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Significant Association of Interleukin-10 Polymorphisms with Childhood Leukemia Susceptibility in Taiwan

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Abstract. *Mounting evidence supports the notion that inflammatory processes play a role in carcinogenesis, and interleukin-10 (IL10) is an important inflammatory cytokine. This study aimed to evaluate the contribution of IL10 A-1082G (rs1800896), T-819C (rs3021097) and A-592C (rs1800872) genotypes to the risk of childhood acute lymphoblastic leukemia (ALL) in Taiwan. Associations of these IL10 polymorphic genotypes with ALL risk were analyzed in 266 patients with childhood ALL patients and 266 non-cancer healthy controls by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) methodology. The results showed that CC genotype carriers at IL10 T-819C were at lower risk for childhood ALL (odds ratio=0.33, 95% confidence interval=0.16-0.68). On the contrary, AC and CC genotype carriers at IL10 A-592C were at higher risk for childhood ALL (odds ratio=1.73 and 6.34, 95% confidence interval=1.19-2.51 and 3.16-12.72, respectively). There was no difference in the distribution of A-1082G genotypes between childhood ALL and control groups. The genotypes at IL10 T-819C and A-592C may serve as predictive biomarkers for childhood ALL in Taiwan.*

Acute lymphoblastic leukemia (ALL), a type of pediatric leukemia, accounts for 25-30% of all childhood malignancies (1). Worldwide, the annual incidence of childhood ALL is approximately 10 cases per 100,000, with a peak incidence occurring at approximately 2 to 5 years of age (2). Although the pathological, immunophenotypic and diagnostic features of ALL are well documented, the genetic contribution to ALL has not been fully clarified (1). Accumulating evidence suggests that genetic factors may play a significant role in the development of childhood ALL. For instance, inherited genetic human diseases, such as Down syndrome and Fanconi anemia, have been associated with enhanced ALL risk (3, 4). Additionally, genetic mutations in several oncogenes and tumor-suppressor genes, such as p53, neuroblastoma RAS viral (v-ras) oncogene homolog (*NRAS*), and PHD finger protein 6 (*PHF6*), have frequently been identified in patients with ALL (5); and finally, only a small fraction of children who are exposed to ionic radiation developed ALL, indicating individual susceptibility.

Interleukin-10 (*IL10*), also known as human cytokine synthesis inhibitory factor, is produced mainly by monocytes and lymphocytes. The genetic polymorphisms found in the regulatory sites, especially the promoter region of *IL10* gene, are believed to affect the expression of IL10 protein and possibly be associated with leukemia susceptibility and prognosis. In literature, Yao *et al*. found the T allele of *IL10* T-819C was not only associated with higher risk of AML, but also synergistically acts with the A allele of *IL10* A-592C, contributing to enhanced expression of *IL10* at mRNA level among the patients (6). In 2015, Fei and colleagues confirmed that the genotypes of *IL10* T-819C and A-592C have synergistic effects on determining personal AML susceptibility (7). However, both reports investigated the contribution of *IL10* genotypes to adult AML, not ALL, and the investigated patients were all adults. In addition, the sample size of their cases were rather small (less than 200) and their findings need to be validated in other populations as well.

To validate whether *IL10* genotype is a risk factor for childhood leukemia, we sought to investigate the association

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Table I. *Demographic data for the 266 patients with childhood acute lymphoblastic leukemia and 266 controls investigated in this study.*

Characteristic	Controls (n=266)			Cases (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]*p*-Value based on Chi-square test.

Table II. *The primer sequences, polymerase chain reaction and restriction fragment length polymorphism conditions for identifying the interleukin-10 (IL10) A-1082G, T-819C and A-592C genotypes among the investigated individuals.*

Polymorphism (locations)	Primer sequences	Restriction enzyme	SNP sequence	DNA fragment size (bp)
A-1082G (rs1800896)	F: 5'-CTCGCTGCAACCCAACTGGC-3'	*Mnl* I	A	139
	R: 5'-TCTTACCTATCCCTACTTCC-3'		G	106 + 33
T-819C (rs3021097)	F: 5'-TCATTCTATGTGCTGGAGAT-3'	*Mae* III	T	209
	R: 5'-TGGGGGAAGTGGGTAAGAGT-3'		C	125 + 84
A-592C (rs1800872)	F: 5'-GGTGAGCACTACCTGACTAG-3'	*Rsa* I	C	412
	R: 5'-CCTAGGTCACAGTGACGTGG-3'		A	236 + 176

F and R indicate forward and reverse primers, respectively.

of polymorphisms in the promoter region of *IL10* A-1082G, T-819C and A-592C with childhood leukemia in a Taiwanese population.

Materials and Methods

Study population and sample collection. Two hundred and sixty-six patients diagnosed with childhood ALL (all under 18 years of age) were recruited during 2005-2010 from the General Surgery Outpatient Clinics within the Pediatric Departments at China Medical University Hospital and the National Taiwan University Hospital, Taiwan, Republic of China. All clinical characteristics of these individuals, including their histological details, were identified and recorded by expert surgeons. All participants completed a questionnaire with the assistance and approval of their parents and provided peripheral blood samples. An equal number of age-matched individuals without cancer were selected for use as a control group following initial random sampling from the Health Examination Cohort. Our study was approved by The Institutional Review Board of China Medical University Hospital, and all participants provided written informed consent. Selected characteristics of all the participants are provided in Table I.

Genotyping assays. Genomic DNA was prepared from peripheral blood leucocytes using a QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and further processed according to previous methods (8-10). Briefly, the polymerase chain reaction (PCR) cycling conditions for *IL10* genotyping included the following: 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, along with a final extension at 72˚C for 10 min as described in our previous publications (11-14). The success rate of PCR-restriction fragment length polymorphisms (RFLP) is 100%, and the genotypes of 5% of the participants in both the control and patient groups were analyzed by direct sequencing PCR (Genomics BioSci & Tech Co., Taipei, Taiwan) and the consistency was 100% between PCR-RFLP and direct sequencing. Pairs of PCR primer sequences and the restriction enzyme for each DNA product are all listed in Table II.

Statistical analyses. To ensure that the controls used were representative of the general population and to exclude the possibility of genotyping error, the deviation of the genotypic frequencies of *IL10* SNPs in the controls from those expected under the Hardy–Weinberg equilibrium was assessed using the goodness-of-fit test. All of them fit Hardy–Weinberg equilibrium ($p>0.05$). 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 *IL10* genotypes between cases and controls. Leukemia risk associated with the genotypes was estimated as odds ratio (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression. Data were recognized as significant when the statistical *p*-value was less than 0.05.

Results

The distributions of age and gender among the patients with ALL and the controls are shown in Table I. The age and gender of the patients and controls were all well-matched and none of the differences between the groups was statistically significant ($p>0.05$) (Table I).

Table III. *Distribution of interleukin-10 (IL10) genotypes among the 266 patients with childhood acute lymphoblastic leukemia and 266 controls investigated in this study.*

Genotype	Controls		Cases		OR (95% CI)	*p*-Value[a]
	n	%	n	%		
A-1082G						
AA	202	75.9%	205	77.1%	1.00 (reference)	
AG	52	19.6%	50	18.8%	0.95 (0.61-1.46)	0.8943
GG	12	4.5%	11	4.1%	0.90 (0.39-2.09)	0.9817
AG+GG	64	24.1%	61	22.9%	0.94 (0.63-1.40)	0.8379
p_{trend}						0.9489
T-819C						
TT	142	53.4%	170	63.9%	1.00 (reference)	
TC	96	36.1%	85	32.0%	0.76 (0.52-1.09)	0.1289
CC	28	10.5%	11	4.1%	0.33 (0.16-0.68)	0.0034*
TC+CC	124	46.6%	96	36.1%	0.65 (0.46-0.92)	0.0175*
p_{trend}						5.01×10^{-3}*
A-592C						
AA	170	63.9%	117	44.0%	1.00 (reference)	
AC	85	32.0%	101	38.0%	1.73 (1.19-2.51)	0.0053*
CC	11	4.1%	48	18.0%	6.34 (3.16-12.72)	0.0001*
AC+CC	96	36.1%	149	56.0%	1.49 (1.07-2.08)	0.0001*
p_{trend}						3.45×10^{-8}*

OR: Odds ratio; CI: confidence interval. [a]Based on Chi-square test with Yate's correction; *$p<0.05$.

The genotypic frequencies of *IL10* promoter A-1082G (rs1800896), T-819C (rs3021097) and A-592C (rs1800872) polymorphisms among the patients and the controls are shown in Table III. Using wild-type -819T as the reference group, there was an obvious association between homozygosity for -819C of *IL10* and childhood ALL risk (OR=0.33, $p=0.0034$). Combination of the homozygotes and heterozygotes for the C allele (TC + CC) showed that the C allele at *IL10* T-819C conferred a 0.65-fold risk for childhood ALL ($p=0.0175$) (Table III). Using wild-type -592A as the reference group, heterozygosity and homozygosity of -592C for *IL10* conferred an increased risk of childhood ALL (OR=1.73 and 6.34, $p=0.0053$ and 0.0001, respectively). Combination of the homozygotes and heterozygotes for the C allele (AC + CC) showed that the C allele at *IL10* T-592C conferred an increased risk for childhood ALL of 1.49-fold ($p=0.0001$) (Table III). On the contrary, neither hetero- nor homozygosity for -1082G of *IL10* seemed to be risky for childhood ALL (Table III). Overall, among the three *IL10* polymorphisms investigated, -819C of *IL10* seems to be protective, while -592C seems to be a genotypic marker indicating increased risk for childhood ALL.

The distributions of allelic frequencies for *IL10* promoter A-1082G (rs1800896), T-819C (rs3021097) and A-592C (rs1800872) polymorphisms among the patients and the controls are shown in Table IV. Consistent with the findings in Table III, C alleles at *IL10* T-819C and A-592C were significantly associated with childhood ALL risk ($p=0.0013$ and 1.01×10^{-9}, respectively). In contrast, allele A or G at *IL10* A-1082G were not differently distributed between the childhood ALL and control groups ($p>0.05$).

Discussion

IL10, which is produced by activated T-cells, monocytes, B-cells and thymocytes, was found to be an important immunoregulatory cytokine playing a modulating role in activating and suppressing immunoresponses (15). IL10 not only regulates the differentiation and proliferation of several types of immune cells, but has both tumor-promoting and tumor-inhibiting properties, and thus may play a critical role in tumor development and metastasis (16). Polymorphisms in *IL10*, especially those in the promoter region, can alter the function of this cytokine, altering the downstream signaling and cellular behaviors, and greatly regulate the development of human disorders. In the literature, the genotype at *IL10* A-1082G was associated with osteosarcoma (17), lymphoma (18), gastric cancer (13, 19, 20), oral cancer (14), nasopharyngeal carcinoma (21) and papillary thyroid cancer (22). In addition, the genotype at *IL10* A-1082G has been implicated in development of chronic lymphoid leukemia among Russians, especially in the late stages (23). For *IL10* T-819C, genotype was associated with lung cancer (12, 24), gastric cancer (25), and adult leukemia (6, 7), while

Table IV. *Allelic frequencies for interleukin-10 (IL10) polymorphisms in childhood acute lymphoblastic leukemia and control groups investigated in this study.*

Polymorphic site Allele	Controls, n (%) N=532	Cases, n (%) N=532	OR (95% CI)	*p*-Value[a]
A-1082G				
Allele A	456 (85.7)	460 (86.5)	1.00 (reference)	0.7231
Allele G	76 (14.3)	72 (13.5)	0.94 (0.66-1.33)	
T-819C				
Allele T	380 (71.4)	425 (79.9)	1.00 (reference)	0.0013*
Allele C	152 (28.6)	107 (20.1)	0.63 (0.47-0.84)	
A-592C				
Allele A	425 (79.9)	335 (63.0)	1.00 (reference)	1.01×10^{-9}*
Allele C	107 (20.1)	197 (37.0)	2.34 (1.77-3.08)	

OR: Odds ratio; CI: confidence interval; [a]*p*-Value based on Chi-square test with Yate's correction; *$p<0.05$.

genotype for *IL10* A-592C was associated with lung (24), gastric (26, 27) and esophageal (28) cancer, and adult leukemia (6). Little is known about the contribution of *IL10* genotypes to childhood leukemia.

The present study investigated the role of genotypes of *IL10* gene, a gene which has not been reported to be associated with childhood leukemia risk. Among the three *IL10* promoter polymorphisms examined in this study, T-819C (rs3021097) and A-592C (rs1800872), but not A-1082G (rs1800896), were found to be associated with childhood ALL (Tables III and IV). This is the first study to examine the contribution of *IL10* genotypes to childhood cancer. The sample size of the patient group is relatively large among childhood cancer studies, and concise data were analyzed without any modification, strengthening the accuracy and reliability of the current study.

Similarly among adults in a Han population, *IL10* T-819C and A-592C genotypes were found to be associated with adult AML (6, 7). However, there are many differences between ALL and AML, and more differences in the development for childhood and adult leukemia. From the molecular perspective, polymorphisms at *IL10* T-819C and A-592C may determine subtle differences in the capacity of IL10 to alter the functions of IL10 itself and its downstream signaling and cellular behaviors, contributing to individual susceptibilities to ALL and AML among children and adults. It was reported that the T allele of *IL10* T-819C was not only associated with higher risk of AML, but also synergistically acts with A allele of *IL10* A-592C, contributing to enhanced expression of *IL10* at the mRNA level among patients (7). In the future, the joint effects of *IL10* genotypes with other genes need to be further investigated in other populations.

In conclusion, to our knowledge, this is the first report to investigate the association between *IL10* gene polymorphisms and childhood leukemia. Our findings suggest that genotypes at *IL10* promoter T-819C (rs3021097) and A-592C (rs1800872) are potentially predictive of risk for childhood leukemia.

Conflict of Interest

The Authors declare no conflicts of interest.

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References

1 Parkin DM: International variation. Oncogene *23*: 6329-6340, 2004.
2 Franekova M, Halasova E, Bukovska E, Luptak J and Dobrota D: Gene polymorphisms in bladder cancer. Urol Oncol *26*: 1-8, 2008.
3 Cohen SM, Shirai T and Steineck G: Epidemiology and etiology of premalignant and malignant urothelial changes. Scand J Urol Nephrol Suppl: 105-115, 2000.
4 Pryor WA, Hales BJ, Premovic PI and Church DF: The radicals in cigarette tar: their nature and suggested physiological implications. Science *220*: 425-427, 1983.
5 Hung RJ, Hall J, Brennan P and Boffetta P: Genetic polymorphisms in the base excision repair pathway and cancer risk: a HuGE review. Am J Epidemiol *162*: 925-942, 2005.
6 Yao CJ, Du W, Chen HB, Xiao S, Wang CH and Fan ZL: Associations of *IL10* gene polymorphisms with acute myeloid leukemia in Hunan, China. Asian Pac J Cancer Prev *14*: 2439-2442, 2013.
7 Fei C, Yao XM, Sun Y, Gu XZ, Yu LQ and Lai X: Interleukin-10 polymorphisms associated with susceptibility to acute myeloid leukemia. Genet Mol Res *14*: 925-930, 2015.

8 Su CH, Chang WS, Hu PS, Hsiao CL, Ji HX, Liao CH, Yueh TC, Chuang CL, Tsai CW, Hsu CM, Lane HY and Bau DT: Contribution of DNA double-strand break repair gene XRCC3 genotypes to triple-negative breast cancer risk. Cancer Genomics Proteomics *12*: 359-367, 2015.
9 Chang WS, Wang SC, Chuang CL, Ji HX, Hsiao CL, Hsu CM, Tsai CW, Liu SP, Hsu PC, Lo YL and Bau DT: Contribution of interleukin-4 genotypes to lung cancer risk in Taiwan. Anticancer Res *35*: 6297-6301, 2015.
10 Chang WS, Tsai CW, Wang JY, Ying TH, Hsiao TS, Chuang CL, Yueh TC, Liao CH, Hsu CM, Liu SP, Gong CL, Tsai CH and Bau DT: Contribution of X-Ray repair complementing defective repair in Chinese Hamster Cells 3 (XRCC3) genotype to leiomyoma risk. Anticancer Res *35*: 4691-4696, 2015.
11 Hsia TC, Chang WS, Wang S, Shen TC, Hsiao WY, Liu CJ, Liang SJ, Chen WC, Tu CY, Tsai CW, Hsu CM and Bau DT: The Contribution of interleukin-10 promoter genotypes to susceptibility to asthma in adults. In Vivo *29*: 695-699, 2015.
12 Hsia TC, Chang WS, Liang SJ, Chen WC, Tu CY, Chen HJ, Yang MD, Tsai CW, Hsu CM, Tsai CH and Bau DT: Interleukin-10 (*IL10*) promoter genotypes are associated with lung cancer risk in Taiwan males and smokers. Anticancer Res *34*: 7039-7044, 2014.
13 Kuo WH, Huang CY, Fu CK, Hsieh YH, Liao CH, Hsu CM, Huang YK, Tsai CW, Chang WS and Bau DT: Effects of interleukin-10 polymorphisms and smoking on the risk of gastric cancer in Taiwan. In Vivo *28*: 967-971, 2014.
14 Tsai CW, Chang WS, Lin KC, Shih LC, Tsai MH, Hsiao CL, Yang MD, Lin CC and Bau DT: Significant association of interleukin-10 genotypes and oral cancer susceptibility in Taiwan. Anticancer Res *34*: 3731-3737, 2014.
15 Helminen M, Lahdenpohja N and Hurme M: Polymorphism of the interleukin-10 gene is associated with susceptibility to Epstein-Barr virus infection. J Infect Dis 180: 496-499, 1999.
16 Couper KN, Blount DG and Riley EM: IL10: the master regulator of immunity to infection. J Immunol *180*: 5771-5777, 2008.
17 Cui Y, Zhu JJ, Ma CB, Cui K, Wang F, Ni SH and Zhang ZY: Interleukin 10 gene -1082A/G polymorphism is associated with osteosarcoma risk and poor outcomes in the Chinese population. Tumour Biol, 2015.
18 Yu X, Chen B, Cheng J, Gao C, Zhang X and Bao W: The interleukin-10-1082A>G polymorphism and lymphoma risk: a meta-analysis. Cancer Biomark *14*: 381-388, 2014.
19 Li C, Tong W, Liu B, Zhang A and Li F: The -1082A>G polymorphism in promoter region of interleukin-10 and risk of digestive cancer: a meta-analysis. Sci Rep *4*: 5335, 2014.
20 Ni P, Xu H, Xue H, Lin B and Lu Y: A meta-analysis of interleukin-10 -1082 promoter polymorphism associated with gastric cancer risk. DNA Cell Biol *31*: 582-591, 2012.
21 Tsai CW, Tsai MH, Shih LC, Chang WS, Lin CC and Bau DT: Association of interleukin-10 (*IL10*) promoter genotypes with nasopharyngeal carcinoma risk in Taiwan. Anticancer Res *33*: 3391-3396, 2013.
22 Cil E, Kumral A, Kanmaz-Ozer M, Vural P, Dogru-Abbasoglu S, Altuntas Y and Uysal M: Interleukin-10 -1082 gene polymorphism is associated with papillary thyroid cancer. Mol Biol Rep *41*: 3091-3097, 2014.
23 Ovsepyan VA, Gabdulkhakova A, Shubenkiva AA and Zotina EN: Role of interleukin-10 gene promoter region polymorphism in the development of chronic lymphoid leukemia. Bull Exp Biol Med *160*: 275-277, 2015.
24 Lan X, Lan T and Faxiang Q: Interleukin-10 promoter polymorphism and susceptibility to lung cancer: a systematic review and meta-analysis. Int J Clin Exp Med *8*: 15317-15328, 2015.
25 Xue H, Lin B, An J, Zhu Y and Huang G: Interleukin-10 -819 promoter polymorphism in association with gastric cancer risk. BMC Cancer *12*: 102, 2012.
26 Qi M, Liu DM, Pan LL and Lin YX: Interleukin-10 gene -592C>A polymorphism and susceptibility to gastric cancer. Genet Mol Res *13*: 8954-8961, 2014.
27 Xue H, Wang YC, Lin B, An J, Chen L, Chen J and Fang JY: A meta-analysis of interleukin-10 -592 promoter polymorphism associated with gastric cancer risk. PLoS One *7*: e39868, 2012.
28 Sun JM, Li Q, Gu HY, Chen YJ, Wei JS, Zhu Q and Chen L: Interleukin 10 rs1800872 T>G polymorphism was associated with an increased risk of esophageal cancer in a Chinese population. Asian Pac J Cancer Prev *14*: 3443-3447, 2013.

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