의학석사 학위논문

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박철범의 의학석사 학위논문을 인준함

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Association between *CASP7* and *CASP14* genetic polymorphisms and the risk of childhood leukemia

by

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A Thesis Submitted to the Department of Biomedical Sciences in Partial Fulfillment of the Requirements for the Degree of Master of Biomedical Sciences at the Seoul National University College of Medicine

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Abstract

Leukemia is the most common childhood cancer, which is the major cause of morbidity and mortality among pediatric cancers in Korea as well as worldwide. Genetic variation related to apoptosis and cell cycle system may increase the risk of childhood leukemia.

To identify susceptible genetic biomarkers of apoptosis and cell cycle mechanisms for childhood leukemia, a hospital based case-control study has been performed including 136 childhood leukemia cases and 254 controls matched on sex and 5-year interval age. After quality control of biospecimen, a total of 63 patients and 148 controls were included in this study. 304 SNPs in 31 gene regions were selected according to CGEMS, CGAP, SNP500 database and the International HapMap Project. Genotyping was performed using an Illumina GoldenGate oligonucleotide pool assay (OPA) panel. Genetic factors associated with childhood leukemia were assessed by both an additive model and dominant model using unconditional logistic regression models adjusting for age and birth weight. The minimum *P*-value (*minP*) test and the false discovery rate (FDR) test were used to evaluate statistically significant association at gene level and to minimize the false positive rate.

Both SNP and gene-based analyses presented associations with the risk of childhood leukemia for 5 genes: *CASP7, CASP14, CASP8AP2, MYC, and RIPK1* ($P_{trend} < 0.05$ in additive models). Furthermore, in the gene level test two genes represented statistically significant associations: *CASP7* (rs12416109 and rs3814231, $P_{trend} = 0.002$ and 0.009, respectively, *minP* = 0.013, FDR = 0.042) and *CASP14* (rs8110862, $P_{trend} < 0.001$, *minP* = 0.002, FDR = 0.027). In dominant model *CASP7* rs12416109 represented increase risk of childhood leukemia (AG+GG vs AA; OR=4.30, 95% CI 1.70-10.87). On the other hand, *CASP7* rs3814231 and *CASP14* rs8110862 represented decrease risk of childhood leukemia (CT+TT vs CC; OR=0.46, 95% CI 0.24-0.87 and AC+CC vs AA; OR=0.34, 95% CI 0.18-0.63, respectively). When stratified by subtype groups (ALL and AML), *CASP14* was still statistically significant in each subtype.

This study suggests that genetic polymorphisms in apoptosis and cell cycle related genes might play a role in childhood leukemia development.

Keywords: Childhood leukemia, genetic variation, apoptosis, *CASP7* and *CASP14*

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Introduction

Cancer develops by several biological capabilities. The hallmark of cancer comprise sustaining proliferative signaling, resisting cell death, evading growth suppressors, enabling replicative immortality, inducing angiogenesis and activating invasion and metastasis [1]. Leukemia is a type of cancer which develops in the bone marrow which forms abnormal blood cells. These unusual cells produce immature blood cells and accumulate in the blood as well as of the body. The proportion of normal blood cell decrease and the immune system will not function normally.

Korean Statistical Information Service (KOSIS) 2009 annual report found that the first rank in cause of death among children is ‘injury and poisoning’ in Korea. The second, childhood cancer composes the high proportion in cause of death for childhood. Incidence of childhood cancer was total 1137 cases in Korea in 2009. From them childhood leukemia was 377 (37.16%) cases which compose first rank in childhood cancer. This is consistent with SEER cancer incidence rate. According to KOSIS data in Korea, 5 year survival of childhood leukemia have improved since 1993 (Figure 1).

Despite great improvements in survival, mortality of childhood leukemia is

maintained steady (Figure 2). Childhood leukemia is the most common cancer and is the major cause of morbidity and mortality in pediatric cancer [2].

Although exposure to ionizing radiation, non-ionizing electromagnetic fields, hydrocarbon, infectious diseases, chemotherapy, birth weight and chromosomal abnormalities were claimed as the possible etiological risk factors in childhood leukemia, the etiology of childhood leukemia is fully unknown [2-8]. Recently not only environmental risk factors but also genetic susceptible risk factors are studied in several biochemical and genetic mechanisms (Table 1). Since 1999, there have been 37 case-control studies that analyzed the association between genetic polymorphisms and childhood leukemia. Those studies analyzed 49 genes in xenobiotics metabolism, DNA repair, apoptosis, cell cycle and one carbon metabolisms. Most of the results were non-significant or controversial but the gene *MTHFR* which was related to one carbon metabolism represented significant result in most studies (Table 1).

Apoptosis and cell cycle are some of the biochemical and genetic mechanisms contributing to cancer susceptibility. Apoptosis plays a pivotal role in the elimination of DNA damaged cells. Some subsets of damaged cells may escape the DNA repair system, and abort apoptosis causing DNA rearrangement and further chromothripsis [9]. The association between the genetic variation related to apoptosis and the risk of cancer has been described in different human

cancers, such as the bladder, stomach, renal, and prostate cancers and non-Hodgkin lymphoma [10-14]. In cell cycle the tumor suppressor protein, p53, and the cyclin dependent kinase inhibitor, p21, plays a central role in regulating cell damage signals. It regulates the G1-phase and induces growth arrest of the cell cycle by binding to cyclinD-CDK2/4 complexes [15].

In this study, we hypothesized that genetic variation in apoptosis and cell cycle related genes might be associated with the carcinogenesis of childhood leukemia. Here, we report the analysis of genetic polymorphisms for 304 SNPs in 31 genes related to apoptosis and cell cycle in a Korean hospital based case-control study.

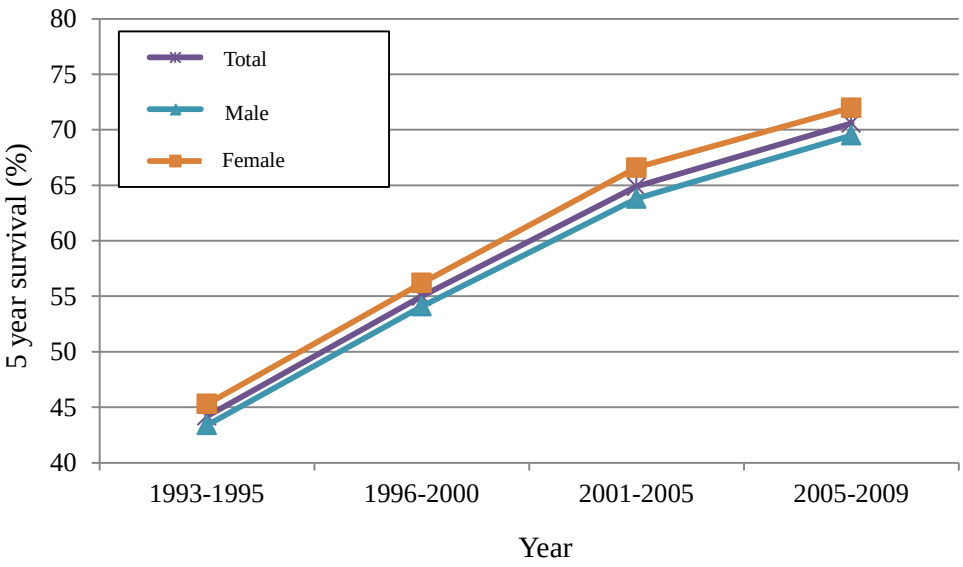

Figure 1. Time trends in childhood leukemia 5-year survival by sex in Korea from 1993 to 2009

* Modified from KOSIS 2009 annual report

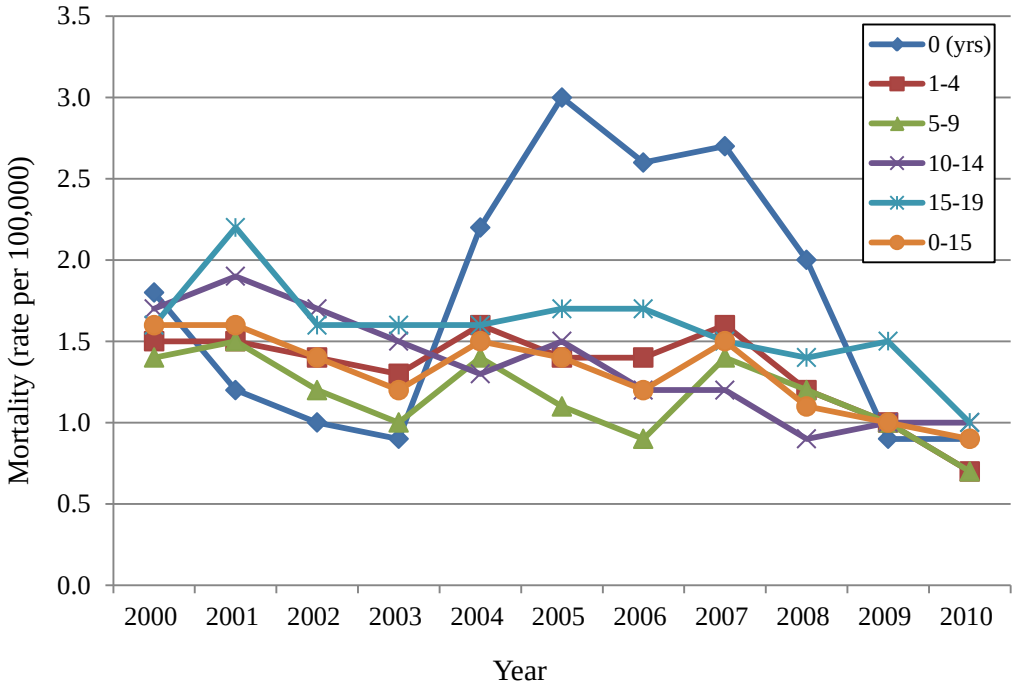

Figure 2. Time trends in childhood leukemia mortality rate by age group in Korea from 2000 to 2010

* Modified from KOSIS data (KOSIS, the number of deaths and mortality) [15]

Table 1. List of genetic susceptible risk factors in childhood leukemia

Genes	Polymorphisms	Subtype	Case No.	Control No.	Country	Significant polymorphism	OR (95% CI)	Reference
Xenobiotics metabolism								
ABCB1	C3435T	ALL	531	756	Chinese and Malay	-	NS	Yeoh (2010) [16]
CYP1A1	T6235C, A4889G, C4887A	ALL	177	304	French Canadian	A4889G	1.8 (1.1-3.1)	Krajinovic (1999) [17]
CYP1A1	*2A, *2B, *4	ALL	176	306	French Canadian	*2A	1.8 (1.1-3.1)	Sinnett (2000) [18]
CYP1A1	*1, *2	ALL	113	221	Brazilian	-	NS	Canalle (2004) [19]
CYP1A1	*1, *2A, *2B, *4	ALL	107	320	Thai	-	NS	Pakakasama (2005) [20]
CYP2D6	*3, *4	ALL	177	304	French Canadian	-	NS	Krajinovic (1999) [17]
CYP2D6	*3, *4, PM	ALL	176	306	French Canadian	-	NS	Sinnett (2000) [18]
CYP2E1	*5	ALL	174	337	French Canadian	*5	2.8 (1.2-6.4)	Krajinovic (2002) [21]
CYP2E1	*3	ALL	113	221	Brazilian	-	NS	Canalle (2004) [19]
CYP3A4	*1, *1B	ALL	107	320	Thai	-	NS	Pakakasama (2005) [20]

Genes	Polymorphisms	Subtype	Case No.	Control No.	Country	Significant polymorphism	OR (95% CI)	Reference
CYP3A5	*3, *6	ALL	107	320	Thai	-	NS	Pakakasama (2005) [20]
CYP3A5	A6986G	ALL	617	203	Denmark and Norway	A6986G	1.6 (1.0-2.7)	Borst (2011) [22]
SLC19A1	G80A	ALL	531	756	Chinese and Malay	-	NS	Yeoh (2010) [16]
SLC19A1	rs1051298 rs1051266	ALL	377	448	Mixed	-	NS	Metayer (2011) [23]
GSTM1	deletion	ALL	177	304	French Canadian	deletion	1.8 (1.2-2.6)	Krajinovic (1999) [17]
GSTM1	deletion	ALL	176	306	French Canadian	-	1.8 (1.2-2.6)	Sinnett (2000) [18]
GSTM1	deletion	ALL	113	221	Brazilian	-	NS	Canalle (2004) [19]
GSTM1	deletion	ALL	107	320	Thai	deletion	1.7 (1.0-2.7)	Pakakasama (2005) [20]
GSTM1	deletion	ALL	531	756	Chinese and Malay	-	NS	Yeoh (2010) [16]
GSTO1	*A140D	ALL	99	100	Thai	*A140D	2.2 (1.2-4.4)	Pongstaporn (2009) [24]
GSTO2	*N142D	ALL	99	100	Thai	*N142D	5.5 (1.7-17.7)	Pongstaporn (2009) [24]
GSTP1	Ile105	ALL	113	221	Brazilian	Ile105	2.7 (1.1-6.8)	Canalle (2004) [19]

Genes	Polymorphisms	Subtype	Case No.	Control No.	Country	Significant polymorphism	OR (95% CI)	Reference
GSTT1	deletion	ALL	177	304	French Canadian	-	NS	Krajinovic (1999) [17]
GSTT1	deletion	ALL	176	306	French Canadian	-	NS	Sinnett (2000) [18]
GSTT1	deletion	ALL	113	221	Brazilian	-	NS	Canalle (2004) [19]
GSTT1	deletion	ALL	107	320	Thai	-	NS	Pakakasama (2005) [20]
GSTT1	deletion	ALL	531	756	Chinese and Malay	-	NS	Yeoh (2010) [16]
MPO	*2	ALL	174	337	French Canadian	-	NS	Krajinovic (2002) [25]
NAT1	*3, *4, *10, *11, *14A, *15	ALL	176	306	French Canadian	*4	1.4 (1.0-1.9)	Krajinovic (2000) [26]
NAT1	*3, *4, *10, *11, *14, *15	ALL	176	306	French Canadian	-	NS	Sinnett (2000) [18]
NAT2	*4, *12A, *5A, *5B, *5C, *6A, *7B	ALL	176	306	French Canadian	slow acetylator	1.5 (1.0-2.2)	Krajinovic (2000) [26]
NAT2	*4, *5A, *5B, *5C, *6A, *7B	ALL	176	306	French Canadian	slow acetylator	1.6 (1.1-2.5)	Sinnett (2000) [18]
NAT1+ *NAT2*	NAT1*4+NAT 2 slow	ALL	176	306	French Canadian	slow acetylator+*4	1.9 (1.1-3.4)	Krajinovic (2000) [26]
NQO1	*2, *3	ALL	174	337	French Canadian	*2+*3	1.8 (1.2-2.4)	Krajinovic (2002) [25]

Genes	Polymorphisms	Subtype	Case No.	Control No.	Country	Significant polymorphism	OR (95% CI)	Reference
NQO1	C609T	ALL	531	756	Chinese and Malay	C609T in malay boy	0.5 (0.4-0.8)	Yeoh (2010) [16]
DNA repair								
APEX1	rs11160711, rs3120073	ALL	377	448	Mixed	haplotype AA	1.9 (1.3-2.9)	Chokkalingam (2011) [27]
EPHX1	Tyr113His, His139Arg	ALL	167	190	Turkish	113His/His	2.3 (1.2-4.4)	Tumer (2012) [28]
ERCC2	codon 312, 751	ALL	206	364	Brazilian	-	NS	Canalle (2011) [29]
ERCC2	rs3916874, rs238416, rs171140	ALL	377	448	Mixed	haplotype GAA	0.6 (0.4-0.9)	Chokkalingam (2011) [27]
hOGG1	Ser326Cys (rs1052133)	ALL	415	511	Chinese	Ser/Ser and Ser/Cys	0.7 (0.5-0.9)	Li (2011) [30]
hOGG1	Ser326Cys	ALL	97	131	Polish	Cys/Cys Ser/Ser	5.4 (1.9-15.1) 0.5 (0.3-0.8)	Skoczen (2011) [31]
MUTYH	Tyr165Cys	ALL	97	131	Polish	-	NS	Skoczen (2011) [31]
NBN	rs12680687, rs6470522, rs7840099, rs1805812, rs709816	ALL	377	448	Mixed	rare haplotype	0.4 (0.2-0.9)	Chokkalingam (2011) [27]

Genes	Polymorphisms	Subtype	Case No.	Control No.	Country	Significant polymorphism	OR (95% CI)	Reference
RAD51	rs2304579, rs7177265, rs2304580	ALL	377	448	Mixed	haplotype AAA, AGA	1.6 (1.0-2.4) 1.5 (1.0-2.3)	Chokkalingam (2011) [27]
TNFα	C850T	ALL	58	87	Greek	-	NS	Fidani (2004) [32]
XPD	codon 312, 715	ALL	108	317	Thai	-	NS	Pakakasama (2007) [33]
XPD	Asp312Asn, Lys751Gln	ALL	70	75	Turkish	-	NS	Batar (2009) [34]
XRCC1	codon 194, 280, 399	ALL	117	117	Indian	codon 399 (Gln/Gln)	2.4 (1.0-6.0)	Joseph (2005) [35]
XRCC1	codon 194, 280, 399	ALL	108	317	Thai	194 399	0.2 (0.1-0.9) 1.7 (1.2-2.3)	Pakakasama (2007) [33]
XRCC1	Arg194Trp, Arg399Gln	ALL	70	75	Turkish	Arg194Trp/Trp 194Trp in female	5.5 (1.5-20.1)	Batar (2009) [34]
XRCC1	Arg194Trp, Arg399Gln	ALL	167	190	Turkish	Gln/Gln Arg/Gln + Gln/Gln	2.0 (1.0-3.8) 1.6 (1.0-2.4)	Tumer (2010) [36]
XRCC1	codon 194, 399	ALL	206	364	Brazilian	-	NS	Canalle (2011) [29]
XRCC1	Arg399Gln	ALL	97	131	Polish	-	NS	Skoczen (2011) [31]
XRCC1	Arg399Gln	ALL	167	190	Turkish	EPHX1 Tyr113His +	2.1 (1.2-5.1)	Tumer (2012) [28]

Genes	Polymorphisms	Subtype	Case No.	Control No.	Country	Significant polymorphism	OR (95% CI)	Reference
						XRCC1 Arg399Gln		
XRCC4	rs7727691, rs6869366, rs2075685, rs2075686, rs28360071, rs3734091, rs28360317	All types	266	266	Taiwan	rs6869366+rs28360071	4.9 (1.0-24.3)	Wu (2010) [37]
XRCC4	rs7711825, rs1193695, rs301276, rs301287, rs3777018	ALL	377	448	Mixed	haplotype CGGGA	0.4 (0.2-1.0)	Chokkalingam (2011) [27]
Apoptosis & cell cycle								
CCND1	A870G	ALL	183	190	Chinese	AA	3.3 (2.0-9.0)	Hou (2005) [38]
CDKN1A	T1284C, T899G, T791C	ALL	240	277	French Canadian	-	NS	Healy (2007) [39]
CDKN1B	C1857T, G1608A, G373T	ALL	240	277	French Canadian	-	NS	Healy (2007) [39]

Genes	Polymorphisms	Subtype	Case No.	Control No.	Country	Significant polymorphism	OR (95% CI)	Reference
CDKN2A	T222A	ALL	240	277	French Canadian	T222A	2.2 (1.2-4.0)	Healy (2007) [39]
CDKN2B	C1270T, A593T, C287G	ALL	240	277	French Canadian	593T	0.7 (0.6-1.0)	Healy (2007) [39]
One carbon metabolism								
AKAP5	rs2230491	ALL	377	448	Mixed	-	NS	Metayer (2011) [23]
CBS	rs400660 rs8128028 rs11909493 rs719037 rs11700748 rs760124 rs6586281 rs4920037 rs234705 rs2851391 rs234715 rs9982015	ALL	377	448	Mixed	rs400660 rs11909493	1.4 (1.1-1.9) 1.4 (1.1-1.8)	Metayer (2011) [23]
DHFR	rs836788 rs1232027 rs12517451 rs1650723	ALL	377	448	Mixed	-	NS	Metayer (2011) [23]

Genes	Polymorphisms	Subtype	Case No.	Control No.	Country	Significant polymorphism	OR (95% CI)	Reference
FOLH1	rs6485963 rs11040270 rs617528	ALL	377	448	Mixed	-	NS	Metayer (2011) [23]
MS	A2756G	ALL	73	128	Iran	MS GG + TS 2R2R (combine)	1.3 (0.6-2.7)	Rahimi (2012) [40]
MTHFD1	G401A, G1958A	ALL	460	552	German	-	NS	Gast (2007) [41]
MTHFD1	G1958A	ALL	531	756	Chinese and Malay	-	NS	Yeoh (2010) [16]
MTHFD1	rs2983733 rs1956545 rs3783731 rs1950902 rs11627525 rs8016556 rs8012229 rs3818239	ALL	377	448	Mixed	-	NS	Metayer (2011) [23]
MTHFR	C677T, A1298C	AML	253	200	UK	C677T+MLL translocation	0.4 (0.2-0.9)	Wiemels (2001) [42]
MTHFR	C677T, A1298C	AML	253	200	UK	C677T homozygote TT	0.5 (0.2-1.2)	Wiemels (2001) [42]
MTHFR	C677T, A1298C	AML	253	200	UK	A1298C homozygote	0.3 (0.1-0.8)	Wiemels (2001) [42]

Genes	Polymorphisms	Subtype	Case No.	Control No.	Country	Significant polymorphism	OR (95% CI)	Reference
						CC		
MTHFR	C677T, A1298C	ALL	270	300	French Canadian	T677T/A1298A C677C/C1298C	0.4 (0.2-0.9) 0.3 (0.1-0.6)	Krajinovic (2004) [43]
MTHFR	C677T, A1298C	ALL	103	111	Portuguese	-	NS	Oliveira (2005) [44]
MTHFR	C677T, A1298C	ALL	52	88	Greek	677T	0.4 (0.2-0.8)	Chatzidakis (2006) [45]
MTHFR	C677T, A1298C	ALL	66	100	Korean	A1298C	2.2 (1.1-4.5)	Kim (2006) [46]
MTHFR	C677T, A1298C	ALL	66	100	Korean	1298AC+CC	2.1 (1.1-4.2)	Kim (2006) [46]
MTHFR	C677T, A1298C	ALL	531	756	Chinese and Malay	C677T in Malay boy	0.6 (0.5-0.9)	Yeoh (2010) [16]
MTHFR	C677T, A1298C	ALL+ AML	939	824	UK	A1298C	0.8 (0.7-1.0)	Lightfoot (2010) [47]
MTHFR	C677T, A1298C	ALL	361	508	Chinese	C677T	0.6 (0.4-0.9)	Tong (2010) [48]
MTHFR	rs1537515 rs1801131 rs12121543 rs6541003 rs1801133	ALL	377	448	Mixed	rs1537515	1.6 (1.1-2.3)	Metayer (2011) [23]

Genes	Polymorphisms	Subtype	Case No.	Control No.	Country	Significant polymorphism	OR (95% CI)	Reference
	rs17421462 rs4846052 rs9651118							
MTHFR	C677T, A1298C	AL	764	1681	French	-	NS	Amigou (2012) [49]
MTHFR	C677T, A1298C	ALL	72	109	Iran	-	NS	Azhar (2012) [50]
MTR	A2756G	ALL	460	552	German	-	NS	Gast (2007) [41]
MTR	A2756G	ALL+ AML	939	824	UK	A2756G	1.3 (1.1-1.6)	Lightfoot (2010) [47]
MTR	rs10925235 rs12759827 rs12567062 rs1805087	ALL	377	448	Mixed	-	NS	Metayer (2011) [23]
MTRR	A66G, C524T, A1049G, C1783T	ALL	460	552	German	A66G	0.8 (0.7-0.9)	Gast (2007) [41]
MTRR	rs1801394 rs3776465 rs6555501 rs162031 rs162033 rs161871 rs162037	ALL	377	448	Mixed	-	NS	Metayer (2011) [23]

Genes	Polymorphisms	Subtype	Case No.	Control No.	Country	Significant polymorphism	OR (95% CI)	Reference
	rs2287779 rs10380 rs1802059							
MTRR	A66G, C524T	AL	764	1681	French	-	NS	Amigou (2012) [49]
RFC	G80A	ALL	460	552	German	-	NS	Gast (2007) [41]
SHMT1	C1420T	ALL	460	552	German	-	NS	Gast (2007) [41]
SHMT1	C1420T	ALL+ AML	939	824	UK	-	NS	Lightfoot (2010) [47]
SHMT1	rs11868708 rs9909104 rs2273027 rs9901160	ALL	377	448	Mixed	-	NS	Metayer (2011) [23]
TS	1494del6, 28bp repeat	ALL+ AML	939	824	UK	-	NS	Lightfoot (2010) [47]
TS	28-bp repeat	ALL	73	128	Iran	MS GG + TS 2R2R (combine)	1.3 (0.6-2.7)	Rahimi (2012) [40]
TYMS	1494del6, 2R>3R	ALL	460	552	German	-	NS	Gast (2007) [41]
TYMS	3 -TYMS 6 bp deletion	ALL	531	756	Chinese and Malay	-	NS	Yeoh (2010) [16]

Genes	Polymorphisms	Subtype	Case No.	Control No.	Country	Significant polymorphism	OR (95% CI)	Reference
TYMS	5 -TYMS 28 bp repeats	ALL	531	756	Chinese and Malay	-	NS	Yeoh (2010) [16]
TYMS	2R-3R	ALL	206	364	Brazilian	2R-3R	0.6 (0.4-1.0)	Canalle (2011) [29]
TYMS	rs502396 rs2847153 rs2853524 rs2853532 rs1059393	ALL	377	448	Mixed	-	NS	Metayer (2011) [23]
Others								
BRCA2	rs4942448, rs9943876	ALL	377	448	Mixed	haplotype GA	1.8 (1.1-2.9)	Chokkalingam (2011) [27]
HFE	rs1800562, rs1799945, rs1800730	ALL	117	414	Welsh and Scottish	rs1800562 in male	0.4 (0.2-0.6)	Davis (2010) [51]
mTOR	rs2536, rs2295080	ALL	417	554	Chinese	rs2536	0.7 (0.5-1.0)	Huang (2012) [52]

* Of the 74 items retrieved by the PUBMED search (keyword: childhood[title] AND leukemia[title] AND risk AND (polymorphisms OR polymorphism OR snp) AND (apoptosis OR "cell cycle" OR "dna repair" OR metabolism)). 37 papers were remained after screening of the title or abstract.

* AL acute leukemia, ALL acute lymphoblastic leukemia, AML acute myeloid leukemia

Methods

1. Subjects

The eligible patients were recruited from three medical centers in Korea between 2003 and 2006. The cases were diagnosis of leukemia. The controls were clinic-based selected in the department of pediatrics in the same hospitals with no medical history of childhood cancer. Total of 300 childhood leukemia patients and 558 controls were included in this study. For the Illumina OPA chip analysis cases were random selected and all subjects met the following criteria for inclusion: DNA sample of at least 750ng and a 260/280 ratio of 1.5-2.0 for the DNA. Because of a low yield of DNA from the controls, 10% more controls were enrolled in this study. All controls were frequency matched by sex and 5-year interval age (≤5, 6-9, 10-14, and 15≤ years), and 136 cases and 254 controls (about 1:2) were used for the genotyping. We excluded no genotyped results and low completion rate of genotyping for the analysis. After quality control, a total of 63 childhood leukemia patients and 148 controls were finally included for the analysis (Figure 3).

A total of 136 childhood leukemia patients were comprised of acute lymphoblastic leukemia (ALL) (62.5%), acute myeloid leukemia (AML) (27.2%),

acute biphenotypic leukemia (8.1%), chronic myeloid leukemia (CML) (1.5%), and juvenile myelomonocytic leukemia (JMML) (0.7%). The clinical diagnoses of the control patients can be found in the reference article [53]. There was no difference between distribution of leukemia subtype in 300 and 136 cases. Informed consent was obtained from each of the participant subjects, and the study was approved by the Institutional Review Board (IRB) for Human Research of Seoul National University Hospital (IRB No. H-0407-128-001).

Blood samples and informed consents were obtained from all the cases and the controls. The administered, structured questionnaire included information on the characteristics of the child, parental smoking habits and alcohol consumption, and maternal medication used during pregnancy. The information on the subject selection and data collection procedure are described in detail elsewhere [54].

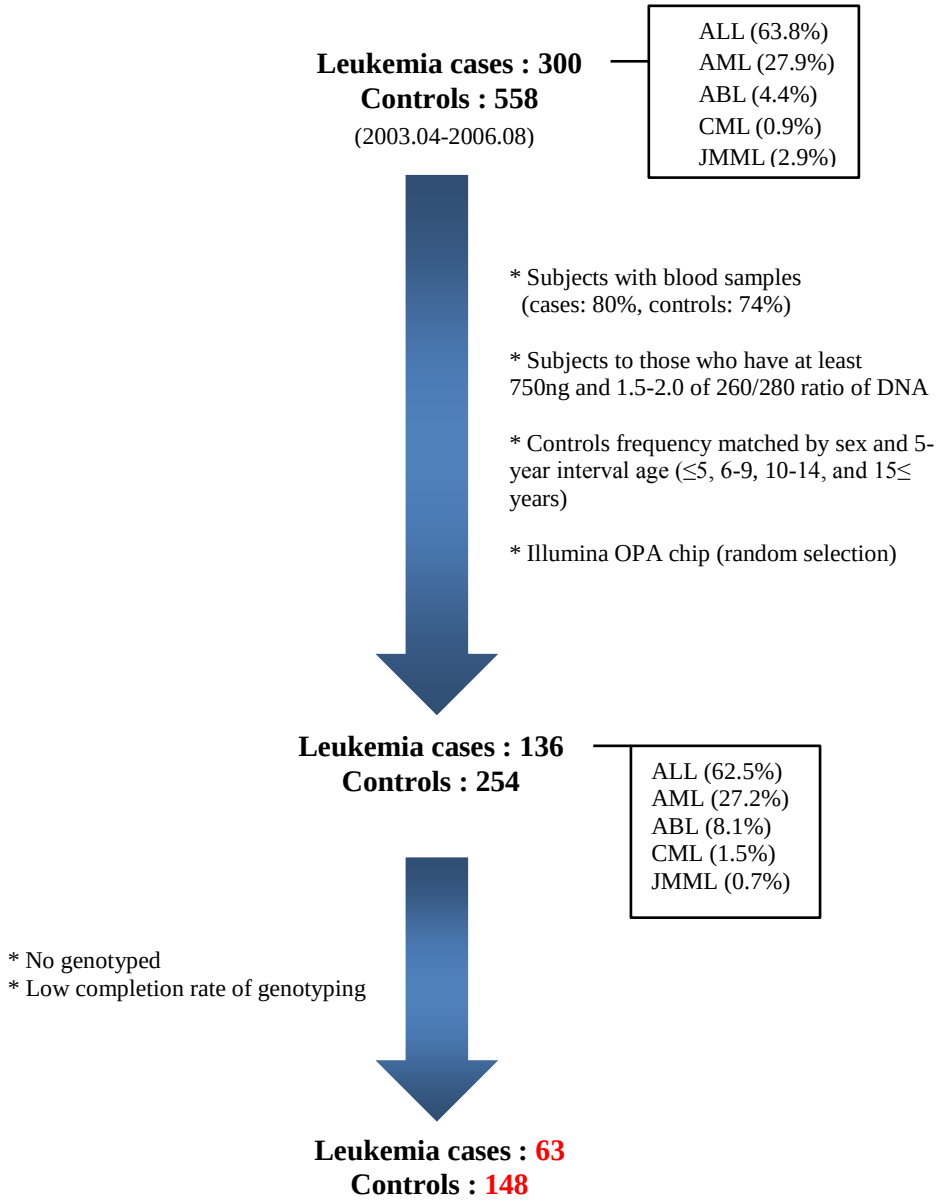

Figure 3. The flow of subject selection

2. SNP selection and genotyping

Genomic DNA was extracted from peripheral blood using the Gentra Puregene Blood Kit (Gentra, USA).The DNA concentration was measured by Picogreen assay.

Genotyping was performed at the NCI Core Genotyping Facility (CGF: Advanced Technology Corporation, Gaithersburg, MD) using an Illumina GoldenGate OPA panel designed to tag 203 candidate genes or gene regions. The panel was composed of the immunity, apoptosis and cell cycle related genes. Initial candidate genes were proposed by CGEMS in NCI/NIH. SNPs included in those genes were chosen according to some criteria: first, initial SNPs were selected by CGEMS in NCI/NIH, the second, additional SNPs were added based on relevant literature and SNP database (Cancer Genome Anatomy Project (CGAP) and SNP500 database [55]). And the last, the tag SNPs were selected from the designable set of SNPs that were genotyped as part of the international HapMap Project. For Illumina chip analysis, tag SNPs were selected based on the following parameters: 1) minor allele frequency (MAF) > 5% in the controls, 2) linkage disequilibrium (LD) threshold of $r^2 > 0.8$, and 3) SNPs with a design score of 1.1 were weighted higher and SNPs with a design score less than 0.6 were excluded.

The detailed criteria about selecting gene regions and SNPs were described

by Rajaraman et al., who reported the association between innate immunity and adult glioma using the same platform [56]. Among the 1,536 SNPs, virtually half (55%) of them were located in introns, 22% in promoter regions (flanking sequence, UTR), 15% in 3'UTRs, and 9% in exons. Synonymous and nonsynonymous changes were 73% and 27%, respectively, among SNPs located on exons.

Quality control (QC) was performed using the results of 82 overlapping SNPs between innate immunity OPA panel and NHL OPA panel. The duplicate results for 272 subjects showed >99% concordance for each SNP (data not shown).

Among a total 1,536 SNPs, 304 SNPs were related to apoptosis and cell cycle in 31 genes (Appendix I). Fifty-six SNPs were considered unusable because of failure to genotype or monomorphism (46 SNPs), due to the Hardy-Weinberg equilibrium (HWE) $P < 0.001$ (3 SNPs), and a minor allele frequency (MAF) < 0.05 (7 SNPs) in both the cases and controls, which were excluded from the analysis. Finally, 248 SNPs in 31 gene regions were used in the analysis.

3. Statistical analysis

A Pearson chi-square (χ^2) test was used to estimate the genotype distribution of the deviation from Hardy–Weinberg equilibrium (HWE) in the control group. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated to determine factors associated with the risk of childhood leukemia using unconditional logistic regression models adjusting for age (continuous; years) and birth weight (<3.25, 3.25-3.70, and >3.70; kg). Tests for the linear trend in risk were done by treating categorical values as a continuous variable. For the genetic factor, the homozygote of the most common genotype was used as the referent group. In genotyping result the risk of childhood leukemia was estimated by both additive and dominant model.

A gene-level *minP* [57] was calculated with a permutation-based re-sampling procedure (10,000 permutation) for all SNPs in each gene considering the significance with a $P_{trend} < 0.05$. To evaluate accurate associations, false discovery rate (FDR) was used with a $P < 0.05$ [58]. Linkage disequilibrium (LD) between SNPs in our study and SNPs in previous reports were tested using Haploview version 4.2 (www.broad.mit.edu/mpg/haploview) based on the International HapMap Project data markers [59, 60].

Additionally, associations between genetic factors and leukemia risk were

estimated after stratified by the histological subtype: ALL (n = 41) and AML (n = 15), which is the most common subtype of childhood leukemia.

All statistical procedures were done with SAS software version 9.2 (SAS Institute, Cary, NC, USA).

Results

1. Selected characteristics of subjects

The distributions of the selected characteristics between the childhood leukemia patients and the controls were presented in Table 1. There were no statistically significant differences in parental characteristics, including smoking habits, alcohol consumption, and medication during pregnancy (data not shown). Among child characteristics, only the birth weight was statistically associated with a high risk of childhood leukemia (P_{trend} = 0.019, Table 2). There were no significant differences for the distribution of the basic characteristics between the subjects with DNA and subjects without DNA, except for the education level of the parents in the control group (Appendix II-IV).

Table 2. Selected characteristics of childhood leukemia patients and controls

	Cases (%) (n=63)	Controls (%) (n=148)	OR[a] (95% CI)
Sex			
Male	45 (71.4)	99 (66.9)	1.00
Female	18 (28.6)	49 (33.1)	0.82 (0.43-1.56)
P-value			0.517
Age at diagnosis (years)			
≤5	27 (42.9)	78 (52.7)	1.00
6-9	22 (34.9)	34 (23.0)	1.87 (0.94-3.74)
10-14	13 (20.6)	30 (20.3)	1.25 (0.57-2.74)
15-19	1 (1.6)	6 (4.0)	0.48 (0.06-4.18)
P_{trend}[b]			0.692
Birth weight (kg)			
< 3.25	27 (42.9)	89 (60.9)	1.00
3.25-3.70	24 (38.1)	42 (28.8)	1.85 (0.95-3.58)
> 3.70	12 (19.0)	15 (10.3)	2.51 (1.04-6.04)
P_{trend}[b]			0.019
Breast feeding			
No	30 (47.6)	72 (49.0)	1.00
Yes	33 (52.4)	75 (51.0)	1.02 (0.56-1.86)
P-value			0.239
Education levels for both father and mother			
High school or less	21 (33.3)	48 (32.6)	1.00
University graduate	15 (23.8)	26 (17.7)	1.41 (0.62-3.22)
Graduate school or more	27 (42.9)	73 (49.7)	0.91 (0.46-1.80)
P_{trend}[b]			0.726
Family history of cancer			
No	30 (47.6)	80 (54.4)	1.00
Yes	33 (52.4)	67 (45.6)	1.23 (0.68-2.23)
P-value			0.213

[a] ORs and [b] P_{trend} are adjusted for age (continuous) and birth weight (< 3.25, 3.25-3.70, and > 3.70; kg).

2. Association between genes related to apoptosis, cell cycle and childhood leukemia risk

Among the 31 genes related to apoptosis and cell cycle, 10 SNPs (rs714920, rs8110862, rs11196422, rs12416109, rs3814231, rs6585241, rs17764308, rs292240, rs4733550, and rs7765221) in 5 genes (*CASP7, CASP8AP2, CASP14, MYC,* and *RIPK1*) were significantly associated with the risk of childhood leukemia ($P_{trend} < 0.05$) (Table 3).

At a genetic level, two genes (*CASP7* and *CASP14*) had statistically significant associations to the risk of childhood leukemia (*minP* = 0.013 and 0.002, respectively) and the FDR results also support the permutation test results (*P* = 0.042 and 0.027, respectively) (Table 4).

Table 3. List of the 10 significant SNPs found in 5 genes

Gene	Chr	Gene region	SNP (rs#)	[a]*P*-value
CASP7	10	IVS2+3353G>A	rs11196422	0.029
	10	IVS6-461G>A	rs12416109	0.002
	10	IVS5+91C>T	rs3814231	0.009
	10	IVS9+417G>A	rs6585241	0.042
CASP8AP2	6	-1919C>T	rs17764308	0.048
	6	*511G>A	rs292240	0.044
CASP14	19	-11344T>C	rs714920	0.018
	19	*9276A>C	rs8110862	<0.001
MYC	8	*9957A>T	rs4733550	0.031
RIPK1	6	IVS8+2003A>G	rs7765221	0.035

[a] *P*-value are adjusted for age (continuous) and birth weight (<3.25, 3.25-3.70, and >3.70; kg)

Table 4. Associations between genetic polymorphisms in apoptosis and cell cycle related genes and childhood leukemia risk

Gene	No. of SNP	No. of SNP with $P < 0.05$	Lowest P_{trend}[a]	*minP*[a]	FDR[a]
CASP7	12	4	0.002	0.013	0.042
CASP8AP2	12	2	0.044	0.195	0.171
CASP14	8	2	<0.001	0.002	0.027
MYC	9	1	0.030	0.194	0.171
RIPK1	8	1	0.034	0.192	0.171

[a] *P*-value are adjusted for age (continuous) and birth weight (<3.25, 3.25-3.70, and >3.70; kg).

3. Association between *CASP7* and childhood leukemia risk

Among 12 SNPs (rs11196418, rs11196422, rs11196444, rs11196454, rs12358301, rs12358524, rs12416109, rs3124737, rs3814231, rs4497356, rs6585241, and rs7907519) of *CASP7* gene, 4 SNPs (rs11196422, rs12416109, rs3814231, and rs6585241) were significantly associated with risk of childhood leukemia. In SNPs rs11196422, rs12416109, and rs6585241, there was a trend toward higher risk of childhood leukemia in patients with variant allele compared to wild-type carriers (GG vs GA+AA; OR=2.29, 95% CI 1.21-4.32, AA vs AG+GG; OR=2.42, 95% CI 1.23-4.77, and AA vs AG+GG; OR=2.06, 95% CI 1.05-4.02, respectively) (Table 5). On the other hand, one SNP rs3814231 represented a trend toward lower risk of childhood leukemia in patients with T allele carriers compared with wild-type carriers (CC vs CT+TT; OR=0.46, 95% CI 0.24-0.87) (Table 5).

Table 5. Odds ratios and 95% confidence interval for selected SNPs in *CASP7* gene

SNP(rs#)	Cases (%)	Controls (%)	OR[a]	(95% CI)	P_{trend} [a]
rs11196418 (-13264A>G)					
GG	54 (85.7)	121 (81.8)	1.00		
GA	8 (12.7)	25 (16.9)	0.74	(0.31-1.77)	
AA	1 (1.6)	2 (1.4)	2.06	(0.12-34.41)	0.719
GA+AA			0.79	(0.34-1.84)	
rs11196422 (IVS2+3353G>A)					
GG	36 (57.1)	112 (75.7)	1.00		
GA	25 (39.7)	32 (21.6)	2.43	(1.26-4.68)	
AA	2 (3.2)	4 (2.7)	1.30	(0.22-7.61)	0.029
GA+AA			2.29	(1.21-4.32)	
rs11196444 (IVS4+11325G>C)					
GG	50 (80.7)	123 (83.7)	1.00		
GC	12 (19.4)	23 (15.7)	1.28	(0.58-2.80)	
CC	0 (0)	1 (0.68)	-		0.724
GC+CC			1.22	(0.56-2.66)	
rs11196454 (*4433C>T)					
CC	42 (67.7)	83 (56.1)	1.00		
CT	16 (25.8)	57 (38.5)	0.63	(0.32-1.24)	
TT	4 (6.5)	8 (5.4)	0.99	(0.27-3.74)	0.365
CT+TT			0.68	(0.36-1.28)	
rs12358301 (*8018C>T)					
TT	49 (79.0)	129 (87.8)	1.00		
TC	13 (21.0)	17 (11.6)	1.94	(0.86-4.39)	
CC	0 (0)	1 (0.7)	-		0.189
TC+CC			1.84	(0.82-4.12)	
rs12358524 (Ex3-49C>T)					
CC	54 (85.7)	122 (82.4)	1.00		
CT	8 (12.7)	24 (16.2)	0.78	(0.32-1.87)	
TT	1 (1.6)	2 (1.4)	2.08	(0.12-34.63)	0.804
CT+TT			0.83	(0.36-1.94)	

rs12416109 (IVS6-461G>A)					
AA	17 (27.9)	66 (44.9)	1.00		
AG	29 (47.5)	64 (43.5)	1.99	(0.97-4.08)	
GG	15 (24.6)	17 (11.6)	4.30	(1.70-10.87)	0.002
AG+GG			2.42	(1.23-4.77)	
rs3124737 (*7795G>A)					
AA	23 (37.1)	53 (36.3)	1.00		
AG	28 (45.2)	68 (46.6)	1.18	(0.59-2.34)	
GG	11 (17.7)	25 (17.1)	1.35	(0.55-3.32)	0.500
AG+GG			1.22	(0.64-2.33)	
rs3814231 (IVS5+91C>T)					
CC	27 (42.9)	43 (29.1)	1.00		
CT	30 (47.6)	77 (52.0)	0.52	(0.26-1.02)	
TT	6 (9.5)	28 (18.9)	0.28	(0.10-0.79)	0.009
CT+TT			0.46	(0.24-0.87)	
rs4497356 (IVS4-3352T>A)					
TT	54 (87.1)	114 (77.0)	1.00		
AT	7 (11.3)	32 (21.6)	0.49	(0.20-1.19)	
AA	1 (1.6)	2 (1.4)	1.28	(0.11-14.89)	0.208
AA+AT			0.53	(0.23-1.23)	
rs6585241 (IVS9+417G>A)					
AA	40 (63.5)	110 (74.3)	1.00		
AG	21 (33.3)	34 (30.0)	2.05	(1.03-4.07)	
GG	2 (3.2)	4 (2.7)	2.19	(0.37-12.88)	0.042
AG+GG			2.06	(1.05-4.02)	
rs7907519 (IVS2+1946A>C)					
CC	32 (50.8)	88 (59.5)	1.00		
CA	25 (39.7)	53 (35.8)	1.38	(0.72-2.62)	
AA	6 (9.5)	7 (4.7)	2.83	(0.85-9.40)	0.087
CA+AA			1.53	(0.83-2.82)	

[a]ORs and P_{trend} are adjusted for age (continuous) and birth weight (<3.25, 3.25-3.70, and >3.70; kg)

4. Association between *CASP14* and childhood leukemia risk

Among 8 SNPs (rs10425745, rs16980286, rs3181163, rs3181309, rs4808901, rs5021087, rs714920, rs8110862) of *CASP14* gene, 2 SNPs (rs714920 and rs8110862) were significantly associated with risk of childhood leukemia. In SNPs rs714920, there was a trend toward higher risk of childhood leukemia in patients with variant allele compared to wild-type carriers (TT vs CT+CC; OR=1.83, 95% CI 0.98-3.43) (Table 6). On the other hand, one SNP rs8110862 represented a trend toward lower risk of childhood leukemia in patients with C allele carriers compared with wild-type carriers (AA vs AC+CC; OR=0.34, 95% CI 0.18-0.63) (Table 6).

In the number of 8 SNPs, the most significant SNP was in *CASP14* rs8110862 (Table 6).

Table 6. Odds ratios and 95% confidence interval for selected SNPs in *CASP14* gene

SNP(rs#)	Cases (%)	Controls (%)	OR[a]	(95% CI)	P_{trend} [a]
rs10425745 (*2935A>T)					
TT	45 (72.6)	97 (65.6)	1.00		
TA	17 (27.4)	45 (72.6)	0.82	(0.42-1.62)	
AA	0 (0.0)	6 (4.1)	-		0.208
TA+AA			0.73	(0.38-1.43)	
rs16980286 (*8052C>T)					
TT	50 (79.4)	121 (82.3)	1.00		
TC	13 (20.6)	22 (15.0)	1.40	(0.64-3.04)	
CC	0 (0.0)	4 (2.7)	-		0.919
TC+CC			1.21	(0.57-2.59)	
rs3181163 (IVS4+77T>C)					
TT	49 (77.8)	103 (70.1)	1.00		
TC	14 (22.2)	39 (26.5)	0.77	(0.38-1.58)	
CC	0 (0)	5 (3.4)	-		0.182
TC+CC			0.69	(0.34-1.39)	
rs3181309 (*894C>T)					
TT	28 (44.4)	60 (40.5)	1.00		
TC	27 (42.9)	67 (45.3)	0.78	(0.40-1.51)	
CC	8 (12.7)	21 (14.2)	0.94	(0.36-2.45)	0.694
TC+CC			0.82	(0.44-1.51)	
rs4808901 (-5786C>T)					
CC	21 (33.3)	63 (42.6)	1.00		
CT	29 (46.0)	65 (43.9)	1.15	(0.58-2.28)	
TT	13 (20.6)	20 (13.5)	1.88	(0.78-4.50)	0.192
CT+TT			1.32	(0.70-2.49)	
rs5021087 (*13181G>T)					
GG	54 (85.7)	122 (82.4)	1.00		
GT	8 (12.7)	24 (16.2)	1.00	(0.52-1.94)	
TT	1 (1.6)	2 (1.4)	0.30	(0.10-0.88)	0.061
GT+TT			0.76	(0.41-1.43)	

rs714920 (-11344T>C)					
TT	23 (36.5)	78 (52.7)	1.00		
TC	28 (44.4)	58 (39.2)	1.55	(0.79-3.03)	
CC	12 (19.1)	12 (8.1)	3.12	(1.21-7.99)	0.018
TC+CC			1.83	(0.98-3.43)	
rs8110862 (*9276A>C)					
AA	37 (58.7)	47 (31.8)	1.00		
AC	22 (34.9)	73 (49.3)	0.40	(0.21-0.76)	
CC	4 (6.4)	28 (18.9)	0.19	(0.06-0.61)	<0.001
AC+CC			0.34	(0.18-0.63)	

[a]ORs and P_{trend} are adjusted for age (continuous) and birth weight (<3.25, 3.25-3.70, and >3.70; kg)

5. Association between *CASP7, CASP14* and childhood leukemia risk in subgroup analysis

Additionally we analyzed the risk of childhood leukemia in subtype groups of leukemia. When stratified by the subtypes, *CASP7* and *CASP14* were still significant in AML subtype group (P_{trend} = 0.007 and 0.022, *minP* = 0.001 and 0.037, respectively) (Table 7). In ALL subtype analysis, *CASP14* was also statistically significant (P_{trend} = 0.001, *minP* = 0.002) (Table 7 and 8). However, *CASP7* was not significant in ALL group.

Other genes were not significantly associated according to the subtypes of leukemia.

Table 7. Associations between genetic polymorphisms in apoptosis and cell cycle related genes and childhood acute myelocytic leukemia

Gene	No. of SNP	No. of SNP with $P < 0.05$	Lowest P_{trend}[a]	*minP*[a]	Lowest P_{trend} SNP
CASP5	13	2	0.041	0.267	rs507879
CASP7	12	1	0.007	0.037	rs12416109
CASP14	8	1	0.022	0.001	rs8110862
RIPK1	8	1	0.039	0.210	rs7765221

[a]P_{trend} and *minP* adjusted for age (continuous) and birth weight (<3.25, 3.25-3.70, and >3.70; kg)

Table 8. Associations between genetic polymorphisms in apoptosis and cell cycle related genes and childhood acute lymphocytic leukemia

Gene	No. of SNP	No. of SNP with $P < 0.05$	Lowest P_{trend}[a]	*minP*[a]	Lowest P_{trend} SNP
CASP7	12	2	0.027	0.186	rs12416109
CASP8	11	1	0.047	0.285	rs3769825
CASP14	8	2	<0.001	0.002	rs8110862
MYC	9	1	0.025	0.153	rs4733550

[a]P_{trend} and *minP* adjusted for age (continuous) and birth weight (<3.25, 3.25-3.70, and >3.70; kg)

Discussion

This study employed an *a priori* candidate gene approach to estimate the role of the common genetic variation associated with childhood leukemia. In this study, we found that polymorphisms of *CASP7* and *CASP14* genes increased the risk of childhood leukemia among 31 apoptosis and cell cycle control related genes by candidate gene selection. Until now, there has been no data reported on the polymorphisms of apoptosis and cell cycle related genes in childhood leukemia, and this is the first report on the polymorphisms of the *CASP7* and *CASP14* genes in childhood leukemia.

Caspases participate in apoptosis as initiators and executioners. Changes in their expression may cause an improper homeostatic death-proliferation balance and contribute to the development of proliferative disorders such as tumors.

The *CASP7* gene is a well-known effector caspase that is critical for inducing apoptosis [61]. The association between *CASP7* and human cancer has been reported in some studies [61-66]. Soung et al. proposed that inactivating mutation of the *CASP7* gene, the 70 Cys to Tyr mutant, might lead to the loss of its apoptotic function and cause to the carcinogenesis of human cancers [61]. In several studies, the *CASP7* genetic polymorphisms were associated with the risk of other cancers (i.e., endometrial and colorectal cancers), that consistent with our

findings [65, 66]. From the Haplotype analysis, the rs11196422 in *CASP7* that statistically associated SNP in presented study was in LD relationship with reported significant SNPs in endometrial (rs11196418, rs11593766, and rs10787498) and colorectal (rs2227310) cancer development (Figure 4). On the other hand, rs12416109 and rs6585241 showed no significant results in endometrial cancer [65, 66]. We hypothesized that three SNPs in *CASP7* acts as a strong apoptosis signal that block or delay the apoptosis of cancer cells. Further functional studies of these SNPs on blood cancer are required to confirm our findings.

The *CASP14* was distinguished from the usual caspases, which is involved in cell differentiation [67]. Our study showed that rs8110862 of *CASP14* was the most significantly related with increasing risk of childhood leukemia. From previous reports, several tumors had increased expression of *CASP14* (lung, breast, and cervical tumors) and its overexpression showed a poor prognosis (tumor stage, cell differentiation, and lymph vascular involvement) [68, 69]. However, in some studies, genetic variation of *CASP14* did not show significant association with the risk of tumors such as epithelial malignancies and salivary cancer [68, 70]. Although there are some limitations including a lack of functional information and established epidemiological studies supporting the results for selected SNPs, this study identified the mutation of *CASP14* might lead the abnormal

differentiate function and contribute to the childhood leukemia.

Additionally, the most significant variant, rs8110862 of *CASP14* was associated with the total population for childhood leukemia patients as well as for each subtype of leukemia.

The main limitations of this study were the small sample size and lack of replication for the results. Although we did re-sampling based on the permutation and FDR tests to minimize the false positive findings, the findings should be cautiously interpreted.

In summary, the results suggest that apoptosis and cell cycle system related genes might play a central role in the development of childhood leukemia. However, further replication studies and a larger sample size for the subtype group analyses are needed to confirm the findings of these genes in childhood leukemia patients.

Figure 4. Linkage disequilibrium of *CASP7* gene

* HapMap Genome Browser release #27, CHB+JBT

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Appendix

Appendix I. Candidate genes and SNPs in apoptosis and cell cycle pathway analyzed in this study

Gene region	SNP (rs #)	Gene information	Chr	Chr position
AICDA	rs11046349	Ex5+143A>C	12	8648058
	rs12306110	IVS1-401G>C	12	8651276
	rs2028373	Ex4+38C>T	12	8648748
	rs2518144	IVS2+16G>A	12	8650712
	rs2580873	IVS1-2066T>C	12	8652941
	rs3794318	IVS2-462T>C	12	8649810
	rs714629	-997C>G	12	8657628
BAX	rs1042265	Ex16-439C>T	19	54163632
	rs11667200	-2260A>T	19	54147737
	rs11667229	-2184C>T	19	54147813
	rs11667351	-2031T>G	19	54147966
	rs1805419	IVS3+14A>G	19	54150916
	rs2270938	IVS13-61T>A	19	54165839
	rs2270939	Ex5-26T>C	19	54134745
	rs3765148	Ex7-52G>A	19	54139525
	rs4645900	Ex6-157C>T	19	54156175
	rs4802527	*1652C>G	19	54141655
BCL2	rs1026825	IVS2-24283T>C	18	58971255
	rs10503078	IVS2-4196C>T	18	58951168
	rs11663788	*1236A>G	18	59148758
	rs12454712	IVS2-49892A>G	18	58996864
	rs12457700	IVS2-64254A>G	18	59011226
	rs12458289	IVS2+6601C>A	18	59129566
	rs12605881	IVS2+43746T>A	18	59092421
	rs12957119	IVS2-85163T>G	18	59032135
	rs12961672	IVS2-86861C>G	18	59033833
	rs1381548	IVS2+27791C>T	18	59108376
	rs1481031	IVS2-56093A>G	18	59003065
	rs1542578	IVS2-18923T>G	18	58965895
	rs1564483	Ex3+1339G>A	18	58945634
	rs17070659	*14834G>A	18	58932003
	rs17678177	IVS2-21887C>G	18	58968859

Gene region	SNP (rs #)	Gene information	Chr	Chr position
	rs17679032	IVS2-61168A>G	18	59008140
	rs17749561	*12646T>C	18	58934191
	rs17756266	IVS2-20061A>G	18	58967033
	rs17757541	IVS2-83694G>C	18	59030666
	rs17759659	IVS2+26543T>C	18	59109624
	rs1807999	IVS2+83015G>C	18	59053152
	rs1944419	IVS2+60574T>A	18	59075593
	rs1982673	IVS2-2023T>G	18	58948995
	rs2062011	IVS2-80122A>T	18	59027094
	rs2448806	*14257A>G	18	58932580
	rs2551408	IVS9+568A>T	18	59152902
	rs2849377	IVS2+13543T>A	18	59122624
	rs2849380	IVS2+5827G>A	18	59130340
	rs2850762	IVS2+18984G>A	18	59117183
	rs2850764	*5499G>C	18	59144495
	rs2850767	IVS9+1376C>A	18	59152094
	rs2850768	IVS9+1080T>C	18	59152390
	rs3744948	IVS2+62955A>G	18	59073212
	rs4941185	IVS2-27562T>C	18	58974534
	rs4941189	IVS2+77260G>A	18	59058907
	rs4987721	IVS2+31124A>G	18	59105043
	rs4987764	IVS2+77055T>C	18	59059112
	rs4987768	IVS2+80670G>T	18	59055497
	rs4987808	IVS2-31594C>A	18	58978566
	rs4987827	IVS2-10906C>A	18	58957878
	rs4987852	Ex3+2072A>G	18	58944901
	rs4987853	Ex3+2338A>G	18	58944635
	rs4987873	*5860A>T	18	58940977
	rs6567326	IVS2-44616C>A	18	58991588
	rs720321	IVS2-86658C>T	18	59033630
	rs7230970	IVS2-25084A>G	18	58972056
	rs7234941	IVS2+62336G>A	18	59073831
	rs7236090	IVS2+38076A>G	18	59098091
	rs7242402	IVS2+43229G>A	18	59092938

Gene region	SNP (rs #)	Gene information	Chr	Chr position
	rs7243091	IVS2-84570C>T	18	59031542
	rs8083946	IVS2+79266T>C	18	59056901
	rs8085707	IVS2+55407A>G	18	59080760
	rs8086404	IVS2+68063C>G	18	59068104
	rs8089538	IVS2+41636T>C	18	59094531
	rs8094315	IVS2+49140T>C	18	59087027
	rs8096380	IVS2+64706C>T	18	59071461
	rs8096471	IVS2+36794T>C	18	59099373
	rs949037	IVS2+6174C>T	18	59129993
	rs956572	IVS2-24579T>C	18	58971551
	rs9807663	IVS2+65857T>A	18	59070310
	rs9972996	IVS2+63297T>C	18	59072870
	rs9989529	*14480G>A	18	58932357
BCL2A1	rs1138357	Ex1+238G>A	15	78050461
	rs11631974	-8538T>C	15	78059055
	rs11636338	*7818T>G	15	78032645
	rs12372938	-7282G>A	15	78057799
	rs17215263	IVS1-2740A>C	15	78043311
	rs17287997	-11578T>C	15	78062095
	rs2562754	IVS1-1693G>A	15	78042264
	rs6495460	IVS1-3839A>G	15	78044410
BCL2L1	rs6060563	*4948A>G	20	29712464
	rs6060843	IVS2+12411C>G	20	29760708
	rs7270207	-24396C>G	20	29784544
	rs7354225	IVS2+12577G>A	20	29760542
BCL2L2	rs1955559	-2711A>G	14	22857807
BCL2L10	rs11637028	*9535T>C	15	50179801
	rs12396	Ex13-194C>G	15	50200608
	rs12909161	IVS9-50C>A	15	50207783
	rs2398	-2485G>A	15	50194701
	rs2414133	*2390T>C	15	50199855
	rs3751601	IVS1+219C>T	15	50191508
BCL2L11	rs11681263	-13312C>A	2	111584481
	rs13388646	*13839C>T	2	111581386

Gene region	SNP (rs #)	Gene information	Chr	Chr position
	rs1470053	IVS6+4465G>T	2	111632417
	rs17041883	IVS5-1615C>A	2	111626213
	rs17484848	IVS3-1505T>C	2	111617031
	rs2289321	-11102T>C	2	111586691
	rs3761704	IVS4+1601A>G	2	111620169
	rs3789068	IVS5+1523A>G	2	111625718
	rs6727356	IVS6+1248A>G	2	111629200
	rs6760053	*11188C>G	2	111649468
	rs686952	IVS6-2364A>C	2	111635817
	rs726430	*9612T>C	2	111647892
	rs7567444	*12362C>T	2	111579909
BCL6	rs1005099	IVS10-117G>T	3	188923200
	rs1474326	IVS10+202G>T	3	188925221
	rs1523465	*10737A>C	3	188912202
	rs1523474	IVS1+3701A>G	3	188942191
	rs1523475	IVS8+309A>G	3	188926904
	rs17797517	-14000G>A	3	188948176
	rs3172469	IVS1+4110A>C	3	188941782
	rs4686467	IVS1+902T>C	3	188944990
	rs6799313	-18888T>C	3	188953064
	rs9827569	IVS3+175C>A	3	188935176
BCL7A	rs11043307	IVS5+1950C>T	12	120979228
	rs12827036	IVS3-502G>T	12	120965673
	rs1880030	IVS4+2581G>A	12	120968923
	rs1916334	IVS4+2335G>A	12	120968677
	rs745327	-3473C>T	12	120940907
BCL7C	rs4889653	*3472G>A	16	30803214
	rs9933843	IVS4+228G>A	16	30811180
BCL10	rs11161586	-19750G>A	1	85534374
	rs11576939	Ex2-34C>G	1	85514600
	rs12087340	-4957G>A	1	85519581
	rs12744565	IVS2+2201T>C	1	85512366
	rs12757160	-17632T>C	1	85532256
	rs2647395	*4645C>G	1	85501252

Gene region	SNP (rs #)	Gene information	Chr	Chr position
	rs2735592	-1705A>C	1	85516329
	rs4949927	IVS2+1955A>G	1	85512612
	rs6693365	-7679G>C	1	85522303
	rs962409	Ex4+725T>G	1	85505529
CASP2	rs10500136	IVS1+759T>C	7	142724366
	rs3181165	IVS1-6G>A	7	142696806
	rs3181166	IVS2+149T>G	7	142697020
	rs4647322	IVS10+488T>C	7	142708143
	rs7806162	-1245A>C	7	142691927
	rs7810486	*4946A>G	7	142717233
CASP3	rs1049253	Ex8-102T>C	4	185785945
	rs2696057	IVS4+614G>C	4	185792828
	rs2705897	IVS5-4A>C	4	185790092
	rs2720376	IVS4-750G>A	4	185791294
	rs2720378	IVS2+1506G>C	4	185805107
	rs2720380	IVS4-523A>T	4	185819399
	rs4647610	IVS2+1688G>A	4	185804925
	rs4862401	IVS6+1129G>C	4	185825341
	rs870825	IVS6+827A>G	4	185825039
CASP4/CASP5/CASP1	rs10791740	*5825G>A	11	104314864
	rs11226565	IVS7-374T>C	11	104323503
	rs12800151	*11326G>A	11	104430188
	rs17446518	IVS3+451T>A	11	104378760
	rs1785882	-17234T>A	11	104428285
	rs1785883	*12058T>C	11	104429456
	rs2282657	IVS2+7T>C	11	104383013
	rs3181174	IVS2+1086A>T	11	104381934
	rs3181175	IVS2-1151A>G	11	104380471
	rs3181330	IVS6+352T>C	11	104374470
	rs4121642	*9404C>T	11	104362276
	rs492859	-5645T>G	11	104390522
	rs501626	-12291A>G	11	104423342
	rs507879	Ex2-118A>G	11	104383137
	rs508760	*12353A>C	11	104429161

Gene region	SNP (rs #)	Gene information	Chr	Chr position
	rs528076	-2347A>G	11	104413398
	rs540819	IVS8+6T>A	11	104371671
	rs571407	IVS5+380G>A	11	104326482
	rs609092	IVS1-1531C>T	11	104334163
	rs620080	IVS4-352G>A	11	104327387
	rs7123277	IVS1-1315A>G	11	104333947
	rs9326349	-9128G>C	11	104420179
	rs9651713	IVS2-647C>T	11	104379967
CASP6	rs1541373	-4342G>A	4	110848343
	rs1800627	IVS5-1493T>C	4	110833107
	rs2285714	Ex3+60G>A	4	110858259
	rs3181345	IVS4-57G>A	4	110835362
	rs3733611	Ex5+47T>C	4	110823233
	rs5030552	IVS2-82A>G	4	110838455
	rs5030606	*218G>A	4	110828416
	rs739733	IVS4-1702A>G	4	110821485
	rs768063	IVS1-958A>G	4	110839905
CASP7	rs11196418	-13264A>G	10	115428456
	rs11196422	IVS2+3353G>A	10	115433080
	rs11196444	IVS4+11325G>C	10	115458677
	rs11196454	*4433C>T	10	115483723
	rs12358301	*8018C>T	10	115487308
	rs12358524	Ex3-49C>T	10	115441771
	rs12416109	IVS6-461G>A	10	115474650
	rs3124737	*7795G>A	10	115487085
	rs3814231	IVS5+91C>T	10	115471008
	rs4342983	IVS2-1209C>G	10	115440504
	rs4497356	IVS4-3352T>A	10	115467429
	rs6585241	IVS9+417G>A	10	115476600
	rs7907519	IVS2+1946A>C	10	115431673
CASP8AP2	rs11754332	IVS1+4328G>C	6	90581618
	rs11755610	-6759A>G	6	90654149
	rs11967579	IVS1+183C>A	6	90596729
	rs12215515	-21353C>T	6	90591676

Gene region	SNP (rs #)	Gene information	Chr	Chr position
	rs12661230	-20841A>G	6	90592188
	rs17764308	-1919C>T	6	90658989
	rs192655	IVS1-5005C>T	6	90574999
	rs2585018	*1189G>C	6	90641439
	rs292240	*511G>A	6	90663052
	rs292241	-682G>A	6	90660226
	rs441577	*8694T>C	6	90648944
	rs456671	-17226G>C	6	90595803
	rs466321	*9077G>C	6	90649327
	rs6913036	IVS1-4016G>A	6	90608986
	rs7744538	IVS1-7575C>T	6	90577569
	rs9362695	IVS6-462C>T	6	90628104
CASP9	rs12130370	IVS5+729T>C	1	15683211
	rs2020902	IVS3+8T>C	1	15706947
	rs3766160	Ex4-17G>A	1	15681459
	rs4646047	IVS5-530A>G	1	15704370
	rs4646092	IVS7+95G>A	1	15694260
	rs4661636	IVS6-1114G>A	1	15695648
	rs7516435	IVS4-2202A>G	1	15741279
CASP10/CASP8	rs1035140	*1173A>T	2	201860736
	rs1045485	Ex13+51G>C	2	201857834
	rs10931936	IVS11+2101T>C	2	201852173
	rs11679181	IVS11+1623G>A	2	201870583
	rs11899004	IVS2-8753G>A	2	201822271
	rs12613347	IVS3-2395C>T	2	201763557
	rs12693932	IVS11-261T>C	2	201801640
	rs2293554	IVS5+73T>G	2	201839832
	rs3731714	IVS5+149C>T	2	201769065
	rs3769821	IVS3+325C>T	2	201831675
	rs3769825	IVS2-11399A>G	2	201819625
	rs6736233	IVS2-3805G>C	2	201827219
	rs700636	*1934A>C	2	201861497
CASP14	rs10425745	*2935A>T	19	15030836
	rs16980286	*8052C>T	19	15035953

Gene region	SNP (rs #)	Gene information	Chr	Chr position
	rs3181163	IVS4+77T>C	19	15027162
	rs3181309	*894C>T	19	15028795
	rs4808901	-5786C>T	19	15018276
	rs5021087	*13181G>T	19	15008113
	rs714920	-11344T>C	19	15012718
	rs8110862	*9276A>C	19	15004208
CCND1	rs2450254	-6297A>T	11	69158965
	rs592483	-10908C>T	11	69154354
	rs603965	Ex4-1G>A	11	69172091
	rs649392	IVS4-1093G>A	11	69173974
LIG3	rs3744355	Ex1+471G>C	17	30313159
	rs8249	Ex1-269A>T	17	30314050
LMO2	rs10128650	-41118T>G	11	33888627
	rs10836123	IVS5-1348A>C	11	33839045
	rs10836126	IVS1-2768G>A	11	33862777
	rs10836127	IVS1-4365G>T	11	33864374
	rs10836129	IVS1+894A>C	11	33869024
	rs1885524	IVS1-476G>A	11	33860485
	rs3740617	Ex6+106A>G	11	33837592
	rs3758638	IVS2+3414G>T	11	33856532
	rs3758640	-23495T>C	11	33871004
	rs3758642	-24958T>C	11	33872467
	rs3781575	IVS5+829A>G	11	33841895
	rs3781578	IVS1-3393C>T	11	33863402
	rs3824848	IVS4-184G>A	11	33843123
	rs4007	IVS4-1179A>T	11	33844118
	rs4756077	IVS1-4522A>C	11	33864531
	rs746481	IVS1-1938C>T	11	33861947
	rs750781	-26533A>T	11	33874042
	rs7941248	-35042G>A	11	33882551
	rs911817	IVS1+4795G>A	11	33865123
	rs941940	-33854C>T	11	33881363
	rs941941	-33790A>G	11	33881299
MRE11A	rs11020806	*12273A>G	11	93883620

Gene region	SNP (rs #)	Gene information	Chr	Chr position
MYC	rs10505505	-9068G>T	8	128808953
	rs10956383	*10090C>T	8	128832477
	rs11782002	*9659C>T	8	128832046
	rs16902357	-10780C>T	8	128807241
	rs16902359	-5988C>T	8	128812033
	rs16902364	-3573A>C	8	128814448
	rs17187428	-2495G>A	8	128815526
	rs3891248	IVS1-355T>A	8	128819321
	rs4645943	-1368T>C	8	128816653
	rs4645956	IVS1-282C>T	8	128819394
	rs4733550	*9957A>T	8	128832344
PIM1	rs10507	Ex6+713C>T	6	37250400
RIPK1	rs10498658	IVS7+147C>T	6	3049695
	rs12200314	NC_*9223C>T	6	3003689
	rs2326173	-1504A>G	6	3020552
	rs6596945	-5144A>C	6	3016912
	rs6920337	-3145A>G	6	3018911
	rs7739011	IVS9-757C>T	6	3057529
	rs7765221	IVS8+2003A>G	6	3053287
	rs7775816	NC_*7946A>G	6	3002412
	rs9391981	IVS5+1364C>G	6	3032005
	rs9392454	IVS5+1873G>A	6	3032514
RIPK2	rs13250228	-5811A>T	8	90833612
	rs13276910	-12480G>T	8	90826943
	rs16900429	IVS2+1083T>C	8	90845430
	rs218932	-17348A>G	8	90822075
	rs390993	IVS1-1848G>A	8	90842346
	rs39508	IVS7-976G>A	8	90864443
TP53	rs12951053	IVS7+92T>G	17	7518132
TP53I3	rs10200844	IVS2-2034T>G	2	24146867
	rs10495746	IVS3-370A>T	2	24156384
	rs2303287	IVS4+68G>A	2	24155750
	rs6733127	*12647T>C	2	24179309
	rs7604723	-6646G>A	2	24167347

Appendix II. Comparison of distribution between the included and the excluded total population in this study

	Included (%) (n=211)	Excluded (%) (n=179)	[a]*P*-value
Sex			0.104
Male	144 (68.25)	108 (60.34)	
Female	67 (31.75)	71 (39.66)	
Age at diagnosis (years)			0.125
≤5	76 (42.46)	105 (49.76)	
6-9	43 (24.02)	56 (26.54)	
10-14	47 (26.26)	43 (20.38)	
15-19	13 (7.26)	7 (3.32)	
Birth weight (kg)			0.183
< 3.25	88 (49.16)	116 (55.50)	
3.25-3.70	56 (31.28)	66 (31.58)	
> 3.70	35 (19.55)	27 (12.92)	
Breast feeding			0.410
No	79 (44.38)	102 (48.57)	
Yes	99 (55.62)	108 (51.43)	
Education levels for both father and mother			0.083
High school or less	52 (29.21)	69 (32.86)	
University graduate	52 (29.21)	41 (19.52)	
Graduate school or more	74 (41.57)	100 (49.62)	
Family history of cancer			0.198
No	110 (52.38)	102 (52.38)	
Yes	100 (47.62)	71 (41.04)	

[a]Two-sided *P*-value based on chi-square test

Appendix III. Comparison of distribution between the included and the excluded case population in this study

	Included (%) (n=63)	Excluded (%) (n=73)	[a]*P*-value
Sex			0.173
Male	45 (71.43)	44 (60.27)	
Female	18 (28.57)	29 (39.73)	
Age at diagnosis (years)			0.062
≤5	27 (42.86)	35 (47.95)	
6-9	22 (34.92)	13 (17.81)	
10-14	13 (20.63)	19 (26.03)	
15-19	1 (1.59)	6 (8.22)	
Birth weight (kg)			0.809
< 3.25	27 (42.86)	31 (42.47)	
3.25-3.70	24 (38.10)	25 (34.25)	
> 3.70	12 (19.05)	17 (23.29)	
Breast feeding			0.547
No	30 (47.62)	31 (42.47)	
Yes	33 (52.38)	42 (57.53)	
Education levels for both father and mother			0.621
High school or less	21 (33.33)	28 (38.89)	
University graduate	15 (23.81)	19 (26.39)	
Graduate school or more	27 (42.86)	25 (34.72)	
Family history of cancer			0.550
No	30 (47.62)	38 (52.78)	
Yes	33 (52.38)	34 (47.22)	

[a]Two-sided *P*-value based on chi-square test

Appendix IV. Comparison of distribution between the included and the excluded control population in this study

	Included (%) (n=148)	Excluded (%) (n=106)	[a]*P*-value
Sex			0.286
Male	99 (66.89)	64 (60.38)	
Female	49 (33.11)	42 (39.62)	
Age at diagnosis (years)			0.165
≤5	78 (52.70)	105 (49.76)	
6-9	34 (22.97)	56 (26.54)	
10-14	30 (20.27)	43 (20.38)	
15-19	6 (4.05)	7 (3.32)	
Birth weight (kg)			0.264
< 3.25	89 (60.96)	57 (53.77)	
3.25-3.70	42 (28.77)	31 (29.25)	
> 3.70	15 (10.27)	18 (16.98)	
Breast feeding			0.609
No	72 (48.98)	48 (45.71)	
Yes	75 (51.02)	57 (54.29)	
Education levels for both father and mother			0.029
High school or less	48 (32.65)	24 (22.64)	
University graduate	26 (17.69)	33 (31.13)	
Graduate school or more	73 (49.66)	49 (46.23)	
Family history of cancer			0.160
No	80 (54.42)	64 (63.37)	
Yes	67 (45.58)	37 (36.63)	

[a]Two-sided *P*-value based on chi-square test

Abstract in Korean

*CASP7, CASP14*의 유전적 다형성과 소아 백혈병의 위험도에 관한 연관성 연구

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의과학과

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백혈병은 소아암 중에서 가장 빈번하게 발생하는 암 종이다. 한국뿐만 아니라 세계적으로 소아 사망률의 주요 사망 원인은 백혈병이다. 세포자살 signaling 기전과 세포주기 G0/G1 phase 기전의 비정상적인 발현과 변이는 혈구세포 이상 증식을 일으켜 암 발생에 관여한다. 세포자살 기전과 세포주기 기전에 관여하는 유전자의 유전적 변이가 소아백혈병의 위험도를 높일 수 있다.

소아백혈병 발생에 관계 있는 유전적 바이오마커를 세포자살과 세포주기 기전 내에서 확인하기 위해 병원 기반 환자 대조군 연구를 진행하였다. 본 연구는 동일 기관에서 136 명의 소아 백혈병 환자와 254 명의 대조군을 수집하였다. 대조군은 성별과 나이를 대조하여 수집되었다. 검체의 정도 관리 후에 총 63 명의 백혈병 환자와 148 명의 대조군이 본 연구에 포함되었다. 미국 NIH 의 CGEMS 에서 후보 유전자를 선정하고, CGAP, SNP500 database, International HapMap Project 를 통해서 tag SNP 을 추가하였다. 총 304 개의 SNP 과 31 개의 세포자살, 세포주기 관련 유전자를 선택하였다. Genotyping 은 NIH 에서 시행하였으며 Illumina GoldenGate OPA panel 을 사용하였다. 소아 백혈병과 유전적 요인의 관련성을 보기 위해 additive, dominant 모델을 이용하여 로지스틱 회귀분석을 시행하였다. 분석을 시행할 때 각각의 값은 성별과 출생 시 몸무게로 보정하였다. 유전자 수준에서의 유의성을 확인하기 위해 *minP* test 와 다중비교의 문제점을 오류를 줄이기 위해 FDR 을 사용하였다.

유전자 기반 SNP 분석을 시행한 결과 5 개 유전자 (*CASP7, CASP14, CASP8AP2, MYC, RIPK1*)가 소아 백혈병의 위험도와 관련이 있는 것으로 나타났다. 추가적으로 유전자 수준의 *minP* test 와 FDR 을 하였을

때도 통계적으로 유의함을 보였다. 특히 *CASP7* (rs12416109, rs3814231; P_{trend} = 0.002, 0.009; *minP* = 0.013; FDR = 0.042)와 *CASP14* (rs8110862; P_{trend} < 0.001; *minP* = 0.002; FDR = 0.027)가 소아 백혈병과 관련이 있음을 나타냈다. Dominant model 에서는 *CASP7* 의 SNP rs12416109 가 소아 백혈병의 위험도 증가와 관련이 있었고 (AG+GG vs AA; OR=4.30 CI 1.70-10.87), 반대로, *CASP7* SNP rs3814231 과 *CASP14* SNP rs8110862 는 소아백혈병의 위험도 감소와 관련이 있었다 (CT+TT vs CC; OR=0.46 CI 0.24-0.87 와 AC+CC vs AA; OR=0.34 CI 0.18-0.63). 추가적으로 ALL 과 AML 로 구분하여 아형 분석을 시행하였을 때, *CASP14* 유전자는 통계적으로 여전히 유의한 결과를 보였다.

결론적으로 본 연구는 세포자살, 세포주기 기전과 관련된 유전자의 유전적 다형성이 소아 백혈병의 발생과 관련이 있음을 시사한다.

주요 단어: 소아 백혈병, 유전적 변이, 세포자살(apoptosis), *CASP7, CASP14*

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