

## Association of Cyclooxygenase 2 Polymorphic Genotypes with Prostate Cancer in Taiwan

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**Abstract.** The aim of this study was to evaluate the association of polymorphic genotypes in the cyclooxygenase 2 gene (*COX2*), which is reported to be overexpressed in prostate tumors, with Taiwan prostate cancer patients. **Materials and Methods:** Six polymorphic variants of *COX2* were analyzed for their association with prostate cancer susceptibility. A total of 218 patients with prostate cancer and 436 healthy controls in central Taiwan were enrolled in this investigation. *P*-values and odds ratios with 95% confidence intervals were used to assess the strength of the association. **Results:** Among the six polymorphic sites examined, only the *Cox-2* promoter G-765C (rs14133) genotypes were distributed differently between the prostate cancer and control groups. The *COX2* -765GG genotype was associated with higher prostate cancer risk than -765GC. **Conclusion:** These findings provide evidence that the G allele of *COX2* promoter G-765C may be associated with the development of prostate cancer and may be a useful marker for early detection of prostate cancer.

Prostate cancer is one of the most important cancers globally. In Taiwan, although the incidence of prostate cancer is much lower compared with other countries, prostate cancer still takes sixth place in the cancer causes of death for male

Taiwanese (1). Prostate cancer has become a serious issue in Taiwan public health since the number of patients and the death rate have kept increasing in the past two decades (1).

Cyclooxygenases (COXs, also known as prostaglandin endoperoxide synthases or PTGSs) are key enzymes that convert arachidonic acid to prostaglandin H<sub>2</sub>, a precursor to all of the other prostanoids (2). There are two forms of COXs, namely COX-1 and COX-2; the former may be a housekeeping enzyme involved in cell signaling, whereas the latter is absent from many cell types unless induced by tumor promoters, growth factors, or cytokines (3-5). Accumulating evidence has shown that up-regulation of COX-2 favors malignant progression (6-9). Mounting evidence from the investigations of the mRNA and protein levels of *COX2* showed that the levels may vary dramatically among the individuals, and the variation may be partially determined under different molecular mechanisms, which may depend on single nucleotide polymorphisms (SNPs) of *COX2* itself (10, 11).

In the literature, the association between SNPs of *COX2* and prostate cancer susceptibility has been examined in the Western (12-14), and Africa (15) populations, however, it has never been examined in the Taiwanese population. The present study was motivated by two aims: one was to perform a case-control study in Taiwan, a very genetically conserved Eastern population; and the other was to examine the biological plausibility that genetic variation in *COX2* could alter enzyme expression levels or biochemical function and consequently may have an impact on modifying the individual risk of prostate cancer. To examine the hypothesis that the SNP variants of *COX2* are associated with the risk of prostate cancer, the genetic polymorphisms of six *COX2* SNPs, including G-1195A (rs689466), G-765C (rs20417), T+8473C (rs5275), intron 1 (rs2745557), intron 5

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Table I. The primer sequences, PCR and restriction fragment length polymorphism (RFLP) conditions for COX2 gene polymorphisms.

Polymorphism (location)	Primer sequences (5' to 3')	Restriction enzyme	SNP sequence	DNA fragment size (bp)
G-1195A (rs689466)	F: CCCTGAGCACTACCCATGAT R: GCCCTTCATAGGAGATACTGG	<i>Hha I</i>	A	273
G-765C (rs20417)	F: TATTATGAGGAGAAATTTACCTTTTCGC R: GCTAAGTTGCTTTCAACAGAAGAAT	<i>Pvu II</i>	C	220+53
T+8473C (rs5275)	F: GTTTGAAATTTTAAAGTACTTTTGAT R: TTTCAAATTATTGTTTCATTGC	<i>Bcl I</i>	G	100
Intron 1 (rs2745557)	F: GAGGTGAGAGTGTCTCAGAT R: CTCTCGGTTAGCGACCAATT	<i>Taq I</i>	T	74+26
Intron 5 (rs16825748)	F: GCGGCATAATCATGGTACAA R: CAGCACTTCACGCATCAGTT	<i>BsrG I</i>	C	147
Intron 6 (rs2066826)	F: ACTCTGGCTAGACAGCGTAA R: GCCAGATTGTGGCATAACATC	<i>Aci I</i>	A	124+23
			G	439
			A	353+76
			A	417
			G	314+103
				327
				233+94

F and R indicate forward and reverse primers, respectively.

Table II. Characteristics of prostate cancer patients and controls.

Characteristic	Controls (n=436)			Patients (n=218)			P-value <sup>a</sup>
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			63.9 (6.6)			63.6 (6.9)	0.58
<50	275	63.1%		142	65.1%		
≥50	161	36.9%		76	34.9%		0.67
Habit							
Cigarette smokers	336	77.0%		177	81.2%		
Non-smokers	100	23.0%		41	18.8%		0.27

<sup>a</sup>Based on Chi-square test.

(rs16825748), and intron 6 (rs2066826) were analyzed in a Taiwanese population.

## Materials and Methods

**Study population and sample collection.** Two hundred and eighteen patients diagnosed with prostate 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 participated voluntarily, completed a self-administered questionnaire and provided peripheral blood samples. A total of 436 non-prostate cancer healthy volunteers were recruited as controls, selected by matching for age, gender and habits 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 questions about smoking and alcohol drinking habits. The study was approved by the Institutional Review Board of the China Medical University Hospital and written-informed consent was obtained from all participants.

**Genotyping assays.** Genomic DNA was prepared from peripheral blood leukocytes using a QIAmp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed as described in previous genotyping

studies (16-20). The polymerase chain reaction (PCR) cycling conditions were: one cycle at 94°C for 5 min; 35 cycles of 94°C for 30 sec, 55°C for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. Pairs of PCR primer sequences and restriction enzyme for each DNA product are all listed in Table I.

**Statistical analyses.** Only those individuals with both genotypic and clinical data (control/case: 436/218) were selected for final analysis. 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 COX2 SNPs in the controls from those expected under the Hardy-Weinberg equilibrium was assessed using the goodness-of-fit test. Pearson's  $\chi^2$  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 to be significant when the *p*-value was less than 0.05. Cancer risk associated with the genotypes was estimated as odds ratios (ORs) and 95% confidence intervals (95% CIs) using unconditional logistic regression.

## Results

The frequency distributions of the age, gender and smoking habits of the 218 prostate cancer patients and 436 controls are shown in Table II. The characteristics of the patients and

Table III. Distribution of COX2 genotypes among prostate cancer patients and control groups.

Genotype	Controls	%	Patients	%	P-value <sup>a</sup>	OR (95% CI)
A-1195G (rs689466)					0.7900	
AA	122	28.0%	61	28.0%		1.00 (reference)
AG	210	48.2%	100	45.9%		0.95 (0.65-1.41)
GG	104	23.8%	57	26.1%		1.10 (0.70-1.71)
G-765C (rs20417)					<b>0.0161</b>	
GG	365	83.7%	198	90.8%		1.00 (reference)
GC	71	16.3%	20	9.2%		<b>0.52 (0.31-0.88)</b>
CC	0	0%	0	0%		ND
T+8473C (rs5275)					0.4804	
TT	298	68.3%	143	65.6%		1.00 (reference)
TC	138	31.7%	75	34.4%		1.13 (0.80-1.60)
CC	0	0%	0	0%		ND
Intron 1 (rs2745557)					0.8182	
GG	320	73.4%	165	75.7%		1.00 (reference)
AG	107	24.5%	49	22.5%		0.89 (0.60-1.31)
AA	9	2.1%	4	1.8%		0.86 (0.26-2.84)
Intron 5 (rs16825748)					1.0000	
TT	433	99.3%	217	99.5%		1.00 (reference)
AT	3	0.7%	1	0.5%		0.67 (0.07-6.43)
AA	0	0%	0	0%		ND
Intron 6 (rs2066826)					0.6390	
GG	394	90.4%	192	88.1%		1.00 (reference)
AG	37	8.5%	23	10.5%		1.29 (0.74-2.23)
AA	5	1.1%	3	1.4%		1.24 (0.29-5.26)

<sup>a</sup>P-values are based on Chi-square test, and significant *p*-value is marked in bold; OR, odds ratio; CI, confidence interval; ND, not determined when the observed counts for cases or controls are zero; Significant ORs are marked in bold.

Table IV. COX2 allelic frequencies among the prostate cancer patients and control groups.

Allele	Controls	%	Patients	%	P-value	OR (95% CI)
A-1195G (rs689466)					0.6956	
Allele A	454	52.1%	222	50.9%		1.00 (reference)
Allele G	418	47.9%	214	49.1%		1.05 (0.83-1.32)
G-765C (rs20417)					<b>0.0172</b>	
Allele G	801	91.9%	416	95.4%		1.00 (reference)
Allele C	71	8.1%	20	4.6%		<b>0.54 (0.32-0.90)</b>
T+8473C (rs5275)					0.5251	
Allele T	734	84.2%	361	82.8%		1.00 (reference)
Allele C	138	15.8%	75	17.2%		1.11 (0.81-1.50)
Intron 1 (rs2745557)					0.5343	
Allele G	747	85.7%	379	86.9%		1.00 (reference)
Allele A	125	14.3%	57	13.1%		0.90 (0.64-1.26)
Intron 5 (rs16825748)					0.7233	
Allele T	869	99.7%	435	99.8%		1.00 (reference)
Allele A	3	0.3%	1	0.2%		0.67 (0.07-6.42)
Intron 6 (rs2066826)					0.3371	
Allele G	825	94.6%	407	93.3%		1.00 (reference)
Allele A	47	5.4%	29	6.7%		1.26 (0.78-2.04)

OR, odds ratio; CI, confidence interval; Significant *p*-values and ORs are marked in bold.

controls were all well matched. None of the differences in these characteristics between both groups were statistically significant ( $p > 0.05$ ) (Table II).

The frequencies of the genotypes for the COX2 SNPs in controls and prostate cancer patients are shown in Table III. The genotype distributions of the genetic polymorphisms of

Table V. Summary of previous and current studies of the association between *COX2* G-765C (rs20417) polymorphism and prostate cancer risk.

Reference	Country	Race	Cases	Controls	Association with prostate cancer
13	USA	European/African	90/124	90/164	Associated
12	USA	European/African	416/89	417/88	Not associated
15	South Africa	African	151	134	Associated
Current one	Taiwan	Asian	218	436	Associated

*COX2* promoter G-765C were significantly different between prostate cancer and control groups ( $p=0.0161$ ), while those for other polymorphisms were not significant, ( $p>0.05$ ) (Table III). Compared with those with GG, those with GC genotype may have 0.52-fold ORs of prostate cancer susceptibility (95% CI=0.31-0.88).

The frequencies of the alleles for *COX2* SNPs in controls and prostate cancer patients are shown in Table IV. The C allele of the *COX2* promoter G-765C polymorphism was found to be associated with prostate cancer ( $p=0.0172$ ). Compared with those with G allele, those with C allele at *COX2* promoter G-765C may have 0.54-fold ORs of prostate cancer susceptibility (95% CI=0.32-0.90). Thus from the data in Tables III and IV it can be concluded that the *COX2* promoter -765G allele appears to be associated with higher risk for prostate cancer in the Taiwanese population, while other *COX2* genotypes investigated in this study do not.

## Discussion

In order to understand the role of *COX2* and to find potential biomarkers of prostate cancer, six SNPs of the *COX2* gene were selected from the National Center for Biotechnology Information website and their associations with the susceptibility for prostate cancer in a population of Taiwan was examined. Recently, several studies demonstrated that variants in *COX2* were associated with the risk of prostate cancer (12-15). In one study of African-Americans, Nigerians, and European Americans, four promoter variants in *COX2* were evaluated and divergent patterns of association were observed across the three groups (13). Two variants, -1265G/A (rs20415) and -899G/C (rs20417), were associated with an increased risk of prostate cancer among African-Americans, while the -297 C/G (rs5270) variant was associated with a reduced risk overall and among African-Americans and European Americans (13). In a second study of a Swedish population, five *COX2* variants were examined and two variants, +3100 C/T (rs689470) and +8365C/T (rs2043), were associated with a reduced risk of prostate cancer (14). A third study focused on advanced prostate cancer patients in African-Americans and European Americans, three of the nine examined SNPs demonstrated significant associations with prostate cancer risk, with the

most compelling polymorphism, rs2745557, associated with a lower risk of disease (12). In another Africa case-control study, the -1285G allele and -1265T allele were both associated with increased risk of prostate cancer (15).

In the present study, the C variant genotypes of *COX2* promoter -765 were found to be associated significantly with a lower susceptibility for prostate cancer (Tables III and IV). This finding is important and has been compared with the findings of previous studies investigating other populations (Table V). However, further studies with a larger population in Taiwan and other countries are warranted, and should be compared with updated multi-ethnic studies to elucidate the role of *COX2* in prostate cancer. Moreover, more sophisticated gene-gene and gene-environment interactions (21), together with genotype-phenotype correlation should also be investigated in the near future.

In conclusion, this is the first study which demonstrated that common genetic variation in *COX2* influences the risk of prostate cancer in the Taiwanese population. The presence of the G allele of promoter -765 was found to be associated with a higher risk of prostate cancer and this finding supports previous reports showing an association between *COX2* variants and prostate cancer risk (13, 15). This study provides evidence not only for a potent biomarker for prostate cancer early detection in the Taiwanese population, but also for the genetic basic background for further gene-gene and gene-environment interactions, or genotype-phenotype correlation studies of prostate cancer in Taiwan.

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