Interaction of Cyclooxygenase 2 Genotype and Smoking Habit in Taiwanese Lung Cancer Patients

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Abstract. *Aim: The aim of this study was to evaluate the association and interaction of genotypic polymorphisms in the cyclooxygenase 2 (Cox-2) gene with smoking habits with lung cancer patients in Taiwan. Six polymorphic variants of Cox-2 were analysed in association with their effect on lung cancer susceptibility, and their joint effects with smoking habits on lung cancer risk is discussed. Materials and Methods: Three hundred and fifty-eight patients with lung cancer and 716 healthy controls from the China Medical Hospital in central Taiwan were genotyped. Results: The Cox-2 intron 6 (rs2066826) genotypes were distributed differently between the lung cancer and control groups. The A allele of Cox-2 intrin 6 was found more frequently in the cancer patient group than in the controls. Furthermore, the interactions of smoking with genetic factors were significant for the Cox-2 intron 6 genotypes. Patients who smoked and had the Cox-2 intron 6 AG or AA genotype had an increased risk of 2.21 (95% confidence interval=1.53-3.27) for developing lung cancer. Conclusion: These results provide evidence that the A allele of Cox-2 intron 6 may be associated with the development of lung cancer and may be a useful marker for early detection and treatment of lung cancer.*

Tobacco smoking is the leading cause of lung cancer, which in turn is the leading cause of cancer mortality in the world (1, 2). Although tobacco smoking is the major risk factor in the development of lung cancer, only 10-15% of all smokers develop lung cancer, suggesting that there is a great variation among individuals in their susceptibility to lung carcinogenesis (3, 4). In Taiwan, lung cancer has high incidence, high mortality, and a low 5-year survival rate, especially in female adenocarcinoma cases (5).

Inflammation is an important risk factor for the pathogenesis of lung cancer, and may be due to continuous exposure to tobacco components that result in oxidative stress and thus contribute to tumour promotion and progression in the lung (8, 9). Increasing evidence suggests that chronic inflammation, particularly in smokers, is associated with an increased risk of lung cancer (8-11). Tobacco smoke stimulates bronchial epithelial cells to release pro-inflammatory cytokines such as interleukin-1 beta, and to up-regulate various inflammation-related genes including cyclooxygenases-2 (*Cox-2*) (12-14). Cyclooxygenases (also known as prostaglandin endoperoxide synthases or PTGSs) are key enzymes which convert arachidonic acid to prostaglandin H2, a precursor to all of the other prostanoids (15). There are two forms of human COXs, *Cox-1* and *Cox-2*. *Cox-1* may be a housekeeping enzyme involved in cell signaling, whereas *Cox-2* is absent from many cell types unless induced by tumour promoters, growth factors, or cytokines (16-18). Studies have shown that *Cox-2* can be induced by tobacco smoke condensate *in vitro* and by the tobacco specific carcinogen nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in mice (19, 20), which may play an important role in smoking-related carcinogenesis. Emerging evidence also suggests *Cox-2* plays an important role in lung carcinogenesis (21). However, the mRNA and protein levels of *Cox-2* may vary among individuals, and this variability may be partially genetically determined under different molecular mechanisms, which may depend on single nucleotide polymorphisms (SNPs) of *Cox-2* (22, 23).

In 2005, the association between SNPs of *Cox-2* and lung cancer susceptibility was examined in a Chinese population, and it was found that 8473CT/CC is associated with a

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

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

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

decreased lung cancer risk (24). However, only one SNP was investigated in the study and the finding is contradictory to that of a similar investigation of a Norwegian population (25). The present work was motivated by 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 lung cancer. To further test the hypothesis that the SNP variants of *Cox-2* are associated with the risk of lung 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 analysed in a Taiwanese population (control/case: 716/358), and the interaction of *Cox-2* genotypes and smoking habits in a Taiwanese population was investigated.

Materials and Methods

Study population and sample collection. Three hundred and fifty-eight cancer patients diagnosed with lung cancer were recruited at the outpatient clinics of general surgery between 2005-2008 at the China Medical University Hospital, Taichung, Taiwan. The clinical characteristics of patients including histological details were all graded and defined by expert surgeons. All patients voluntarily participated, completed a self-administered questionnaire and provided peripheral blood samples. Twice as many non-lung cancer healthy volunteers as controls were selected by matching for age, gender and smoking 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 related to smoking 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 QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to previous studies (14-22). The polymerase chain reaction (PCR) cycling conditions were: one cycle at 94°C for 5 min; 35 cycles of 94°C for 30 s, 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: 716/358) 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.

Results

The frequency distributions of selected characteristics (age, gender and smoking habits) of 358 lung cancer patients and 716 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 lung cancer patients are shown in Table III. The genotype distributions of the genetic polymorphisms of *Cox-2* intron 6 were significantly different between lung cancer and control groups ($p=1.54\times10^{-4}$), while those for other polymorphisms were not significant $p>0.05$) (Table III). The frequencies of the alleles for *Cox-2* SNPs in controls and lung cancer patients are shown in Table IV. The A allele of the

Table II. *Characteristics of lung cancer patients and controls.*

Characteristic	Controls (n=716)			Patients (n=358)			*p*-Value[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			64.8 (6.8)			64.0 (6.9)	0.58
Gender							0.36
Male	488	68.1%		254	70.9%		
Female	228	31.9%		104	29.1%		
Habit							
Cigarette smokers	563	78.6%		293	81.8%		0.23
Non-smokers	153	21.4%		65	18.2%		

[a]Based on Chi-square test.

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

Genotype	Controls	%	Patients	%	*p*-Value[a]
A-1195G (rs689466)					0.8200
AA	193	27.0%	102	28.5%	
AG	345	48.2%	172	48.0%	
GG	178	24.8%	84	23.5%	
G-765C (rs20417)					0.4945
GG	622	86.9%	317	88.5%	
GC	94	13.1%	41	11.5%	
CC	0	0%	0	0%	
T+8473C (rs5275)					0.6824
TT	468	65.4%	239	66.8%	
TC	248	34.6%	119	33.2%	
CC	0	0%	0	0%	
Intron 1 (rs2745557)					0.6642
GG	558	77.9%	284	79.3%	
AG	151	21.1%	69	19.3%	
AA	7	1.0%	5	1.4%	
Intron 5 (rs16825748)					0.4521
TT	710	99.2%	356	99.4%	
AT	6	0.8%	2	0.6%	
AA	0	0%	0	0%	
Intron 6 (rs2066826)					1.54×10^{-4}
GG	614	85.8%	274	76.5%	
AG	97	13.5%	74	20.7%	
AA	5	0.7%	10	2.8%	

[a]Based on Chi-square test.

Table IV. *Cox-2 allelic frequencies among the lung cancer patient and control groups.*

Allele	Controls	%	Patients	%	*p*-Value[a]
A-1195G (rs689466)					0.5215
Allele A	731	51.1%	376	52.5%	
Allele G	701	48.9%	340	47.5%	
G-765C (rs20417)					0.4506
Allele G	1338	93.4%	675	94.3%	
Allele C	94	6.6%	41	5.7%	
T+8473C (rs5275)					0.6852
Allele T	1184	82.7%	597	83.4%	
Allele C	248	17.3%	119	16.6%	
Intron 1 (rs2745557)					0.7364
Allele G	1267	88.5%	637	89.0%	
Allele A	165	11.5%	79	11.0%	
Intron 5 (rs16825748)					0.6164
Allele T	1426	99.6%	714	99.7%	
Allele A	6	0.4%	2	0.3%	
Intron 6 (rs2066826)					2.20×10^{-5}
Allele G	1325	92.5%	622	86.9%	
Allele A	107	7.5%	94	13.1%	

[a]Based on Chi-square test.

Cox-2 intron 6 polymorphism seems to be associated with lung cancer ($p=2.2 \times 10^{-5}$). Thus from the data in Tables III and IV it can be concluded that the *Cox-2* intron 6 A allele appears to be associated with higher risk for lung cancer in Taiwan, while other SNPs that were investigated do not.

Since smoking is the predominant risk factor for lung cancer, the interaction between *Cox-2* genotype and individual smoking habits was also analysed by stratified individual smoking status (Table V). It was observed that those with the homozygous AA plus heterozygous AG for *Cox-2* intron 6 had higher risks of lung cancer in smoking group, but not in the case of non-smoking group. The adjusted odds ratios for AG plus AA groups in overall and smoker stratifications are 1.88 and 2.21, respectively (95% confidence intervals are 1.54-2.68 and 1.53-3.27). To summarize, an association was observed between smoking status, *Cox-2* intron 6 genotypes and lung cancer susceptibility.

Discussion

In order to understand the role of *Cox-2* and to find potential biomarkers of lung cancer, six SNPs of the *Cox-2* gene were selected and their association with the susceptibility for lung cancer in a population of central Taiwan was investigated. The A allele-bearing genotypes of *Cox-2* intron 6 were found to be

Table V. *Distribution of Cox-2 intron 6 (rs2066826) genotype and lung cancer after stratification by smoking habit.*

SNP/Genotype	Overall			Never smokers			Ever smokers		
	Controls N (%)	Cases N (%)	Adjusted[a] OR (95% CI)[c]	Controls N (%)	Cases N (%)	Adjusted[b] OR (95% CI)[c]	Controls N (%)	Cases N (%)	Adjusted[b] OR (95% CI)[c]
Intron 6 (rs2066826)									
GG	614 (85.8)	274 (76.5)	1.00 (Ref.[d])	129 (84.3)	52 (80.0)	1.00 (Ref.[d])	485 (86.1)	222 (75.8)	1.00 (Ref.[d])
AG+AA	102 (14.2)	84 (23.5)	1.88 (1.54-2.68)	24 (15.7)	13 (20.0)	1.37 (0.72-2.37)	78 (13.9)	71 (24.2)	2.21 (1.53-3.27)

[a]Adjusted for age, gender and smoking (pack-years); [b]adjusted for age and gender; [c]OR, odds ratio; CI, confidence interval; [d]Ref., reference.

associated significantly with a higher susceptibility for lung cancer (Tables III and IV). This finding is important and should be investigated in other populations. For the important role smoking plays in lung carcinogenesis (5-7, 26), the association between *Cox-2* intron 6 genotype and lung cancer risk in patients and controls who have a cigarette smoking habit was further analysed. Interestingly, the interaction between *Cox-2* intron 6 and cigarette smoking habit indicates that smokers with the AG or AA genotype have a 2.21-fold higher odds ratio of developing lung cancer than those smokers with GG genotype (confidence interval=1.53-3.27), which is not the case in the non-smokers (odds ratio 1.37 and 95% confidence interval=0.72-2.37). The odds ratio is also higher than that in the overall population, which is 1.88-fold (confidence interval=1.54-2.68). It can therefore be proposed that the A allele of intron 6 may affect *Cox-2* activity, slightly influencing its normal function. As smokers with the A allele(s) get older, the alteration towards carcinogenesis may accumulate *via* the decreasing function of *Cox-2* and a lower inflammatory capacity. Cigarette smoking releases many inducers of DNA damage into the respiratory system and thus causes DNA damage to cells. Therefore, in people who have a risky genetic variant, such as the A allele of intron 6, and also have a smoking habit, the combined effect of these factors may synergistically increase their susceptibility to developing lung cancer.

To summarize, this is the first study which focuses on selected SNPs of *Cox-2* and their effects when combined with a smoking habit on lung cancer risk in Taiwan. The presence of the A allele of intron 6 was found to be associated with a higher risk of lung cancer. Future studies should focus on investigating multiple SNPs of other related genes from the same pathway in order to elucidate further gene-gene and gene-environment interactions in susceptibility of lung cancer.

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