cancer patients may be looked upon in the near future.

To sum up, this is the first study to demonstrate that a genetic variation in *Cox-2* may influence the risk of bladder cancer. The presence of the C allele of promoter -765 was found to be associated with a higher risk of bladder cancer and this finding supports previous reports showing an association between *Cox-2* variants and urinary cancer risk (23-27). We have provided evidence for a potent biomarker for bladder cancer early detection in Taiwanese and potentially for other countries.

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