

Association Study of Cyclooxygenase 2 Single Nucleotide Polymorphisms and Childhood Acute Lymphoblastic Leukemia in Taiwan

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Abstract. Aim: The relationship between COX-2 gene and childhood leukemia risk is ambiguous. In this study, the association of genotypic polymorphisms in cyclooxygenase 2 (Cox-2) with childhood leukemia were investigated. Materials and Methods: A total of 266 patients with childhood leukemia and 266 healthy controls recruited from the China Medical Hospital in central Taiwan were genotyped by PCR-RFLP method. Six polymorphic variants of Cox-2 were investigated, including G-1195A, G-765C, T+8473C, intron 1, intron 5, and intron 6, and the associations of specific genotypes with susceptibility to childhood leukemia were analysed. Results: The data showed that although there was no difference in the distribution for each genotype of Cox-2 G-1195A, G-765C, T+8473C, intron 1, intron 5, and intron 6, between the childhood leukemia and control groups ($p>0.05$), the analysis of combined 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: These 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.

Acute lymphoblastic leukemia (ALL) is the most common cancer in childhood, accounting for 30% of the childhood

malignancies (1). The aetiology of childhood ALL is mostly unknown. Infections in the first years of life and some environmental factors such as ionizing radiation and parental alcohol and tobacco use may play a causative role in ALL (2-4). However, the contributing genomic 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 (5-9). 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, MDRI, cyclin D1, CCND1, and XRCC4 with childhood ALL risk (1, 10-13). It is commonly agreed that single environmental or genetic factors can only partly explain a small number of cases that develop child ALL.

Cyclooxygenases (COXs, 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 (14). There are two forms of human COXs: COX-1 and COX-2. It was reported that COX-2 overexpression may contribute to carcinogenesis via its regulation on apoptosis, immunosurveillance, angiogenesis, and also xenobiotic metabolism (15, 16). 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 (17-20). 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 (21, 22).

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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 (rs689466)	F: CCCTGAGCACTACCCATGAT R: GCCCTTCATAGGAGATACTGG	<i>Hha I</i>	A G	273 220+53
G-765C (rs20417)	F: TATTATGAGGAGAATTTACCTTTTCGC R: GCTAAGTTGCTTTCAACAGAAGAAT	<i>PvuII</i>	C G	100 74+26
T+8473C (rs5275)	F: GTTTGAAATTTTAAAGTACTTTTGAT R: TTTCAAATTATTTGTTTCATTGC	<i>Bcl I</i>	T C	147 124+23
intron 1 (rs2745557)	F: GAGGTGAGAGTGTCTCAGAT R: CTCTCGGTTAGCGACCAATT	<i>Taq I</i>	G A	439 353+76
intron 5 (rs16825748)	F: GCGGCATAATCATGGTACAA R: CAGCACTTCACGCATCAGTT	<i>BsrG I</i>	T A	417 314+103
intron 6 (rs2066826)	F: ACTCTGGCTAGACAGCGTAA R: GCCAGATTGTGGCATACATC	<i>Aci I</i>	A G	327 233+94

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

Although COX-2 overexpression and COX-2 inhibitor drugs have been studied extensively in cancer, there were very few studies reporting the effects of COX-2 inhibition in haematologic malignancies, not to mention childhood ALL. In 2002, it was reported that COX-2 overexpression was frequent in patients with chronic myelocytic leukemia (CML) and also found to be associated with shorter survival (23). The present work is motivated by the biological possibility 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 investigate the hypothesis that the SNP variants of COX-2 are associated with the risk of childhood ALL, 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 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 (all under 18 years old) were recruited at the Pediatric Departments at the China Medical University Hospital and National Taiwan University Hospital, Taiwan, between 2005-2009. The average and medium ages of the controls were 6.8 and 4.6 years, respectively. Each patient and healthy subject (matched by gender and age after initial random sampling from the Health Examination Cohort of the two hospitals) completed a self-completed 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 (24-31). The polymerase chain reaction (PCR) cycling conditions were:

one cycle at 94°C for 5 min; 35 cycles of 94°C for 30s, 55°C for 30s, and 72°C for 30s, 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 analysis. Only those individuals with both genotypic and clinical data (controls/cases=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 deemed to be 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. The characteristics of patients and controls were all well matched. None of the differences between the 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 childhood ALL ($p>0.05$).

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

Table II. Characteristics of 266 childhood ALL patients and 266 controls.

Characteristic	Controls (n=266)			Patients (n=266)			<i>p</i> -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%		

^aBased on chi-square test.

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

Genotype	Controls	%	Patients	%	<i>p</i> -Value ^a
A-1195G (rs689466)					0.9793
AA	74	27.8%	75	28.2%	
AG	127	47.7%	128	48.1%	
GG	65	24.5%	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%	

^aBased on chi-square test.

here the results of G-765C and intron genotypes only are 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.

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

Allele	Controls	%	Patients	%	<i>p</i> -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%	

^aBased on chi-square test.

Discussion

In order to elucidate the role of COX-2 and to find potential biomarkers of childhood ALL, in this study, six SNPs of the COX-2 gene were selected and their associations with the susceptibility for childhood ALL was investigated in a population in northern and central Taiwan. It was found that 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 aetiology of childhood ALL, which is an outcome of complex genetic and environmental interactions. Among the SNPs that were analyzed, G-765C (rs20417) was found to be slightly associated with childhood ALL ($p=0.06$), although not statistically significant. The genotypic distribution of GC

Table V. 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

^aBased on Fisher's exact test. OR, Odds ratio; CI, confidence interval.

heterozygotes of G-765C was higher in the childhood ALL group (18%) than the control group (12%) (Table III). The lack of CC homozygote of G-765C in the investigated population of this study may indicate that CC homozygotes bore some fetal defects related to this SNP which lead to apoptosis of the cells or early lethality of the patients. We propose that the C allele of *COX-2* G-765C, via the differential sensitivity to transcription factors, may influence the expression level of COX-2 and be associated with the carcinogenesis of childhood ALL. The supporting evidence comes from a previous study that documented that COX-2 is responsible for many processes such as inflammation, organ development, and carcinogenesis (32). Several studies have also reported that COX-2 overexpression is important in mediating drug resistance to apoptosis in CLL (33, 34). Pharmacological suppression of COX-2 might enhance the effect of chemotherapy-mediated apoptosis in lymphoma patients (35), and COX-2 overexpression in multiple myeloma is closely related to a poor survival rate (36). Therefore, the current non-significant results still meaningfully suggest that individuals who have a risky genetic variant, such as these with the C allele of G-765C, may have an increased susceptibility to childhood ALL.

In conclusion, this is the first study which focuses on the SNPs of *COX-2* and their joint effects on childhood ALL risk. It was found that the presence of the C allele of G-765C may play a minor role, not as strong as *XRCC4* G-1394T which these authors previously reported (13), 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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