RESEARCH COMMUNICATION

Genetic Variants in Interleukin-2 and Risk of Lymphoma among Children in Korea

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Abstract

  To estimate the genetic susceptibility for childhood lymphoma, we conducted an association study for 23 cases and 148 controls. Total 1536 tag single nucleotide polymorphisms (SNPs) were selected in 138 candidate gene regions related to immune responses, apoptosis, the cell cycle, and DNA repair. Twelve SNPs were significantly associated with the risk of lymphoma (P_{trend}<0.05) in six genes (*IL1RN*, *IL2*, *IL12RB1*, *JAK3*, *TNFRSF13B*, and *XRCC3*). The most significant association was seen for *IL2* variant rs2069762 (OR_{TG+GG} vs. TT=3.43 (1.29-9.11), P_{trend}=0.002, min$P$=0.005). These findings suggest that common genetic variants in *IL2* might play a role in the pathogenesis of childhood lymphoma.

Key words: Childhood lymphoma - genetic variation - IL2 - Korea

Introduction

Lymphoma is the third most common cancer of children around the world (Howlader et al., 2011). Although it is known that immune system disorders are main causes of lymphoma, little is known regarding genetic factors involved not only in the immune system but in others like apoptosis, cell cycle, DNA repair (Seidemann et al., 2005).

Although some studies have reported SNPs associated with lymphoma, there are a few studies about immune response, apoptosis, cell cycle and DNA repair genes aimed among children. As gene variations of immune system, *TNF* and *lymphotoxin-a* (*LT-a*) polymorphisms were potential prognostic factors in childhood Non-Hodgkin lymphoma (NHL) (Seidemann et al., 2005). , a DNA repair related gene, variants were associated with decreased Burkitt lymphoma and B-cell lymphoma in children (Baris et al., 2009).

We hypothesized that gene variations of immune response and DNA repair gene may affect childhood lymphoma risk. In order to estimate the genetic susceptibility for childhood lymphoma, we conducted an association study using 1536 SNPs selected in 138 candidate gene regions related to immune response, apoptosis, cell cycle, and DNA repair among Korean children.

Materials and Methods

Subjects

Forty cases and 254 controls were recruited in three hospitals (Seoul National University Hospital, Samsung, Samsung Medical Center, ASAN Medical Center, Boramae Hospital) in Seoul, Korea independently between May 2003 and August 2006. Cases were lymphoma patients aged under 23 and controls were non-cancer patients aged under 23 who recruited from the departments of pediatrics, pediatric surgery, pediatric orthopedic surgery, pediatric urology, and pediatric otolaryngology without a medical history of cancer at the same hospitals. From all cases and controls, informed consent was obtained and questionnaires administered by trained interviewers and peripheral blood samples were collected. The patients comprised both Hodgkin lymphoma (n=4) and non-Hodgkin lymphoma (n=36).

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SNP selection and genotyping

Tag SNPs were selected from common SNPs in candidate gene regions by Cancer Genome Anatomy Project (CGAP) and SNP500 database using the Tagzilla algorithm(Packer et al. 2006)http://taqzilla.nci.nih.gov/. In this process, the chosen tag SNPs were based on minor allele frequency >5% and threshold of r^2>0.8. Analyzed gene regions are comprised of immune response, apoptosis, cell-cycle, DNA repair, and the other genes, and the numbers of tag SNPs are 932, 304, 295, and 5 respectively among total 1536 tag SNPs in each gene region (supplementary table 1). More than half of SNPs (55%) were located in intron, 22% in promoter (flanking region, UTR), 15% in 3' UTR, and 9% in exon among 1536 tag SNPs.

The DNA was extracted from the peripheral blood of cases and controls using the Gentra Puregene Blood Kit (Gentra, USA) and it was quantified using PicoGreen. For genotyping, the GoldenGate™ oligonucleotide pool assay (OPA) was performed among Korean children (Illumina®, San Diego, CA).

Finally, 23 (57.5%) cases and 148 (56.2%) controls were analyzed due to the quality control (QC) of DNA. 1487 SNPs (96.8%) of 1536 tag SNPs were genotyped successfully. Of 1487 SNPs, 373 SNPs were excluded owing to monomorphism (n=200), Hardy-Weinberg Equilibrium (HWE) p<0.001 (n=13), and minor allele frequency (MAF) <0.05 (n=160).

Statistical analysis

A Pearson χ^2 test and Fisher's exact test were used to test whether the genotype conformed to the HWE (P<0.001). Age adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were estimated by unconditional logistic regression model. The sex, age at diagnosis, birth weight, breast feeding, education levels for parents, and family history of cancer were assessed as potential confounding factors of lymphoma risk. Numerical variables like age at diagnosis (<5, 5-9, 10-14, and >14) and birth weight (<3.25 and ≥3.25) were categorized. To calculate the P_{trend} of each SNP, the genotypes was regarded as a continuous variable after coding as 0, 1, and 2. We used the min$P$ test to decrease type I error rate (Chen et al. 2006). The min$P$ test was used to calculate the proportion of p-values that were smaller than the lowest P_{trend} within each gene region with resampling based on 10,000 times permutations of case-control status. The homozygote of the most common allele was set as the reference group of each genotype in dominant and co-dominant models.

All statistical analyses were conducted using the SAS® software version 9.2 (Cary, North Carolina).

Results

The patients consist of 19 male (82.6%) and 4 female (17.4%), and the controls consist of 99 male (66.9%) and 49 female (33.1%). Only age at diagnosis was statistically

Table 1. Immune Response and DNA Repair Gene Regions that are Significantly Associated with Childhood Lymphoma

Genes	SNPs	Lowest P_{trend}*	MinP[†]
IL1RN (Interleukin 1 receptor antagonist)	11	0.005	0.024
IL2 (Interleukin 2)	5	0.002	0.005
IL12RB1 (Interleukin 12 receptor, beta1 subunit)	3	0.004	0.009
JAK3 (Janus kinase3)	12	0.003	0.02
TNFRSF13B (Tumor necrosis factor receptor superfamily, member 13B)	15	0.029	0.019
XRCC3 (X-ray cross-complementing group 3)	4	0.023	0.037

*Adjusted for age; †The proportion of *p*-values that were smaller than the lowest P_{trend} within each gene region with resampling based on 10,000 times permutations of case-control status

Table 2. Tag SNPs in the *IL2* Gene Region Associated with Childhood Lymphoma

Gene and SNP	Cases (%)	Controls(%)	OR* (95% CI)
rs10857092 (-11623T>C)			
CC	12 (52.2)	38 (25.7)	1.00
TC	8 (34.8)	70 (47.3)	0.33 (0.12-0.91)
TT	3 (13.0)	40 (27.0)	0.23 (0.06-0.92)
TC+TT	11 (47.8)	110 (74.3)	0.29 (0.12-0.75)
rs1383048 (-15234G>C)			
GG	18 (78.3)	121 (81.8)	1.00
CG	5 (21.7)	25 (16.9)	1.14 (0.37-3.52)
CC	0 (0.0)	2 (1.4)	-
CG+CC	5 (21.7)	27 (18.2)	1.08 (0.35-3.32)
rs2069762 (-384G>T)			
TT	7 (30.4)	87 (58.8)	1.00
TG	11 (47.8)	54 (36.5)	2.61 (0.92-7.39)
GG	5 (21.7)	7 (4.7)	10.1 (2.37-43.1)
TG+GG	16 (69.6)	61 (41.2)	3.43 (1.29-9.11)
rs2069772 (IVS3-116A>G)			
AA	17 (73.9)	14 (77.0)	1.00
GA	6 (26.1)	27 (18.2)	1.56 (0.54-4.49)
GG	0 0.0	7 (4.7)	-
GA+GG	6 (26.1)	34 (23.0)	1.23 (1.05-1.29)
rs4833248 (-2809T>C)			
CC	7 (31.8)	87 (58.8)	1.00
TC	9 (40.9)	49 (33.1)	2.43 (0.83-7.12)
TT	6 (27.3)	12 (8.1)	6.31 (1.73-23.0)
TC+TT	15 (68.2)	61 (41.2)	3.22 (1.21-8.61)

*adjusted for age

significant between cases and controls. The mean age of controls was younger than that of cases (*P*<0.01). Cases and controls were similar in sex, birth weight, breast feeding, education levels for parents, and family history of cancer (*P*>0.05).

Among gene regions relevant to immune response, apoptosis, cell cycle, and DNA repair, six genes (*IL1RN*, *IL2*, *IL12RB1*, *JAK3*, *TNFRSF13B*, and *XRCC3*) were significantly associated with childhood lymphoma (Table 1). The most significant tag SNP was *IL2* rs2069762 (P_{trend}=0.002, min$P$=0.005). The allele of *IL2* rs2069762

had at increased risk for lymphoma ($OR_{GG\ vs.\ TT}$=10.09, 95% CI=2.37-43.05 in co-dominant model, $OR_{TG+GG\ vs.\ TT}$=3.43, 95% CI=1.29-9.11 in dominant model) (Table 2). The rs2069772 (OR_{GA+GG} vs. AA=1.23, 95% CI=1.05-1.29) and the rs4833248 ($OR_{TT\ vs.\ CC}$=6.31, 95% CI=1.73-23.03 in co-dominant model, $OR_{TC+TT\ vs.\ CC}$=3.22, 95% CI=1.21-8.61 in dominant model) also had an association with risk of lymphoma in *IL2*. On the other hand, a protective effect of *IL2* rs10857092 was observed ($OR_{TT\ vs.\ CC}$=0.23, 95% CI=0.06-0.92 in co-dominant model, $OR_{TC+TT\ vs.\ CC}$=0.29, 95% CI=0.12-0.75 in dominant model).

Discussion

In our association study, there were 12 SNPs in immune response genes (*IL1RN*, *IL2*, *IL12RB1*, *JAK3*, and *TNFRSF13B*) and DNA repair gene (*XRCC3*) to contribute to childhood lymphoma risk. The most significant association with childhood lymphoma was seen in *IL2*.

As a growth factor for T cells and natural killer (NK) cells, IL2 activated lymphoma cells and IL2 levels affected the Hodgkin lymphoma risk (Skinnider et al. 2002; Cozen et al., 2008; Chan et al., 2010). Although *IL2* rs2069762 in our study was not associated with increased risk of NHL in a pooled analysis, the rs2069762 was associated with follicular lymphoma (FL) survival in adult (Rothman et al., 2006; Cerhan et al., 2007). Up to date, most studies about lymphoma and genetic factors were focused on adults. Still there were only a few studies about genetic factors and risk of lymphoma, further study could be needed to understand immunological etiology of lymphoma.

One of the limitations of this study is a small sample size. In order to decrease the possibility of false-positive results, we conducted 10,000 permutation test with lowest P_{trend} at the gene level. We could not perform subtype analysis according to lymphoma subtypes because of the small sample sizes. Although only a proportion of the collected samples were able to be analyzed at the quality control step, there were no significant differences in selected characteristics between subjects included and excluded in the analysis

Our study has a comprehensive candidate gene approach related to immune response, apoptosis, cell cycle, DNA repair with 1536 SNPs in 138 genes for childhood lymphoma. In conclusion, our findings suggest that *IL2* genetic variation might play a role in the pathogenesis of childhood lymphoma. However, additional larger studies are needed to replicate these findings and understand the mechanisms of these genes in childhood lymphoma patients.

Acknowledgement

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