Association Study of Cyclooxygenase 2 Single Nucleotide Polymorphisms and Childhood Acute lymphoblastic leukemia in Taiwan

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Running title: Wang *et al*: *Cox-2* Genotypes in Childhood Leukemia

Abstract. Aim: The relationship between COX-2 gene and childhood leukemia risk is still ambiguous. In this study, the association and interaction of genotypic polymorphisms in *cyclooxygenase 2* (*Cox-2*) gene and smoking habits with childhood leukemia are investigated. **Materials and Methods:** Up to 266 patients with childhood leukemia and 266 healthy controls recruited from the China Medical Hospital in central Taiwan were genotyped by PCR-RFLP method. We investigated six polymorphic variants of *Cox-2,* including G-1195A, G-765C, T+8473C, intron 1, intron 5, and intron 6, and analyzed the association of specific genotype with susceptibility to childhood leukemia. **Results:** The data showed that although for each genotype of *Cox-2* G-1195A, G-765C, T+8473C, intron 1, intron 5, and intron 6, there is no difference in the distribution between the childhood leukemia and control groups ($P > 0.05$), the analysis of joint effect for *Cox-2* G-765C and intron 6 showed that individuals with GC at G-765C and GG or AG+AA at intron 6 present a slightly higher potential for developing childhood leukemia than other groups. **Conclusion:** Our findings suggest that the C allele of *Cox-2* G-765C may be responsible for childhood leukemia and may be useful in early detection of child leukemia.

Key Words*: *Cox-2, polymorphism, childhood leukemia.

Acute lymphoblastic leukemia (ALL) is the most common cancer in childhood, accounting for 30% of the childhood malignancies {Karathanasis, 2009 #3}. The etiology of childhood ALL is mostly unknown. Although infections in the first years and some environmental factors such as ionizing radiation and parental alcohol and tobacco use could play a causative role in ALL {Schmiegelow, 2008 #4; Rubnitz, 1997 #6; Liu, 2008 #7}. However, the genomic contributing factors of leukemia are still largely unknown, both in adult and child leukemia. ALL is known to result from an accumulation of mutations in tumor suppressor genes and oncogenes, and genetic alterations affecting several chromosomes {Kawamata, 2008 #8; Armstrong, 2005 #9; Patterson, 2009 #11; Pui, 2006 #12; Pui, 2004 #13}. Although common genetic variations may play a role in determining individual susceptibility of leukemia development in children, limited studies have evaluated the association between genetic polymorphisms in candidate genes such as *CYP, GST, NAT, MTHFR, NQO1, XRCC1, MDR1, cyclin D1, CCND1, and XRCC4* with childhood ALL risk {Chokkalingam, 2008 #279; Karathanasis, 2009 #270; Kim, 2006 #281; Sinnett, 2006 #280; Wu #282}. Anyway, it is commonly agreeable that single environmental or genetic factor can only ambiguously explain a small part of subjects developed child ALL. Thereafter, the genetic factors may be more comprehensive and less ignorable.

Cyclooxygenases (also known as prostaglandin endoperoxide synthases or PTGSs)

are key enzymes to convert arachidonic acid to prostaglandin H2, a precursor to all of the other prostanoids {DeWitt, 1991 #231}. There are two forms of human COXs, i.e., *Cox-1* and *Cox-2*. It was reported that Cox-2 over-expression may contribute to carcinogenesis via its regulation on apoptosis, immunosurveillance, angiogenesis, and also xenobiotic metabolism {Nishihara, 2003 #11; Gumgumji, 2003 #12}. In several animal and clinical studies, Cox-2 specific inhibitors have both preventive and therapeutic effects as anticancer drugs for breast, bladder, lung and pancreas cancers {Davies, 2003 #13; Sanchez-Alcazar, 2003 #14; Levitt, 2002 #15; Mizutani, 2002 #16}. However, the association of *Cox-2* genotypes with childhood ALL has never been investigated. In addition, 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 depends on single nucleotide polymorphisms (SNPs) of *Cox-2* {Cok, 2001 #239; Papafili, 2002 #238}.

Although COX-2 over-expression and COX-2 inhibitor drugs have been extensively studied in cancer, there were very few studies reporting the effects of COX-2 inhibition in hematologic malignancies, not to mention childhood ALL. In 2002, it was reported that COX-2 over-expression was frequent in patients with chronic myelocytic leukemia (CML) and also found to be associated with shorter survival {Giles, 2002 #17}. The present work is 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 childhood ALL. To clarify the hypothesis that the SNP variants of *Cox-2* are associated with the risk of childhood ALL, we analyzed 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), in a large Taiwanese childhood ALL population (control/case=266/266).

Materials and Methods

Study population and sample collection. Two hundred and sixty-six patients diagnosed with childhood ALL (i.e. the population under 18 years old) were recruited at the Pediatric Departments at the China Medical University Hospital and National Taiwan University Hospital, Taiwan, in 2005-2009. Each patient and non-cancerous healthy person (matched by gender and age after initial random sampling from the Health Examination Cohort of the two hospitals) completed a self-administered questionnaire and provided their peripheral blood samples.

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 {Chang, 2009 #18; Chang, 2009 #19; Chiu, 2008 #20;

Chiu, 2008 #21; Chiu, 2008 #22; Hsu, 2009 #134; Hsu, 2009 #27; Yang, 2009 #43}. 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 with both genotypic and clinical data (control/case=266/266) 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 expected number in any cell was less than five) was used to compare the distribution of the genotypes between cases and controls. Data were recognized as significant when the statistical *P-value* was less than 0.05.

Results

The frequency distributions of selected characteristics of 266 childhood ALL patients and 266 controls are shown in Table II. These characteristics of patients and controls are all well matched. None of the differences between both groups were statistically significant (P>0.05) (Table II).

The frequencies of the genotypes for the *Cox-2* SNPs in controls and childhood ALL patients are shown in Table III. The genotype distributions of the genetic polymorphisms of *Cox-2* of the six polymorphisms investigated were not significant between the two groups (P>0.05) (Table III). The frequencies of the alleles for *Cox-2* SNPs in controls and childhood ALL patients are shown in Table IV. Neither of the allele of the *Cox-2* of the SNPs were found to be associated with lung cancer (P>0.05).

To further investigate the association of *Cox-2* genotype and childhood ALL, the interactions among SNPs were investigated by genotype analysis. Each of the frequencies of combined genotypic polymorphisms was analyzed, and here only the results of G-765C and intron genotypes were shown in Table IV, while other combinations were not significant (data not shown). There were no significant differences in frequencies of the combined genotypes between the two groups for each combined genotype. The odds ratios (ORs) of the GG/AG+AA, GC/GG, GC/

and AG+AA combined genotypes compared with common GG/GG reference genotype were 1.23 (95% confidence interval, CI=0.76-1.98; *P*=0.4639), 1.67 (95% CI=0.97-2.86; *P*=0.0612), and 1.67 (95% CI=0.58-4.79; *P*=0.4315), respectively.

Discussion

In order to know the role of *Cox-2* and to find potential biomarkers of childhood ALL, in this study, we selected six SNPs of the *Cox-2* gene and investigated their associations with the susceptibility for childhood ALL in a population in northern and central Taiwan. We found that as for single SNP, the variant genotypes of *Cox-2* were not significantly associated with the susceptibility for childhood ALL (Tables III and IV). This may not be due to small sample size (it is relatively large in childhood ALL studies), but more likely *Cox-2* may play a minor role in the etiology of childhood ALL, which is an outcome of complex genetic and environmental interactions. Among the SNPs we checked, G-765C (rs20417) was found to be slightly associated with childhood ALL (P=0.06), although not statistically significant. The genotypic distribution of GC at G-765C was higher in the childhood ALL group (18%) than the control group (12%) (Table 3). The lack of CC homozygote at G-765C in the investigated population of this study may indicate that the individuals with CC homozygote at *Cox-2* G-765C were of some fetal defects related to this SNP which

lead to apoptosis of the cells or early lethality of the people. We propose that the C allele of *Cox-2* G-765C, via the differential sensitivity to the transcription factors, may influence the expression level of Cox-2 and associated with the carcinogenesis of childhood ALL. The supporting evidence comes from the study documented that COX-2 is responsible for many processes such as inflammatory, organ development, and carcinogenesis {Tsujii, 1997 #20}. Also, several studies have reported that COX-2 over-expression is important in mediating drug resistance to apoptosis in CLL {Secchiero, 2005 #21}. Pharmacological suppression of COX-2 might enhance the effect of chemotherapy-mediated apoptosis in lymphoma patients {Wun, 2004 #22}, and COX-2 over-expression in multiple myeloma is closed related to a poor survival rate {Ladetto, 2005 #23}. Therefore, our non-significant results still meaningfully suggested that in people who have a risky genetic variant, such as the C allele of G-765C, may increase their childhood ALL susceptibility.

To sum up, this is the first study which focuses on the SNPs of *Cox-2* and their joint effects on childhood ALL risk. We found that the presence of the C allele of G-765C may play a minor role, not as strong as *XRCC4* G-1394T which we previously reported {Wu #282}, in childhood ALL. Further investigations of multiple SNPs of other related genes, gene-gene interactions, and phenotypic assays of the childhood ALL-associated SNPs are needed in the future.

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References

1 Karathanasis NV, Choumerianou DM and Kalmanti M: Gene polymorphisms in childhood ALL. Pediatr Blood Cancer *52*: 318-323, 2009.

2 Schmiegelow K, Vestergaard T, Nielsen SM and Hjalgrim H: Etiology of common childhood acute lymphoblastic leukemia: the adrenal hypothesis. Leukemia *22*: 2137-2141, 2008.

3 Rubnitz JE and Pui CH: Childhood Acute Lymphoblastic Leukemia. Oncologist *2*: 374-380, 1997.

4 Liu CY, Hsu YH, Pan PC, Wu MT, Ho CK, Su L, Xu X, Li Y and Christiani DC: Maternal and offspring genetic variants of AKR1C3 and the risk of childhood leukemia. Carcinogenesis *29*: 984-990, 2008.

5 Kawamata N, Ogawa S, Zimmermann M, Kato M, Sanada M, Hemminki K, Yamatomo G, Nannya Y, Koehler R, Flohr T, Miller CW, Harbott J, Ludwig WD, Stanulla M, Schrappe M, Bartram CR and Koeffler HP: Molecular allelokaryotyping of pediatric acute lymphoblastic leukemias by high-resolution single nucleotide polymorphism oligonucleotide genomic microarray. Blood *111*: 776-784, 2008.

6 Armstrong SA and Look AT: Molecular genetics of acute lymphoblastic leukemia. J Clin Oncol *23*: 6306-6315, 2005.

7 Patterson D: Molecular genetic analysis of Down syndrome. Hum Genet *126*: 195-214, 2009.

8 Pui CH and Evans WE: Treatment of acute lymphoblastic leukemia. N Engl J Med *354*: 166-178, 2006.

9 Pui CH, Relling MV and Downing JR: Acute lymphoblastic leukemia. N Engl J Med *350*: 1535-1548, 2004.

10 Chokkalingam AP and Buffler PA: Genetic susceptibility to childhood leukaemia. Radiat Prot Dosimetry *132*: 119-129, 2008.

11 Kim AS, Eastmond DA and Preston RJ: Childhood acute lymphocytic leukemia and perspectives on risk assessment of early-life stage exposures. Mutat Res *613*: 138-160, 2006.

12 Sinnett D, Labuda D and Krajinovic M: Challenges identifying genetic determinants of pediatric cancers--the childhood leukemia experience. Fam Cancer *5*: 35-47, 2006.

13 Wu KH, Wang CH, Yang YL, Peng CT, Lin WD, Tsai FJ, Lin DT and Bau DT: Significant association of XRCC4 single nucleotide polymorphisms with childhood leukemia in Taiwan. Anticancer Res *30*: 529-533.

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

15 Nishihara H, Kizaka-Kondoh S, Insel PA and Eckmann L: Inhibition of apoptosis in normal and transformed intestinal epithelial cells by cAMP through induction of inhibitor of apoptosis protein (IAP)-2. Proc Natl Acad Sci U S A *100*: 8921-8926, 2003.

16 Gumgumji AA, Dawood T and Parvez T: Role of COX-2 specific inhibitors in oncogenesis. J Coll Physicians Surg Pak *13*: 361-365, 2003.

17 Davies G, Salter J, Hills M, Martin LA, Sacks N and Dowsett M: Correlation between cyclooxygenase-2 expression and angiogenesis in human breast cancer. Clin Cancer Res *9*: 2651-2656, 2003.

18 Sanchez-Alcazar JA, Bradbury DA, Pang L and Knox AJ: Cyclooxygenase (COX) inhibitors induce apoptosis in non-small cell lung cancer through cyclooxygenase independent pathways. Lung Cancer *40*: 33-44, 2003.

19 Levitt RJ and Pollak M: Insulin-like growth factor-I antagonizes the antiproliferative effects of cyclooxygenase-2 inhibitors on BxPC-3 pancreatic cancer cells. Cancer Res *62*: 7372-7376, 2002.

20 Mizutani Y, Kamoi K, Ukimura O, Kawauchi A and Miki T: Synergistic cytotoxicity and apoptosis of JTE-522, a selective cyclooxygenase-2 inhibitor, and 5-fluorouracil against bladder cancer. J Urol *168*: 2650-2654, 2002.

21 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.

22 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.

23 Giles FJ, Kantarjian HM, Bekele BN, Cortes JE, Faderl S, Thomas DA, Manshouri T, Rogers A, Keating MJ, Talpaz M, O'Brien S and Albitar M: Bone marrow cyclooxygenase-2 levels are elevated in chronic-phase chronic myeloid leukaemia and are associated with reduced survival. Br J Haematol *119*: 38-45, 2002.

24 Chang CH, Chang CL, Tsai CW, Wu HC, Chiu CF, Wang RF, Liu CS, Lin CC and Bau DT: Significant association of an XRCC4 single nucleotide polymorphism with bladder cancer susceptibility in Taiwan. Anticancer Res *29*: 1777-1782, 2009.

25 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.

26 Chiu CF, Tsai MH, Tseng HC, Wang CL, Wang CH, Wu CN, Lin CC and Bau DT: A novel single nucleotide polymorphism in XRCC4 gene is associated with oral cancer susceptibility in Taiwanese patients. Oral Oncol *44*: 898-902, 2008.

27 Chiu CF, Wang CH, Wang CL, Lin CC, Hsu NY, Weng JR and Bau DT: A novel single nucleotide polymorphism in XRCC4 gene is associated with gastric cancer susceptibility in Taiwan. Ann Surg Oncol *15*: 514-518, 2008.

28 Chiu CF, Wang HC, Wang CH, Wang CL, Lin CC, Shen CY, Chiang SY and Bau DT: A new single nucleotide polymorphism in XRCC4 gene is associated with breast cancer susceptibility in Taiwanese patients. Anticancer Res *28*: 267-270, 2008.

29 Hsu CF, Tseng HC, Chiu CF, Liang SY, Tsai CW, Tsai MH and Bau DT: Association between DNA double strand break gene Ku80 polymorphisms and oral cancer susceptibility. Oral Oncol *45*: 789-793, 2009.

30 Hsu NY, Wang HC, Wang CH, Chiu CF, Tseng HC, Liang SY, Tsai CW, Lin CC and Bau DT: Lung cancer susceptibility and genetic polymorphisms of Exo1 gene in Taiwan. Anticancer Res *29*: 725-730, 2009.

31 Yang MD, Hsu YM, Kuo YS, Chen HS, Chang CL, Wu CN, Chang CH, Liao YM, Wang HC, Wang MF and Bau DT: Significant association of Ku80 single nucleotide polymorphisms with colorectal cancer susceptibility in Central Taiwan. Anticancer Res *29*: 2239-2242, 2009.

32 Tsujii M, Kawano S and DuBois RN: Cyclooxygenase-2 expression in human colon cancer cells increases metastatic potential. Proc Natl Acad Sci U S A *94*: 3336-3340, 1997.

33 Secchiero P, Barbarotto E, Gonelli A, Tiribelli M, Zerbinati C, Celeghini C, Agostinelli C, Pileri SA and Zauli G: Potential pathogenetic implications of cyclooxygenase-2 overexpression in B chronic lymphoid leukemia cells. Am J Pathol *167*: 1599-1607, 2005.

34 Wun T, McKnight H and Tuscano JM: Increased cyclooxygenase-2 (COX-2): a potential role in the pathogenesis of lymphoma. Leuk Res *28*: 179-190, 2004.

35 Ladetto M, Vallet S, Trojan A, Dell'Aquila M, Monitillo L, Rosato R, Santo L, Drandi D, Bertola A, Falco P, Cavallo F, Ricca I, De Marco F, Mantoan B, Bode-Lesniewska B, Pagliano G, Francese R, Rocci A, Astolfi M, Compagno M, Mariani S, Godio L, Marino L, Ruggeri M, Omede P, Palumbo A and Boccadoro M: Cyclooxygenase-2 (COX-2) is frequently expressed in multiple myeloma and is an independent predictor of poor outcome. Blood *105*: 4784-4791, 2005.

Table I. The primer sequences, polymerase chain reaction and restriction fragment length polymorphism (PCR-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 266 childhood ALL patients and 266 controls

Characteristic	Controls (n = 266)			Patients (n = 266)			*p-value*[a]
	n	%	Mean (SD)	n	%	Mean (SD)	
Age (years)			8.3 (4.8)			7.0 (4.4)	0.64
Gender							1.00
Male	148	55.6%		148	55.6%		
Female	118	44.4%		118	44.4%		

[a] Based on chi-square test.

Table III. Distribution of *Cox-2* genotypes among the childhood leukemia patient and control groups.

Genotype	Controls	%	Patients	%	*p-value*[a]
A-1195G (rs689466)					0.9793
AA	74	27.8%	75	28.1%	
AG	127	47.7%	128	48.1%	
GG	65	24.4%	63	23.7%	
G-765C (rs20417)					0.0684
GG	234	88.0%	218	82.0%	
GC	32	12.0%	48	18.0%	
CC	0	0%	0	0%	
T+8473C (rs5275)					0.7834
TT	178	66.9%	174	65.4%	
TC	88	33.1%	92	34.6%	
CC	0	0%	0	0%	
intron 1 (rs2745557)					0.7575
GG	197	74.1%	204	76.7%	
AG	65	24.4%	59	22.2%	
AA	4	1.5%	3	1.1%	
intron 5 (rs16825748)					1.0000
TT	260	97.7%	261	98.1%	
AT	6	2.3%	5	1.9%	
AA	0	0%	0	0%	
intron 6 (rs2066826)					0.6351
GG	221	83.1%	214	80.5%	
AG	39	14.6%	43	16.1%	
AA	6	2.3%	9	3.4%	

[a] Based on chi-square test.

Table IV. *Cox-2* allelic frequencies among the childhood leukemia patient and control groups.

Allele	Controls	%	Patients	%	*p-value*[a]
A-1195G (rs689466)					0.8539
Allele A	275	51.7%	278	52.3%	
Allele G	257	48.3%	254	47.7%	
G-765C (rs20417)					0.0629
Allele G	500	94.0%	484	91.0%	
Allele C	32	6.0%	48	9.0%	
T+8473C (rs5275)					0.7436
Allele T	444	83.5%	440	82.7%	
Allele C	88	16.5%	92	17.3%	
intron 1 (rs2745557)					0.4654
Allele G	459	86.3%	467	87.8%	
Allele A	73	13.7%	65	12.2%	
intron 5 (rs16825748)					0.7618
Allele T	526	98.9%	527	99.1%	
Allele A	6	1.1%	5	0.9%	
intron 6 (rs2066826)					0.3178
Allele G	481	90.4%	471	88.5%	
Allele A	51	9.6%	61	11.5%	

[a] Based on chi-square test.

Table IV. Frequencies of combined *Cox-2* G-765C and intron 6 genotype polymorphisms among the childhood leukemia and control groups.

Cox-2 G-765C /intron 6 genotype	Control		Patients		OR (95% CI)	*P*-value[a]
	n	%	n	%		
All	266	100.0	266	100.0		
GG/GG	195	73.3	175	65.8	1.00	
GG/AG+AA	39	14.7	43	16.2	1.23 (0.76-1.98)	0.4639
GC/GG	26	9.8	39	14.6	1.67 (0.97-2.86)	0.0612
GC/ AG+AA	6	2.2	9	3.4	1.67 (0.58-4.79)	0.4315

[a] Based on Fisher's exact test. OR, Odds ratio; CI, Confidence interval.