Associations of *Cyclooxygenase 2* Polymorphic Genotypes with Prostate Cancer in Taiwan

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Abstract. Aim: Prostate cancer is the most common cause of man cancer death and a major health problem worldwide. The aim of this study is to evaluate the association of polymorphic genotypes in the *cyclooxygenase 2* (*Cox-2*), which is reported to be over-expressed in prostate tumors, with Taiwan prostate cancer patients. **Materials and Methods:** Six polymorphic variants of *Cox-2* were analyzed of their association with prostate cancer susceptibility, and 218 patients with prostate cancer and 436 healthy controls in central Taiwan were enrolled in this investigation. We used both the *P*-values and odds ratios with 95% confidence intervals 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. Individuals with the *Cox-2* -765GG genotypes were associated with higher prostate cancer risk than those with -765GC. **Conclusion:** Our findings provide evidence that the G allele of *Cox-2* promoter G-765C may be associated with the development of prostate cancer and may be a useful marker for early detection of prostate cancer.

Key Words: *Cox-2*, polymorphism, prostate cancer, carcinogenesis.

Prostate cancer is one of the most important cancers all over the world. In the men of the United States and Western Europe, prostate cancer is the top cause of illness and death (1), while the incidence of prostate cancer widely varies in different races. It is recorded that Asians such as China, Korea, and Japan, have the lowest incidence among the major races, and African—American men have the leading incidence in the world (2). In Taiwan, although the incidence of prostate cancer is much lower compared with other countries, prostate cancer still takes the sixth place in the cancer causes of death for male Taiwanese (3). 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 (3).

Cyclooxygenases (also known as prostaglandin endoperoxide synthases or PTGSs) are key enzymes that convert arachidonic acid to prostaglandin H2, a precursor to all of the other prostanoids (4). 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 later is absent from many cell types unless induced by tumor promoters, growth factors, or cytokines (5-7). Accumulating evidence has shown that up-regulation of Cox-2 favors malignant progression (8-11). Mounting evidence from the investigations of the mRNA and protein levels of *Cox-2* 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 *Cox-2* itself (12, 13).

In the literature, the association between SNPs of *Cox-2* and prostate cancer susceptibility has been examined in the Western population (14-16), and Africa (17), however, never been examined in Taiwanese, an Eastern country. The present work is motivated by two points, one is to perform the case-control study in Taiwan, a very genetic conserved Eastern population; the other is to examine the biological plausibility that genetic variation in the *Cox-2* could alter enzyme expression levels or biochemical function and consequently may have an impact on modifying the individual risk of prostate cancer. To examine our hypothesis that the SNP variants of *Cox-2* are associated with the risk of prostate 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:436/218).

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 voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. Twice as many of non-prostate cancer healthy volunteers as controls were 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 habits and they were recorded. Our 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 QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed as previous genotyping studies (18-22). The polymerase chain reaction (PCR) cycling conditions were: one cycle at 94^{o}C for 5 min; 35 cycles of 94^{o}C for 30 sec, 55^{o}C for 30 sec, and 72^{o}C for 30 sec, and a final extension at 72^{o}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 *Cox-2* SNPs in the controls from those expected under the Hardy-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 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 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 *Cox-2* SNPs in controls and prostate cancer patients are shown in Table III. The genotype distributions of the genetic polymorphisms of *Cox-2* 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 odds ratios of prostate cancer susceptibility (95%CI=0.31-0.88).

The frequencies of the alleles for *Cox-2* SNPs in controls and prostate cancer patients are shown in Table IV. The C allele of the *Cox-2* promoter G-765C polymorphism was found to be associated with prostate cancer (P=0.0172). Compapred with those with G allele, those with C allele at *Cox-2* promoter G-765C may have 0.54-fold odds ratios 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 *Cox-2* promoter -765 G allele appears to be associated with higher risk for prostate cancer in Taiwan, while other *Cox-2* genotypes investigated in this study do not.

Discussion

In order to understand the role of *Cox-2* and to find potential biomarkers of prostate cancer, six SNPs of the *Cox-2* 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 firstly examined. Recently, several studies demonstrated that variants in Cox-2 were associated with the risk of prostate cancer (14-17). In one study of African-Americans, Nigerians, and European Americans, four promoter variants in *Cox-2* were evaluated and divergent patterns of association were observed across the three groups (15). Two variants, -1265 G/A (rs20415) and -899 G/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 (15). In a second study of a Swedish population, five *Cox-2* variants were examined and two variants, +3100 C/T (rs689470) and +8365 C/T (rs2043), were associated with a reduced risk of prostate cancer (16). The 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 (14). In another Africa case-control study, The -1285 G allele and -1265 T allele were both associated with increased risk of prostate cancer (17).

In the present study, the C variant genotypes of *Cox-2* promoter -765 were found to be associated significantly with a lower susceptibility for prostate cancer (Tables III and IV). This finding is important and we have compared the finding with previous studies investigating other populations (Table V). However, further studies with larger population in Taiwan and other countries are warranted, and should be compared with updated multi-ethnic studies to elucidate the role of *Cox-2* in prostate cancer. Moreover, more sophisticated gene-gene and gene-environment interactions (23), together with genotype-phenotype correlation should also be investigated in the near future.

To sum up, this is the first study which demonstrated that common genetic variation in *Cox-2* influences the risk of prostate cancer in Taiwan. 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 *Cox-2* variants and prostate cancer risk (15, 17). We have provided evidence not only for a potent biomarker for Taiwan prostate cancer early detection, 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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References

1 Gronberg H: Prostate cancer epidemiology. Lancet *361*: 859-864, 2003.

2 Hsing AW and Chokkalingam AP: Prostate cancer epidemiology. Front Biosci *11*: 1388-1413, 2006.

3 Department of Health, Executive Yuan, Taiwan. Summary of statistics on causes of death in Taiwan, 2008.

4 DeWitt DL: Prostaglandin endoperoxide synthase: regulation of enzyme expression. Biochim Biophys Acta *1083*: 121-134, 1991.

5 Kujubu DA, Reddy ST, Fletcher BS and Herschman HR: Expression of the protein product of the prostaglandin synthase-2/TIS10 gene in mitogen-stimulated Swiss 3T3 cells. J Biol Chem *268*: 5425-5430, 1993.

6 Kawata R, Reddy ST, Wolner B and Herschman HR: Prostaglandin synthase 1 and prostaglandin synthase 2 both participate in activation-induced prostaglandin D2 production in mast cells. J Immunol *155*: 818-825, 1995.

7 Reddy ST and Herschman HR: Ligand-induced prostaglandin synthesis requires expression of the TIS10/PGS-2 prostaglandin synthase gene in murine fibroblasts and macrophages. J Biol Chem *269*: 15473-15480, 1994.

8 Fujimura T, Ohta T, Oyama K, Miyashita T and Miwa K: Role of cyclooxygenase-2 in the carcinogenesis of gastrointestinal tract cancers: a review and report of personal experience. World J Gastroenterol *12*:

1336-1345, 2006.

9 Marshall SF, Bernstein L, Anton-Culver H, Deapen D, Horn-Ross PL, Mohrenweiser H, Peel D, Pinder R, Purdie DM, Reynolds P, Stram D, West D, Wright WE, Ziogas A and Ross RK: Nonsteroidal anti-inflammatory drug use and breast cancer risk by stage and hormone receptor status. J Natl Cancer Inst *97*: 805-812, 2005.

10 van Rees BP and Ristimaki A: Cyclooxygenase-2 in carcinogenesis of the gastrointestinal tract. Scand J Gastroenterol *36*: 897-903, 2001.

11 Wang W, Bergh A and Damber JE: Cyclooxygenase-2 expression correlates with local chronic inflammation and tumor neovascularization in human prostate cancer. Clin Cancer Res *11*: 3250-3256, 2005.

12 Cok SJ and Morrison AR: The 3'-untranslated region of murine cyclooxygenase-2 contains multiple regulatory elements that alter message stability and translational efficiency. J Biol Chem *276*: 23179-23185, 2001.

13 Papafili A, Hill MR, Brull DJ, McAnulty RJ, Marshall RP, Humphries SE and Laurent GJ: Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. Arterioscler Thromb Vasc Biol *22*: 1631-1636, 2002.

14 Cheng I, Liu X, Plummer SJ, Krumroy LM, Casey G and Witte JS: COX2 genetic variation, NSAIDs, and advanced prostate cancer risk. Br J Cancer *97*:

557-561, 2007.

15 Panguluri RC, Long LO, Chen W, Wang S, Coulibaly A, Ukoli F, Jackson A, Weinrich S, Ahaghotu C, Isaacs W and Kittles RA: COX-2 gene promoter haplotypes and prostate cancer risk. Carcinogenesis *25*: 961-966, 2004.

16 Shahedi K, Lindstrom S, Zheng SL, Wiklund F, Adolfsson J, Sun J, Augustsson-Balter K, Chang BL, Adami HO, Liu W, Gronberg H and Xu J: Genetic variation in the COX-2 gene and the association with prostate cancer risk. Int J Cancer *119*: 668-672, 2006.

17 Fernandez P, de Beer PM, van der Merwe L and Heyns CF: COX-2 promoter polymorphisms and the association with prostate cancer risk in South African men. Carcinogenesis *29*: 2347-2350, 2008.

18 Bau DT, Tseng HC, Wang CH, Chiu CF, Hua CH, Wu CN, Liang SY, Wang CL, Tsai CW and Tsai MH: Oral cancer and genetic polymorphism of DNA double strand break gene Ku70 in Taiwan. Oral Oncol *44*: 1047-1051, 2008.

19 Bau DT, Wu HC, Chiu CF, Lin CC, Hsu CM, Wang CL, Wang RF and Tsai FJ: Association of XPD polymorphisms with prostate cancer in Taiwanese patients. Anticancer Res *27*: 2893-2896, 2007.

20 Chang CH, Chiu CF, Liang SY, Wu HC, Chang CL, Tsai CW, Wang HC, Lee HZ and Bau DT: Significant association of Ku80 single nucleotide polymorphisms with bladder cancer susceptibility in Taiwan. Anticancer Res

29: 1275-1279, 2009.

21 Liu CJ, Hsia TC, Wang RF, Tsai CW, Chu CC, Hang LW, Wang CH, Lee HZ, Tsai RY and Bau DT: Interaction of cyclooxygenase 2 genotype and smoking habit in Taiwanese lung cancer patients. Anticancer Res *30*: 1195-1199, 2010.

22 Tseng HC, Tsai MH, Chiu CF, Wang CH, Chang NW, Huang CY, Tsai CW, Liang SY, Wang CL and Bau DT: Association of XRCC4 codon 247 polymorphism with oral cancer susceptibility in Taiwan. Anticancer Res *28*: 1687-1691, 2008.

23 Wark PA, Van der Kuil W, Ploemacher J, Van Muijen GN, Mulder CJ, Weijenberg MP, Kok FJ and Kampman E: Diet, lifestyle and risk of K-ras mutation-positive and -negative colorectal adenomas. Int J Cancer *119*: 398-405, 2006.

Table I. The primer sequences, PCR and restriction fragment length polymorphism (RFLP) conditions for *Cox-2* gene polymorphisms.

Polymorphism (location)	**Primers sequences (5' to 3')**	**Restriction enzyme**	**SNP sequence**	**DNA fragment size (bp)**
G-1195A (rs689466)	F: CCCTGAGCACTACCCATGAT	*Hha I*	A	273
	R: GCCCTTCATAGGAGATACTGG		G	220 + 53
G-765C (rs20417)	F: TATTATGAGGAGAATTTACCTTTCGC	*Pvu II*	C	100
	R: GCTAAGTTGCTTTCAACAGAAGAAT		G	74 + 26
T+8473C (rs5275)	F: GTTTGAAATTTTAAAGTACTTTTGAT	*Bcl I*	T	147
	R: TTTCAAATTATTGTTTCATTGC		C	124 + 23
intron 1 (rs2745557)	F: GAGGTGAGAGTGTCTCAGAT	*Taq I*	G	439
	R: CTCTCGGTTAGCGACCAATT		A	353 + 76
intron 5 (rs16825748)	F: GCGGCATAATCATGGTACAA	*BsrG I*	T	417
	R: CAGCACTTCACGCATCAGTT		A	314 + 103
intron 6 (rs2066826)	F: ACTCTGGCTAGACAGCGTAA	*Aci I*	A	327
	R: GCCAGATTGTGGCATACATC		G	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[a]
	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%		0.67
≥50	161	36.9%		76	34.9%		
Habit							
Cigarette smokers	336	77.0%		177	81.2%		0.27
Non-smokers	100	23.0%		41	18.8%		

[a] Based on Chi-square test.

Table III. Distribution of *Cox-2* genotypes among prostate cancer patient and control groups.

Genotype	Controls	%	Patients	%	*P*-value[a]	OR (95% CI)[b]
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)					**0.0161**	
GG	365	83.7%	198	90.8%		1.00 (reference)
GC	71	16.3%	20	9.2%		**0.52 (0.31-0.88)**
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)

ND, not determined for the observed counts in case or control are zero; Significant ORs, 95% CIs and *P*-values are bolded marked.

Table IV. *Cox-2* allelic frequencies among the prostate cancer patient 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)					**0.0172**	
Allele G	801	91.9%	416	95.4%		1.00 (reference)
Allele C	71	8.1%	20	4.6%		**0.54 (0.32-0.90)**
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)

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

First author, year	Country	Race	Cases	Controls	Association with prostate cancer
Panguluri et al, 2004	USA	European/African	90/124	90/164	Associated
Cheng et al, 2007	USA	European/African	416/89	417/88	Not associated
Fernandez et al, 2008	South Africa	African	151	134	Associated
Wu HC et al, 2010	Taiwan	Asian	218	436	Associated