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Original Article

Association between single nucleotide polymorphism of N-acetyltransferase 2 and susceptibility to acute lymphoblastic leukemia in Chinese Han children

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Abstract: Aims: To investigate the association of four single nucleotide polymorphisms (SNPs) within NAT2 gene and additional gene-gene interaction with ALL risk in Chinese children. Methods: Generalized multifactor dimensionality reduction (GMDR) was used to screen the best interaction combination among 4 SNPs; Logistic regression was performed to investigate association between 4 SNPs within NAT2 gene and ALL risk and additional gene-gene interaction between rs1799931 and rs1801280. Results: The carriers of homozygous mutant or heterozygous of rs1801280, rs1799930 and rs1799931 were all associated with increased ALL risk than those with wild-type homozygotes, OR (95% CI) were 1.41 (1.12-1.89), 1.54 (1.20-1.95) and 1.76 (1.26-2.32), respectively. GMDR analysis indicated a significant interaction between rs1799931 and rs1801280 on ALL risk. Participants with rs1799931-GA/AA and rs1801280-TC/CC genotype have the highest ALL risk, compared to participants with rs1799931-GG and rs1801280-TT genotype, OR (95% CI) = 3.02 (1.86-4.26). Haplotype containing the rs1799931-A and rs1799931-A allele was associated with increased ALL risk. Conclusions: We found that rs1801280, rs1799930 and rs1799931 within NAT2 gene, interaction between rs1799931 and rs1801280 and haplotype containing the rs1799931-A and rs1799931-A allele were all associated with increased ALL risk.

Keywords: Acute lymphoblastic leukemia, NAT gene, SNP, interaction, haplotype

Introduction

Acute lymphoblastic leukemia (ALL) was one type of leukemia and could account for nearly 25-30% of all cases in all childhood malignancies [1, 2]. However, almost 20% of the children with ALL either relapse or do not respond to treatment [3]. So ALL was a main cause for mortality among children under age 19 years old [4]. Although the clinical, pathological and immunophenotypic features of this disease have been well descripted previously [5], the genetic related mechanism of pediatric ALL were still not well known. Study suggested that genetic factors may play an important role in ALL susceptibility [6]. It is generally considered that the ALL susceptibility is influenced by both environmental and genetic risk factors as well as their synergistic effect with each other [7].

N-acetyltransferase 2 (NAT2), which is a carcinogen-metabolizing gene, play an important role in the detoxification of aromatic and heterocyclic amines and their metabolites in substances [8]. Since the substitution of amino acid residues caused by a single nucleotide change may have a potential effect on biological activity of the gene product [9, 10], and the polymorphisms within the NAT2 gene may modify the some types of cancer risk. Although the NAT2 gene was not the most common variation in several ethnic groups, such as Asians [11], several studies were conducted to investigate the association between NAT2 gene polymorphisms and some types of cancer, including breast cancer [12], bladder cancer [13], pancreatic cancer [14] and lung cancer [15]. However, till now, less study focused on the relationship of NAT2 gene with ALL risk, particu-

Table 1. Description and primers used for genotyping for 4 SNPs

ID	SNP	Chromosome	Functional Consequence	Nucleotide substitution	Probe sequences
rs1801280	T341C	8:18400344	Missense	T>C	GTTCACCTTCTCCTGCAGGTGACCA [C/T] TGACGGCAGGAATTACATTGTCGAT
rs1799930	G590A	8:18400593	Missense	G>A	ATATACTTATTTACGCTTGAACCTC [A/G] AACAATTGAAGATTTTGAGTCTATG
rs1799931	G857A	8:18400860	Missense	G>A	AATCTCGTGCCCAAACCTGGTGATG [A/G] ATCCCTTACTATTTAGAATAAGGAA
rs1208	A803G	8:18400806	Missense	A>G	GAGGAAGAGGTTGAAGAAGTGCTGA [A/G] AAATATATTTAAGATTTCCTTGGGG

Table 2. General characteristics of 673 study participants in cases and controls

Variables	ALL Case group (n = 223)	Control group (n = 450)	*P*-values
Age (year)	9.4±5.6	9.2±5.3	0.651
Males, N (%)	95 (42.6)	203 (45.1)	0.537
Total leukocyte count (*10^9/L)	18.3±6.1	8.6±5.5	<0.001
Hemoglobin concentration (g/dL)	8.5/2.5-13.3	13.5/12.2-14.5	
Platelet count (*10^9/L)	54.6/7.5-641	271.3/151-430	
FAB, N (%)			
L1	41 (18.4)		
L2	158 (70.9)		
L3	24 (10.8)		
Immunophenotype (n/%)			
B-ALL	196 (87.9)		
T-ALL	27 (12.1)		

FAB, French-American-British classification; ALL, acute lymphoblastic leukemia.

larly in Chinese Children. So in current study, we aimed to investigate the association of four single nucleotide polymorphisms (SNPs) within NAT2 gene and additional gene-gene interaction with ALL risk in Chinese Children.

Materials and methods

Subjects

All participants were consecutively recruited between July 2011 and March 2015 from the Second Hospital of Shanxi Medical University. All diagnoses were made in accordance with morphological, cytogenetic/genetic, and immunophenotypic (flow cytometry and immunochemistry) criteria of 2001 WHO classification. The case group included 223 children with newly diagnosed ALL (95 males and 128 females) with age ranging from 3 to 16 years (mean ± standard deviation (SD) = 9.4±5.6 years), including 196 B-ALL and 27 T-ALL patients. Healthy controls were randomly selected from volunteers, who received physical examination in the same hospital and approximate 1:2 matched to ALL cases on the basis of age (±2 years) and sex. Questionnaire investigation was conducted for all participants, and data on demographic information, clinical and biochemical index for all participants were obtained. Blood samples were collected from each participant. Informed consent was obtained from all participants. The protocol of this study was approved by the Ethics Committee of Shanxi Medical University.

Genomic DNA extraction and genotyping

Genomic DNA from participants was extracted from EDTA-treated whole blood, using the DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and stored at 20°C until use. Genomic DNA from participants was extracted from EDTA-treated whole blood, using the DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Four SNPs were detected by Taqman fluorescence probe. Description and probe sequences of these SNPs were shown in **Table 1**. ABI Prism7000 software and allelic discrimination procedure was used for genotyping of fore-mentioned 4 SNPs. A 25 μl reaction mixture including 1.25 μl SNP Genotyping Assays (20×), 12.5 μl Genotyping Master Mix (2×), 20 ng DNA, and

Table 3. Genotype and allele frequencies and analysis on association between 4 SNPs and ALL risk

SNP	Genotypes and Alleles	Frequencies N (%)		OR (95% CI)*	*P*-values in HWE test for controls
		Cases (n = 223)	Controls (n = 450)		
rs1801280 (341T>C)					0.525
	Additive				
	TT	112 (50.2)	287 (63.8)	1.00	
	TC	89 (39.9)	142 (31.6)	1.39 (1.07-1.79)	
	CC	22 (9.9)	21 (4.7)	1.72 (1.20-2.28)	
	Dominant				
	TT	112 (50.2)	287 (63.8)	1.00	
	TC+CC	111 (49.8)	163 (36.2)	1.41 (1.12-1.89)	
	Allele, C (%)	133 (29.8)	184 (20.4)		
rs1799930 (590G>A)					0.967
	Additive				
	GG	115 (51.6)	297 (66.0)	1.00	
	GA	91 (40.8)	137 (30.4)	1.47 (1.13-1.86)	
	AA	17 (7.6)	16 (3.6)	1.74 (1.32-2.21)	
	Dominant				
	GG	115 (51.6)	297 (66.0)	1.00	
	GA+AA	108 (48.4)	153 (34.0)	1.54 (1.20-1.95)	
	Allele, A (%)	125 (28.0)	169 (18.8)		
rs1799931 (857G>A)					0.870
	Additive				
	GG	104 (46.6)	283 (62.9)	1.00	
	GA	93 (41.7)	147 (32.7)	1.70 (1.23-2.25)	
	AA	26 (11.7)	20 (4.4)	1.94 (1.32-2.61)	
	Dominant				
	GG	104 (46.6)	283 (62.9)	1.00	
	GA+AA	119 (53.4)	167 (37.1)	1.76 (1.26-2.32)	
	Allele, A (%)	145 (32.5)	187 (20.8)		
rs1208 (803A>G)					0.647
	Additive				
	AA	126 (56.5)	267 (59.3)	1.00	
	AG	80 (35.9)	157 (34.9)	1.30 (0.83-1.82)	
	GG	17 (7.6)	26 (5.8)	1.44 (0.72-2.16)	
	Dominant				
	AA	126 (56.5)	267 (59.3)	1.00	
	AG+GG	97 (43.5)	183 (40.7)	1.34 (0.80-1.89)	
	Allele, G (%)	114 (25.6)	209 (23.2)		

*Adjusted for gender and age.

the conditions were as follows: initial denaturation for 10 min and 95°C, denaturation for 15 s and 92°C, annealing and extension for 90 s and 60°C, 50 cycles. Genotyping results were confirmed by randomly assaying 10% of the original specimens for replication to exclude genotyping errors. There were no discrepancies between genotypes determined in duplicate.

Statistical analysis

Statistical analyses were performed with the STATA version 12.0 (StataCorp, USA). The means and SDs were calculated for normally distributed continuous variables and compared using Student's t test. Percentages were calculated for categorical variables and compared

Table 4. GMDR analysis on the best gene-gene interaction combinations

Locus no.	Best combination	Cross-validation consistency	Testing accuracy	*p-values**
2	rs1799931 rs1801280	10/10	0.6072	0.0010
3	rs1799931 rs1801280 rs1799930	7/10	0.5399	0.1719
4	rs1799931 rs1801280 rs1799930 rs1208	6/10	0.4958	0.3770

*Adjusted for gender and age.

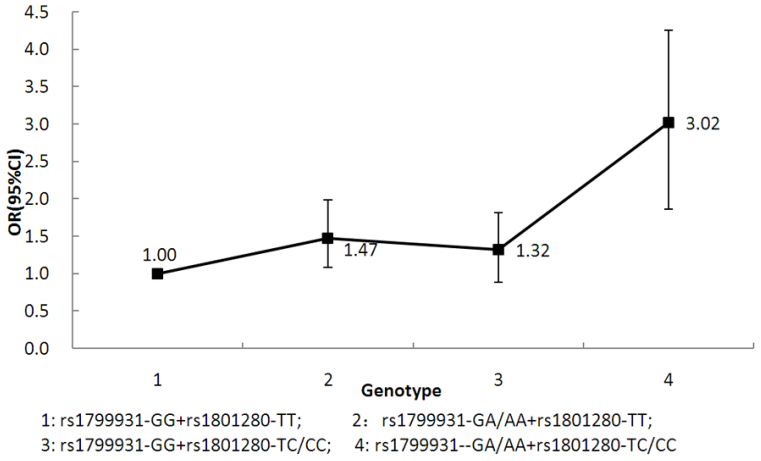

Figure 1. Logistic regression for interaction between rs1799931 and rs1801280.

using χ^2 test. Hardy-Weinberg equilibrium for genotype frequencies and genotype and Pairwise LD analysis were tested using SNPstats (http://bioinfo.iconcologia.net/SNPstats). Generalized multifactor dimensionality reduction (GMDR) was used to screen the best interaction combination among 4 SNPs; some parameters were calculated, such as cross-validation consistency, the testing balanced accuracy, and the sign test. Logistic regression was performed to investigate association between 4 SNPs within NAT2 gene and ALL risk and additional gene-gene interaction between rs1799931 and rs1801280. All reported *p*-values were two-tailed, and those less than 0.05 were considered statistically significant.

Results

Table 2 shows the general demographic characteristics and blood biochemical index in cases and controls. A total of 673 participants (290 males, 343 females) were selected, including 223 ALL patients and 450 controls. The patients included 196 B-ALL and 27 T-ALL patients. The mean age of all participants was 9.3±5.1 years. The rate of males and the mean of age are not significantly different in cases and controls. **Table 3** shows the frequencies of genotypes and alleles for the studied 4 SNPs and analysis on association between SNPs and ALL risk. All genotypes were distributed according to the Hardy-Weinberg equilibrium. Logistic analysis showed that rs1801280, rs1799930 and rs1799931 within NAT2 gene were both associated with increased ALL risk after adjustment for gender and age in additive and dominant models. The carriers of homozygous mutant or heterozygous of rs1801280, rs1799930 and rs1799931 were all associated with increased ALL risk than those with wild-type homozygotes, OR (95% CI) were 1.41 (1.12-1.89), 1.54 (1.20-1.95) and 1.76 (1.26-2.32), respectively. We did not find any relation of rs1208 with ALL risk in both models.

GMDR model was used to screen the best interaction combination among 4 SNPs within NAT2 gene. **Table 4** summarizes the results obtained from GMDR analysis and indicated a significant two-locus model (P = 0.0107) involving rs1799931 and rs1801280, indicating a potential interaction between rs1799931 and rs1801280 on ALL risk, the cross-validation consistency of the two-locus models was 10/10, and the testing accuracy was 60.72%. In order to investigate the interaction effect between the two SNPs, we conducted an interaction analysis by using logistic regression (**Figure 1**), to obtain the odds ratios and 95% CI for the joint effects. We found that participants with rs1799931-GA/AA and rs1801280-TC/CC genotype have the highest ALL risk, compared to participants with rs1799931-GG and rs1801280-TT genotype, OR (95% CI) = 3.02 (1.86-4.26).

Pairwise LD analysis among the 4 SNPs was performed, and just D' value between rs1799931 and rs1799931 was 0.834, which

Table 5. Haplotype analysis on association between NAT2 gene and ALL risk

Haplotypes	rs1799931	rs1799930	Frequencies		OR (95% CI)	*p*-values*
			Case group	Control group		
H1	G	G	0.4505	0.4764	1.00	--
H2	A	G	0.2788	0.2869	1.31 (0.95-1.72)	0.106
H3	G	A	0.1958	0.1999	1.42 (0.91-1.95)	0.221
H4	A	A	0.0749	0.0368	1.76 (1.18-2.36)	<0.001

*Adjusted for gender and age.

was more than 0.80. So we conducted haplotype analysis for the two SNPs, we found that the most common haplotype was rs1799931-G and rs1799931-G haplotype, the frequency of which was 0.4505 and 0.4764 in case group and control group, and a haplotype containing the rs1799931-A and rs1799931-A allele was associated with increased ALL risk, after adjustment for gender and age. (**Table 5**).

Discussion

In this study, we found that rs1801280, rs1799930 and rs1799931 within NAT2 gene were associated with increased ALL risk in Chinese children, but we did not find any relation of rs1208 with ALL risk in both models. Although our study is not the first case-control study to investigate the association between the human NAT2 gene and ALL risk, previously several studies were conducted to investigate this relationship, however, considering the limited number on this topic in Chinese children and inconsistent results from previous studies, perhaps substantially different results, we think the results obtained from this study is necessary and meaningful for understanding the mechanism of ALL, particularly for susceptibility lead by NAT2 gene polymorphisms. Silveira et al [16] found no significant differences between the case and control groups analyzed regarding NAT2 variant polymorphisms. Gra et al [17] indicated that the frequency of NAT2 genotype major alleles were associated with 1.8-folds increased acute leukemia risk in children as compared to healthy controls, it means that minor alleles of these SNPs were associated with decreased ALL risk. Studies also reported that there was no impact of NAT2 acetylator genotypes on the ALL risk both in children [18] and in adults [19]. Also, none of the published GWAS have documented NAT2 as a risk factor in ALL susceptibility [20], yet the studied populations were all of Western origin. All other ethnic groups were deliberately excluded. The inconsistent results were also obtained by Krajinovic et al [21], and they suggested that children carrying NAT2 slow-acetylation genotypes were at increased risk of developing ALL. Recently, Kamel et al [22] indicated that NAT2 gene polymorphisms were generally associated with the increased risk of ALL in children. Another study for children in Brazil suggested that NAT2 SNP 341T>C frequency was higher among both leukemia subtypes compared to controls, and demonstrate that NAT2 slow-acetylation phenotypes are associated with higher susceptibility to childhood acute leukemia, with discrimination between ALL and AML.

Previous studies have demonstrated that the interaction between genetic background, life style, and these environmental factors plays a critical role in development of ALL in children [23]. In this study, we found a significant gene-gene interaction between rs1799931 and rs1801280 on ALL risk; participants with rs1799931-GA/AA and rs1801280-TC/CC genotype have the highest ALL risk. Previous studies [17, 22] have documented the impact of gene-gene interaction between NAT2 gene and other genes, but to date, less study focused on the impact of gene-gene interaction among NAT2 gene on ALL risk in Chinese children. The results of this study suggest that the risk of ALL may be modified by the two genetic variants and they influenced with each other in susceptibility to ALL risk. The implication of LD in association studies is that knowledge of variation at a certain position also gives knowledge of variation at linked loci. In this study we also conducted the haplotype analysis for the two SNPs, we found a haplotype containing the rs1799931-A and rs1799931-A allele was associated with increased ALL risk, after adjustment for gender and age.

Our study has several limitations. Firstly, the sample in present study was relatively small; the results obtained from this study should be

checked by future studies with large sample size. Secondly, some environmental risk factors should be included in the gene-environment interaction analysis. Thirdly, more SNPs within NAT2 gene should been included in the study, not only for these 4 SNPs.

In conclusion, we found that rs1801280, rs1799930 and rs1799931 within NAT2 gene were both associated with increased ALL risk in Chinese children. We also found that gene-gene interaction between rs1799931 and rs1801280 and haplotype containing the rs1799931-A and rs1799931-A allele were also associated with increased ALL risk.

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Disclosure of conflict of interest

None.

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