Received: 21 February 2020 | Revised: 3 May 2020 | Accepted: 5 May 2020

DOI: 10.1002/jcla.23414

RESEARCH ARTICLE

WILEY

Association of *TLX1* gene polymorphisms with the risk of acute lymphoblastic leukemia and B lineage acute lymphoblastic leukemia in Han Chinese children

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Funding information
This work was supported by funds from the Public Projects of Zhejiang Province (No. 2016C37012) and New Seedling Plan (No. 2019R46022, No. 2018R406036).

Abstract

Background: Studies on gene polymorphism association are centered on childhood acute lymphoblastic leukemia (ALL), a common hematological malignancy in children younger than 16 years. Single-nucleotide polymorphisms (SNPs) in some genes, such as *ARID5B* and *CDKN2B*, are associated with the risk of childhood ALL. T-cell leukemia homeobox 1 (*TLX1*), a member of the *HOX* gene family, was identified based on its abnormal expression in T-lineage leukemia. This study aimed to determine whether *TLX1* is associated with B-ALL and which SNP plays a significant role in ALL.

Methods: A total of 217 cases of ALL and 241 controls were included in this study. Six tag SNPs (rs75329544, rs946328, rs12415670, rs2075879, rs17113735, and rs1051723) were selected, and genotyping was carried out on Sequenom MassARRAY platform.

Results: Rs17113735 was possibly the risk locus associated with increased risk for ALL, whereas rs946328 was possibly associated with decreased risk for ALL. Moreover, rs17113735 was likely to be the risk locus for B-cell ALL (B-ALL), and rs2075879 was associated with decreased risk for B-ALL ($P < .05$). All SNPs in the two sample types (ALL and B-ALL samples) demonstrated linkage disequilibrium except between rs75329544 and rs2075879. Haplotype analysis showed no significant difference between the cases and controls in the two sample types.

Conclusion: *TLX1* gene polymorphisms are associated with ALL (rs17113735 and rs946328) and possibly play a significant role in B-ALL (rs17113735 and rs2075879). This work provides a reference for the diagnosis and therapy of this disease.

KEYWORDS
acute lymphoblastic leukemia, B-cell acute lymphoblastic leukemia, single-nucleotide polymorphism, T-cell leukemia homeobox 1

Endian Mei and Xubin Wei contributed equally to this work.

1 | INTRODUCTION

Acute lymphoblastic leukemia (ALL) is a common hematological malignancy that results from the disorder of lymphoid progenitor cells. The fastigium of ALL occurs between the ages of 2 and 5 years, although it also occurs in children and adults.[1,2] The two cancer types of ALL are T-cell ALL (T-ALL) and B-cell ALL (B-ALL); the former is less common than the latter but is more aggressive.[3-5] Specific gene mutations are possibly associated with abnormalities in the signaling pathway, whose abnormal activation could propel oncogenic alterations in ALL.[6] Chromosomal rearrangement induces the formation of fusion genes, such as *BCR-ABL* and *ETV6-RUNX1*, which are oncogenes that cause ALL.[7,8]

T-cell leukemia homeobox 1 (*TLX1*)/*HOX11*, a member of the *HOX* gene family, is identified through its abnormal expression in T-lineage leukemia; it is a DNA-binding homeodomain protein, but its function remains poorly understood.[9] *TLX1* could be aberrantly activated through the translocation of either t(7;10) or t(10;14), and it usually synergizes with *NOTCH1* activation during malignant T-cell transformation.[10,11] The aberrant expression of *TLX1* in T-cell progenitors not only influences the development of normal T-cells but also contributes to the development of aneuploidy during T-cell transformation, and the disruption of the mitotic checkpoint in *TLX1*-induced tumors may be linked to the acquisition of secondary genetic alterations in T-ALL.[12] In the mouse model from Keersmaecker's[13] study, a typical aneuploid and apparent imperfection in the activation of the mitotic checkpoint were observed in *TLX1* tumors. Moreover, it was demonstrated that *STAT5*, a downstream effector of *BUP214-ABL1*, can co-bind the poised enhancer region with *TLX1*, thereby activating the expression of crux proto-oncogenes, such as *MYC* and *BCL2*, and driving the development of T-ALL.[14]

Analysis of single-nucleotide polymorphism (SNP) predicts the risk of ALL. The SNPs of *ARID5B* were confirmed to be significant determinants of the susceptibility and treatment outcomes of childhood ALL, and contributed to racial disparities in this cancer.[15,16] Healy et al[17] also confirmed the association of 5 SNPs (rs7073837, rs10994982, rs10740055, rs10821936, and rs7089424) in the *ARID5B* gene with childhood acute lymphoblastic leukemia. Identified as novel locus, *PIP4K2A* SNPs (rs7088318 and rs10828317) are significantly associated with ALL susceptibility.[18,19] On the basis of functional analysis, Hungate et al[20] discovered that rs662463 could adjust *CDKN2B* expression through *CEBPB* signaling to affect the risk of BCP-ALL.

This study aimed to determine whether *TLX1* is associated with B-ALL and which SNP plays a significant role in ALL. The SNPs of *TLX1* in ALL and B-ALL were examined to explore *TLX1* susceptibility in this disease and provide a reference for diagnosis and therapy.

2 | MATERIALS AND METHODS

2.1 | Case and control groups

The case group consisted of patients diagnosed with ALL at the Children's Hospital of Zhejiang University School of Medicine from 2014 to 2016. Every diagnosis of ALL was based on MICM. All patients diagnosed with ALL exhibited a change in blood and bone marrow, which met the French-American-British (FAB) classification.[21] A total of 241 healthy children from the same hospital were studied under the control group. All children involved were of Chinese Han ethnicity. Data of these children were collected from the above-mentioned children's hospital and are shown in Table 1. The sample size for this study was set to 217 because the immunophenotype of the two cases is unknown. This study was approved by the Ethical Committee of the School of Life Sciences and Medicine, Zhejiang Sci-Tech University. The ethical number of this study is 1601-05. All the families of patients and controls provided an informed consent to genetic analysis for investigational studies.

2.2 | SNP prediction and genomic DNA extraction

TLX1 SNPs were predicted using Haploview. Six tag SNPs (rs75329544, rs946328, rs12415670, rs2075879, rs17113735, and rs1051723) were selected, and their MAF was higher than 0.05. DNA was extracted from EDTA-anticoagulated blood samples by using a DNA extraction kit (Sangon Biotech Co. Ltd.) following the manufacturer's instructions and then stored at −80°C.

2.3 | SNP genotyping

The primers for the six SNPs are shown in Table 2. SNP genotyping was carried out on Sequenom MassARRAY platform (Sequenom) by applying a 384-well plate format. Genotype calling was conducted in real time on MassARRAY RT software version 3.0.0.4. The results were analyzed via MassARRAY Typer software version 3.4 (Sequenom).

2.4 | Statistical analysis

Data were statistically analyzed using chi-square ($\chi 2$) test to ascertain differences in alleles, genotype, the haplotype frequencies, and the Hardy-Weinberg equilibrium between the case and control groups. The odds ratio (OR) and 95% confidence interval (CI) were calculated for comparison. Logistic regression analysis was conducted to test the association between the risk of ALL and *TLX1* polymorphisms. All statistical analyses were performed using SPSS software version 22.0 (SPSS Inc.). Haploview 4.2 software was utilized to calculate the haplotype block and linkage disequilibrium (LD).

3 | RESULTS

3.1 | ALL Association

Six *TLX1* SNPs (rs75329544, rs946328, rs12415670, rs2075879, rs17113735, and rs1051723) in the ALL and control groups were

TABLE 1 Demographics and clinical characteristics of patients with ALL and controls

Characteristics	Cases (n = 219)	Controls (n = 241)	*P*
Age (years), mean ± SD	6.39 ± 3.93	3.61 ± 3.50	.051
Range	0-16	0-14	
Gender (male/female)	142/77	145/96	.319
Immunophenotype			
B-ALL	173		
T-ALL	17		
Pre–B-ALL	17		
Pro–B-ALL	7		
Early Pre–T-ALL	2		
Pre–T-ALL	1		
No Date	2		
Risk			
Low	55		
Middle	80		
High	83		
No Date	1		
Classification			
L1	13		
L2	182		
L3	15		
No Date	9		
MRD			
Low	59		
Middle	81		
High	71		
No Date	8		
Chromosomal type			
Normal	155		
Hypodiploid	9		
Hyperdiploid	17		
High hyperdiploid	37		
No Date	1		
Relapse	13		

Abbreviations: ALL, acute lymphoblastic leukemia; MRD, minimal residual disease; SD, standard deviation.

analyzed (Table 3). All SNPs were in HWE in the two groups ($P > .05$). The genotype A/A of rs17113735 in the case group was significantly higher than that in the control (OR 3.01, 95% CI 1.33-6.79, $P = .006 < .01$). Moreover, rs17113735 accorded with the recessive model (OR 2.91, 95% CI 1.31-6.46, $P = .006 < .01$) and log-additive model (OR 1.37, 95% CI 1.02-1.84, $P = .038 < .05$). The frequency of the mutation type (A allele) was distinctly higher in the ALL group (OR 1.37, 95% CI 1.02-1.84, $P = .039 < .05$) than in the control. The inherited models and allele analysis showed that the rs17113735 polymorphism possibly increased the risk for ALL. The rs946328 genotype C/T in the ALL group was significantly lower than that in the control group (OR 0.64, 95% CI 0.42-0.98, $P = .039 < .05$), and this locus was consistent with the overdominant model (OR 0.64, 95% CI 0.42-0.98, $P = .037 < .05$). These findings indicated that rs946328 might be associated with decreased risk for ALL. In this analysis, the rs17113735 and rs946328, which belong to *TLX1*, were significantly associated with ALL.

The B-ALL cases and controls were analyzed to determine the association of *TLX1* SNPs with B-ALL (Table 4). Rs17113735 still conformed to the recessive model (OR 2.91, 95% CI 1.29-6.55, $P = .007 < .01$), and its genotype (allele A/A) was higher in the B-ALL group than in the control group (OR 2.94, 95% CI 1.29-6.72, $P = .008 < .01$) but not with the log-additive model. This finding showed that *TLX1* possibly affected the morbidity of B-ALL, and rs17113735 could increase the risk of B-ALL. Moreover, rs2075879 decreased the risk for B-ALL because its genotype (Allele A/G) was higher in the healthy people group (OR 0.66, 95% CI 0.44-0.99, $P = .044 < .05$) than in the B-ALL group.

3.2 | LD and Haplotype

All SNPs in the two sample types (ALL and B-ALL samples) demonstrated LD except between rs75329544 and rs2075879 in the block (Figure 1). The haplotype analysis indicated that both blocks for the two sample types (ALL and B-ALL samples) containing rs75329544, rs946328, rs12415670, rs2075879, s17113735, and rs1051723 showed no significant difference between the two groups (Table 5).

4 | DISCUSSION

TLX1 is a homeobox transcription factor oncogene of T-ALL in humans. *TLX1* expression can be detected in mice during the embryonic phase and is significantly associated with the fate of splenic cells under normal conditions.[22-24] However, the specific mechanisms remain unclear, especially in T-ALL.[13] Bergeron showed that T-ALLs with high expression of *TLX1* harbor molecular *TLX1* locus abnormalities, whereas T-ALLs that express *TLX1* at low levels do not share these characteristics.[25] In the cell cycle, the variation in *TLX1* might contribute to the abnormal proliferation of lymphocytes and promote the development of ALL.[11] *TLX1* can alter the cell cycle, including that of G1/S and G2/M, by interacting with *PP2* and *PP2A*.[26,27] Integrative genomics was used by Durinck et al to study the role of *TLX1* in T-ALL; the results showed that ectopic *TLX1* expression inhibits T cell–specific enhancers and mediates an unexpected transcriptional antagonism with *NOTCH1* at critical target genes, including *IL7R* and *NOTCH3*.[11] Riz et al found that *TLX1* and *NOTCH* cooperate to regulate the transcription in T-ALL, and the *TLX1/NOTCH/MYC* transcriptional network coregulates genes involved in T-cell development.[28] Heidari et al[9] utilized whole-genome PCR and found that the *TLX1* protein interacts with pericentromeric human satellite 2 DNA sequences, which could be related to its roles

TABLE 2 Primers of the six tag SNPs

SNP	Forward Primer	Reverse Primer
rs2075879	5′- ACGTTGGATGTTGGAATGGCACCTGGTCTC-3′	5′- ACGTTGGATGAAACAGCTGGGACTCGCATC-3′
rs12415670	5′-ACGTTGGATGTTGTCGCTGAGGGCTAACG-3′	5′- ACGTTGGATGAGGCAAGCAGCAGAGCGTCA-3′
rs17113735	5′-ACGTTGGATGTAACAGTTCAGACAGGTGCG-3′	5′- ACGTTGGATGCATTTGTGCCGACACTGTTC-3′
rs1051723	5′-ACGTTGGATGGCTGTCATCTGAATTTGCCC-3′	5′- ACGTTGGATGCCTATGGGTTTCCATGTGTG-3′
rs946328	5′-ACGTTGGATGAGCCATACACTCGCTGAAAC-3′	5′- ACGTTGGATGAAAGGTACTCGGTTTAGGGC-3′
rs75329544	5′-ACGTTGGATGACAAGGCGAGGCTTAAAAGG-3′	5′- ACGTTGGATGGAAGACAGTTGACTTCACCC-3′

TABLE 3 Associations between *TLX1* SNPs and ALL

TLX1	Control n = 241(n[%])	ALL n = 217(n[%])	OR (95% CI)	*P*
rs75329544				
genotype				
T/T	210 (87.1%)	184 (84.8%)	1.00	
A/T	31 (12.9%)	32 (14.8%)	1.18 (0.69-2.01)	.546
A/A	0 (0%)	1 (0.5%)	NA (0.00–NA)	.286
Dominant model				
T/T	210 (87.1%)	184 (84.8%)	1.00	
A/T-A/A	31 (12.9%)	33 (15.2%)	1.21 (0.72-2.06)	.47
Recessive model				
T/T-A/T	241 (100%)	216 (99.5%)	1.00	
A/A	0 (0%)	1 (0.5%)	NA (0.00–NA)	.22
Overdominant model				
T/T-A/A	210 (87.1%)	185 (85.2%)	1.00	
A/T	31 (12.9%)	32 (14.8%)	1.17 (0.69-1.99)	.56
Log-additive model				
—	—	—	1.25 (0.74-2.09)	.4
Allele				
T	451 (93.6%)	400 (92.2%)	1.00	
A	31 (6.4%)	34 (7.8%)	1.24 (0.75-2.05)	.409
rs946328				
genotype				
C/C	161 (66.8%)	163 (75.1%)	1.00	
C/T	74 (30.7%)	48 (22.1%)	**0.64 (0.42-0.98)**	**.039**
T/T	6 (2.5%)	6 (2.8%)	0.99 (0.31-3.13)	.983
Dominant model				
C/C	161 (66.8%)	163 (75.1%)	1.00	
C/T-T/T	80 (33.2%)	54 (24.9%)	0.67 (0.44-1.00)	.05
Recessive model				
C/C-C/T	235 (97.5%)	211 (97.2%)	1.00	
T/T	6 (2.5%)	6 (2.8%)	1.11 (0.35-3.51)	.85
Overdominant model				
C/C-T/T	167 (69.3%)	169 (77.9%)	1.00	
C/T	74 (30.7%)	48 (22.1%)	**0.64 (0.42-0.98)**	**.037**

(Continues)

TABLE 3 (Continued)

TLX1	Control n = 241(n[%])	ALL n = 217(n[%])	OR (95% CI)	*P*
Log-additive model				
—	—	—	0.74 (0.52-1.06)	.097
Allele				
T	86 (17.8%)	60 (13.8%)	1.00	
C	396 (82.2%)	374 (86.2%)	1.35 (0.95-1.94)	.254
rs12415670				
genotype				
G/G	126 (52.3%)	107 (49.3%)	1.00	
A/G	93 (38.6%)	85 (39.2%)	1.08 (0.73-1.59)	.713
A/A	22 (9.1%)	25 (11.5%)	1.34 (0.71-2.51)	.362
Dominant model				
G/G	126 (52.3%)	107 (49.3%)	1.00	
A/G-A/A	115 (47.7%)	110 (50.7%)	1.13 (0.78-1.63)	.52
Recessive model				
G/G-A/G	219 (90.9%)	192 (88.5%)	1.00	
A/A	22 (9.1%)	25 (11.5%)	1.30 (0.71-2.37)	.4
Overdominant model				
G/G-A/A	148 (61.4%)	132 (60.8%)	1.00	
A/G	93 (38.6%)	85 (39.2%)	1.02 (0.70-1.49)	.9
Log-additive model				
—	—	—	1.13 (0.86-1.48)	.39
Allele				
G	345 (71.6%)	299 (68.9%)	1.00	
A	137 (28.4%)	135 (31.1%)	1.14 (0.86-1.51)	.655
rs2075879				
genotype				
G/G	110 (45.6%)	118 (54.4%)	1.00	
A/G	109 (45.2%)	81 (37.3%)	0.69 (0.47-1.02)	.063
A/A	22 (9.1%)	17 (7.8%)	0.72 (0.36-1.43)	.346
NA		1 (0.5%)		
Dominant model				
G/G	110 (45.6%)	118 (54.6%)	1.00	
A/G + A/A	131 (54.4%)	98 (45.4%)	0.70 (0.48-1.01)	.055
Recessive model				
G/G + A/G	219 (90.9%)	199 (92.1%)	1.00	
A/A	22 (9.1%)	17 (7.9%)	0.85 (0.44-1.65)	.63
Overdominant model				
G/G-A/A	132 (54.8%)	135 (62.5%)	1.00	
A/G	109 (45.2%)	81 (37.5%)	0.73 (0.50-1.06)	.094
Log-additive model				
—	—	—	0.78 (0.58-1.04)	.088
Allele				
G	329 (68.3%)	317 (73.4%)	1.00	
A	153 (31.7%)	115 (26.6%)	0.78 (0.59-1.04)	.089

(Continues)

TABLE 3 (Continued)

TLX1	Control n = 241(n[%])	ALL n = 217(n[%])	OR (95% CI)	*P*
rs17113735				
genotype				
G/G	139 (57.7%)	113 (52.1%)	1.00	
A/G	93 (38.6%)	82 (37.8%)	1.08 (0.74-1.60)	.681
A/A	9 (3.7%)	22 (10.1%)	**3.01 (1.33-6.79)**	**.006**
Dominant model				
G/G	139 (57.7%)	113 (52.1%)	1.00	
A/G-A/A	102 (42.3%)	104 (47.9%)	1.25 (0.87-1.81)	.23
Recessive model				
G/G-A/G	232 (96.3%)	195 (89.9%)	1.00	
A/A	9 (3.7%)	22 (10.1%)	**2.91 (1.31-6.46)**	**.006**
Overdominant model				
G/G-A/A	148 (61.4%)	135 (62.2%)	1.00	
A/G	93 (38.6%)	82 (37.8%)	0.97 (0.66-1.41)	.86
Log-additive model				
—	—	—	**1.37 (1.02-1.84)**	**.038**
Allele				
G	371 (77.0%)	308 (71.0%)	1.00	
A	111 (23.0%)	126 (29.0%)	**1.37 (1.02-1.84)**	**.039**
rs1051723				
genotype				
C/C	169 (70.1%)	167 (77%)	1.00	
C/T	68 (28.2%)	47 (21.7%)	0.70 (0.46-1.07)	.102
T/T	4 (1.7%)	3 (1.4%)	0.76 (0.17-3.44)	.720
Dominant model				
C/C	169 (70.1%)	167 (77%)	1.00	
C/T-T/T	72 (29.9%)	50 (23%)	0.70 (0.46-1.07)	.098
Recessive model				
C/C-C/T	237 (98.3%)	214 (98.6%)	1.00	
T/T	4 (1.7%)	3 (1.4%)	0.83 (0.18-3.75)	.81
Overdominant model				
C/C-T/T	173 (71.8%)	170 (78.3%)	1.00	
C/T	68 (28.2%)	47 (21.7%)	0.70 (0.46-1.08)	.11
Log-additive model				
—	—	—	0.73 (0.50-1.08)	.11
Allele				
T	76 (15.8%)	53 (12.2%)	1.00	
C	406 (84.2%)	381 (87.8%)	1.35 (0.92-1.96)	.122

Note: Of the 217 ALL cases, 197 were B-ALL and 20 were T-ALL; significant values ($P < .05$) are in bold.

Abbreviations: ALL, acute lymphoblastic leukemia; CI, confidence interval; OR, odds ratio; SNPs, single-nucleotide polymorphisms; *TLX1*, T-cell leukemia homeobox 1.

in transcriptional repression and T-cell immortalization. However, the relationship between *TLX1* and B-ALL needs further researches because the studies on the association between *TLX1* and B-ALL are limited and the pathogenic mechanism is unknown.

Single-nucleotide polymorphisms research on ALL has been conducted for many years, and some ALL-associated gene or SNPs have been extensively investigated.[20,29-33] Papaemmanuil et al[34] identified the risk locus for ALL at 7p12.2 (*IKZF1*, rs4132601, OR = 1.69,

TABLE 4 Associations between *TLX1* SNPs and B-ALL

TLX1	Control n = 241(n[%])	ALL n = 197(n[%])	OR (95% CI)	*P*
rs75329544				
genotype				
T/T	210 (87.1%)	168 (85.3%)	1.00	
A/T	31 (12.9%)	28 (14.2%)	1.13 (0.65-1.96)	.665
A/A	0 (0%)	1 (0.5%)	NA (0.00–NA)	.264
Dominant model				
T/T	210 (87.1%)	168 (85.3%)	1.00	
A/T-A/A	31 (12.9%)	29 (14.7%)	1.17 (0.68-2.02)	.57
Recessive model				
T/T-A/T	241 (100%)	196 (99.5%)	1.00	
A/A	0 (0%)	1 (0.5%)	NA (0.00–NA)	.21
Overdominant model				
T/T-A/A	210 (87.1%)	169 (85.8%)	1.00	
A/T	31 (12.9%)	28 (14.2%)	1.12 (0.65-1.94)	.68
Log-additive model				
—	—	—	1.21 (0.71-2.06)	.49
Allele				
T	451 (93.6%)	364 (92.4%)	1.00	
A	31 (6.4%)	30 (7.6%)	1.20 (0.71-2.02)	.49
rs946328				
genotype				
C/C	161 (66.8%)	145 (73.6%)	1.00	
C/T	74 (30.7%)	46 (23.4%)	0.69 (0.45-1.06)	.091
T/T	6 (2.5%)	6 (3%)	1.11 (0.35-3.52)	.859
Dominant model				
C/C	161 (66.8%)	145 (73.6%)	1.00	
C/T-T/T	80 (33.2%)	52 (26.4%)	0.72 (0.48-1.09)	.12
Recessive model				
C/C-C/T	235 (97.5%)	191 (97%)	1.00	
T/T	6 (2.5%)	6 (3%)	1.23 (0.39-3.88)	.72
Overdominant model				
C/C-T/T	167 (69.3%)	151 (76.7%)	1.00	
C/T	74 (30.7%)	46 (23.4%)	0.69 (0.45-1.06)	.085
Log-additive model				
—	—	—	0.80 (0.55-1.14)	.21
Allele				
C	396 (82.2%)	336 (85.3%)	1.00	
T	86 (17.8%)	58 (14.7%)	1.26 (0.87-1.80)	.215
rs12415670				
genotype				
G/G	126 (52.3%)	96 (48.7%)	1.00	
A/G	93 (38.6%)	78 (39.6%)	1.10 (0.74-1.64)	.639
A/A	22 (9.1%)	23 (11.7%)	1.37 (0.72-2.61)	.333

(Continues)

TABLE 4 (Continued)

TLX1	Control n = 241(n[%])	ALL n = 197(n[%])	OR (95% CI)	*P*
Dominant model				
G/G	126 (52.3%)	96 (48.7%)	1.00	
A/G-A/A	115 (47.7%)	101 (51.3%)	1.15 (0.79-1.68)	.46
Recessive model				
G/G-A/G	219 (90.9%)	174 (88.3%)	1.00	
A/A	22 (9.1%)	23 (11.7%)	1.32 (0.71-2.44)	.38
Overdominant model				
G/G-A/A	148 (61.4%)	119 (60.4%)	1.00	
A/G	93 (38.6%)	78 (39.6%)	1.04 (0.71-1.53)	.83
Log-additive model				
—	—	—	1.15 (0.86-1.52)	.34
Allele				
G	345 (71.6%)	270 (68.5%)	1.00	
A	137 (28.4%)	124 (31.5%)	1.16 (0.86-1.55)	.33
rs2075879				
genotype				
G/G	110 (45.6%)	108 (54.8%)	1.00	
A/G	109 (45.2%)	71 (36.0%)	**0.66 (0.44-0.99)**	**.044**
A/A	22 (9.1%)	17 (8.6%)	0.79 (0.40-1.56)	.493
NA		1 (0.5%)		
Dominant model				
G/G	110 (45.6%)	108 (55.1%)	1.00	
A/G-A/A	131 (54.4%)	88 (44.9%)	0.68 (0.47-1.00)	.049
Recessive model				
G/G-A/G	219 (90.9%)	179 (91.3%)	1.00	
A/A	22 (9.1%)	17 (8.7%)	0.95 (0.49-1.83)	.87
Overdominant model				
G/G-A/A	132 (54.8%)	125 (63.8%)	1.00	
A/G	109 (45.2%)	71 (36.2%)	0.69 (0.47-1.01)	.057
Log-additive model				
—	—	—	0.79 (0.59-1.06)	.11
Allele				
G	329 (68.3%)	287 (73.2%)	1.00	
A	153 (31.7%)	105 (26.8%)	0.79 (0.59-1.06)	.11
rs17113735				
genotype				
G/G	139 (57.7%)	105 (53.3%)	1.00	
A/G	93 (38.6%)	72 (36.5%)	1.02 (0.69-1.53)	.904
A/A	9 (3.7%)	20 (10.2%)	**2.94 (1.29-6.72)**	**.008**
Dominant model				
G/G	139 (57.7%)	105 (53.3%)	1.00	
A/G-A/A	102 (42.3%)	92 (46.7%)	1.19 (0.82-1.74)	.36
Recessive model				
G/G-A/G	232 (96.3%)	177 (89.8%)	1.00	

(Continues)

TABLE 4 (Continued)

TLX1	Control n = 241(n[%])	ALL n = 197(n[%])	OR (95% CI)	*P*
A/A	9 (3.7%)	20 (10.2%)	**2.91 (1.29-6.55)**	**.007**
Overdominant model				
G/G-A/A	148 (61.4%)	125 (63.5%)	1.00	
A/G	93 (38.6%)	72 (36.5%)	0.92 (0.62-1.35)	.66
Log-additive model				
—	—	—	1.33 (0.98-1.80)	.069
Allele				
G	371 (77.0%)	282 (71.6%)	1.00	
A	111 (23.0%)	112 (28.4%)	1.33 (0.98-1.80)	.068
rs1051723				
genotype				
C/C	169 (70.1%)	149 (75.6%)	1.00	
C/T	68 (28.2%)	45 (22.8%)	0.75 (0.49-1.16)	.197
T/T	4 (1.7%)	3 (1.5%)	0.85 (0.19-3.86)	.834
Dominant model				
C/C	169 (70.1%)	149 (75.6%)	1.00	
C/T-T/T	72 (29.9%)	48 (24.4%)	0.76 (0.49-1.16)	.2
Recessive model				
C/C-C/T	237 (98.3%)	194 (98.5%)	1.00	
T/T	4 (1.7%)	3 (1.5%)	0.92 (0.20-4.14)	.91
Overdominant model				
C/C-T/T	173 (71.8%)	152 (77.2%)	1.00	
C/T	68 (28.2%)	45 (22.8%)	0.75 (0.49-1.16)	.2
Log-additive model				
—	—	—	0.79 (0.53-1.16)	.23
Allele				
C	406 (84.2%)	343 (87.1%)	1.00	
T	76 (15.8%)	51 (12.9%)	1.26 (0.86-1.85)	.238

Note: Significant values ($P < .05$) are in bold.

Abbreviations: ALL, acute lymphoblastic leukemia; CI, confidence interval; OR, odds ratio; SNPs, single-nucleotide polymorphisms; *TLX1*, T-cell leukemia homeobox 1.

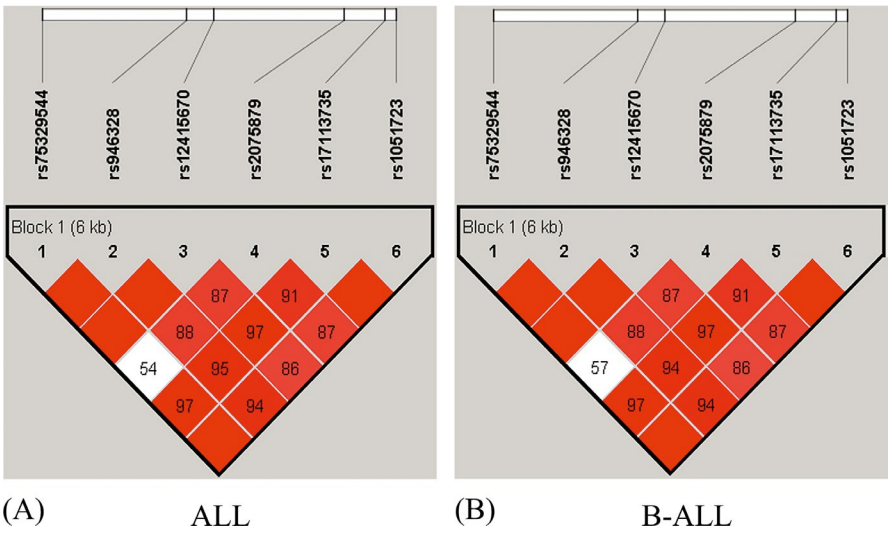

FIGURE 1 Linkage disequilibrium (LD) structure and haplotype blocks of the six Single-nucleotide polymorphisms (SNPs) of the T-cell leukemia homeobox 1 (TLX1) gene. A, LD structure and haplotype blocks of the six SNPs of the TLX1 gene in ALL. B, LD structure and haplotype blocks of the six SNPs of the TLX1 gene in B-ALL

TABLE 5 Haplotype analysis of the six *TLX1* SNPs

Haplotypes	Control	Case	OR (95% CI)	*P*
rs75329544, rs946328, rs12415670, rs2075879, s17113735, rs1051723				
ALL				
TCGAGC	141 (0.293)	110 (0.253)	1	
TCAGGC	127 (0.263)	125 (0.288)	0.19 (0.89-1.79)	.194
TCGGAC	78 (0.162)	88 (0.203)	1.45 (0.98-2.14)	.066
TTGGGT	72 (0.149)	49 (0.113)	0.87 (0.56-1.36)	.543
ACGGAC	27 (0.056)	33 (0.076)	1.57 (0.89-2.76)	.119
TTGGGC	10 (0.021)	7 (0.016)	0.90 (0.33-2.43)	.831
TCGGGC	9 (0.019)	7 (0.016)	1.00 (0.36-2.76)	.995
TCAAGC	6 (0.012)	4 (0.009)	0.86 (0.24-3.10)	.811
B-ALL				
TCGAGC	141 (0.293)	100 (0.254)	1	
TCAGGC	128 (0.266)	114 (0.289)	1.26 (0.88-1.80)	.214
TCGGAC	78 (0.162)	78 (0.198)	1.41 (0.94-2.11)	.096
TTGGGT	72 (0.149)	47 (0.119)	0.92 (0.59-1.44)	.717
ACGGAC	27 (0.056)	29 (0.074)	1.51 (0.85-2.71)	.162
TTGGGC	10 (0.021)	7 (0.018)	0.99 (0.36-2.68)	.980
TCGGGC	9 (0.019)	4 (0.010)	0.63 (0.19-2.09)	.444
TCAAGC	6 (0.012)	4 (0.010)	0.94 (0.26-3.42)	.925

Abbreviations: ALL, acute lymphoblastic leukemia; B-ALL, B-cell acute lymphoblastic leukemia; CI, confidence interval; OR, odds ratio.

$P = 1.20 \times 10^{-19}$), 10q21.2 (*ARID5B*, rs7089424, OR = 1.65, $P = 6.69 \times 10^{-19}$), and 14q11.2 (*CEBPE*, rs2239633, OR = 1.34, $P = 2.88 \times 10^{-7}$) by analyzing 907 ALL cases and 2,398 controls. Ellinghaus studied 474 controls and 419 childhood ALL cases and identified rs17505102 belonging to *TP63* as a novel, genome-wide significant risk locus ($P_{CMH} = 8.94 \times 10^{-9}$, OR = 0.65).[35]

In this study, the association between *TLX1* and ALL was determined. The significance of rs75329544, rs946328, rs12415670, rs2075879, rs1711373, and rs1051723 polymorphisms in the susceptibility to ALL in Chinese children was studied. The results from SPSS and Haploview analysis revealed that the rs17113735 polymorphisms were a novel risk locus correlated with increased risk of ALL, whereas rs946328 prevented ALL in humans. Moreover, *TLX1* was associated with B-ALL. Rs17113735 was a risk locus in the B-ALL samples, and rs2075879 might be associated with decreased risk of B-ALL. The LD analysis showed that all SNPs in the two sample types (ALL and B-ALL samples) demonstrated LD except between rs75329544 and rs2075879. Haplotype analysis found no significant difference between the two groups in both types.

5 | CONCLUSION

This study demonstrated that *TLX1* rs17113735 could be the risk locus associated with increased risk for ALL, including B-ALL. Meanwhile, rs946328 might be associated with decreased risk for ALL. Rs2075879 was associated with decreased risk for B-ALL. These results indicate that *TLX1* possibly plays a significant role in B-ALL. However, the results should be interpreted discreetly due to the relatively small sample size and the homogeneous ethnic origin of the respondents. Further studies should employ larger sample sizes and multifarious populations to thoroughly investigate the association between *TLX1* and ALL, especially B-ALL.

ACKNOWLEDGMENTS

We thank all the participants from Zhejiang Sci-tech University and Children's Hospital of Zhejiang University School of Medicine for greatly helping us on this research.

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How to cite this article: Mei E, Wei X, Gao J, et al. Association of *TLX1* gene polymorphisms with the risk of acute lymphoblastic leukemia and B lineage acute lymphoblastic leukemia in Han Chinese children. *J Clin Lab Anal*. 2020;34:e23414. https://doi.org/10.1002/jcla.23414