Genetic polymorphisms of ARID5B rs7089424 and rs10994982 are associated with B-lineage ALL susceptibility in Chinese pediatric population

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Abstract
Background: Several ARID5B single nucleotide polymorphisms (SNPs) were confirmed to be significantly associated with the susceptibility of childhood acute lymphoblastic leukemia (ALL) based on Caucasian populations in previous studies. Similar investigations in Asian populations were less. The aim of this study is to explore the relationship between ARID5B SNPs rs7089424, rs10994982, and the risk of ALL in Chinese pediatric population.

Methods: A total of 190 pediatric ALL patients and 270 controls were enrolled in this study. PCR amplification combined with mass spectrometry were used to evaluate the genotypes of ARID5B rs7089424 and rs10994982. χ² test was used in allele frequencies and genotype distributions of the SNPs for analyzing statistical differences between patients and controls.

Results: There were significant differences in the risk allele frequencies of ARID5B rs7089424 and rs10994982 between B-lineage ALL (B-ALL) patients and controls (rs7089424, G allele; p = 0.001; rs10994982, A allele; p = 0.000). The genotype distributions of ARID5B rs7089424 and rs10994982 were also statistically different in B-ALL patients compared with controls (rs7089424, p = 0.004; rs10994982, p = 0.001). Further analyzing the relevance of ARID5B rs7089424 and rs10994982 genotypes to clinical risk classification of ALL showed GG genotype of rs7089424 and AA genotype of rs10994982 were strikingly correlated with the medium-risk and low-risk groups of B-ALL. Finally, GG and GT genotypes of rs7089424 and AA genotype of rs10994982 seemed to be responsible for the hyperdiploid subtype susceptibility of childhood B-ALL.

Conclusion: ARID5B rs7089424 and rs10994982 might serve as genetic susceptibility markers for B-ALL in Chinese pediatric population. Moreover, the two ARID5B SNPs are associated with the risk of B-hyperdiploid ALL, which had a better therapeutic response than other ALL subtypes.

Keywords: Acute lymphoblastic leukemia; ARID5B; Children; Single nucleotide polymorphism; Susceptibility

1. INTRODUCTION

Acute lymphoblastic leukemia (ALL), the most common malignancy of childhood, is a genetically complex entity that remains as a major cause of childhood cancer-related mortality. The etiology of ALL is believed to be multifactorial and likely to involve an interplay of environmental and genetic variables. Recent advances in genome-wide association studies (GWASs) substantially improved our understanding of genetic susceptibility to the development of childhood ALL. In 2009, two independent studies reported several single nucleotide polymorphisms (SNPs) located on 10q21.2 (ARID5B), 7p12.2 (IKZF1), and 14q11.2 (CEBPE) had the strongest association with the risk of developing ALL in children. ARID5B, a member of the AT-rich interaction domain family of transcription factors, plays a vital role in the regulation of embryonic development, cell growth, and differentiation through tissue-specific repression of specific gene expression. Several ARID5B SNPs such as rs10824936, rs10994982, rs7089424, rs7073837, and rs10740053 were confirmed to be significantly associated with the susceptibility of childhood ALL in Caucasian populations. However, studies on the relationships between ARID5B SNPs and childhood ALL in Asian populations were relatively less. ARID5B rs10821938 genotype was reported to be significantly associated with B-cell precursor ALL in Thai population. Wang et al. concluded ARID5B rs10821938 could serve as a potential biomarker for assessing the risk of childhood ALL in Chinese children. Another study conducted in Chinese population found that the distribution of genotype rs7073837 in ARID5B significantly differed between ALL and controls. Bhandari et al. unexpectedly provided the first evidence that ARID5B rs10821936 was associated with decreased B-lineage ALL (B-ALL) susceptibility in Indian children.

A meta-analysis emphasized ARID5B SNPs rs7089424 and rs10994982 were indeed significantly associated with increased risk of childhood ALL, and also provided strong evidence that rs10994982 was highly associated with the risk of developing B-hyperdiploid ALL. In spite of this, these two SNPs in ARID5B were never confirmed having relevance to childhood ALL susceptibility in Chinese populations. Therefore, our study aimed to investigate the association of ARID5B SNPs rs7089424 and rs10994982 with the risk of ALL in Chinese pediatric population.
ALL in Chinese populations. In the current study, we conducted a case–control study including 190 ALL cases and 270 controls to validate the relationship between ARID5B SNPs rs7089424, rs10994982 and the risk of ALL in Chinese pediatric population.

2. METHODS

2.1. Study subjects

A total of 190 pediatric patients initially diagnosed with ALL at Children’s Hospital, Zhejiang University School of Medicine and 270 healthy controls excluding cases with history or family history of malignant tumors and hematological system diseases were enrolled in this study from May 2015 to December 2015. All patients were conformed to French–American–British classification, and definitely diagnosed with ALL by morphology, immunology, cytogenetics, and molecular biology. The clinical risk classification and immunophenotype of the ALL patients were determined based on the guideline for diagnosis and treatment of childhood ALL, which was designed by the Chinese Medical Association in 2006.12 This study was approved by the Ethics Committee of Children’s Hospital, Zhejiang University School of Medicine and in accordance with the principles of the Declaration of Helsinki.

2.2. Genomic DNA extraction

EDTA-anticoagulated blood samples (200 µL) were collected from the 190 ALL patients and 270 controls. The genomic DNA was extracted according to the instruction of the Whole Blood Genomic DNA Extraction Kit (Tissuebank Biotechnology Co, Ltd, Shanghai, China). The extracted DNA was then stored at −80ºC for further use.

2.3. PCR amplification

The forward primers and reverse primers of ARID5B rs7089424 and rs10994982 were synthesized by Sangon Biotech (Shanghai) company (Table 1). The PCR reaction system was 5 µL including 0.95 µL of deionized water, 0.625 µL of 10× buffer (containing 15 mM MgCl₂), 0.325 µL of 25 mM MgCl₂, 1 µL of dNTP (2.5 mM each), 1 µL of PCR primers, 0.1 µL of 5 U/µL HotStar Taq (Qiagen), and 1 µL of DNA sample. PCR reaction was carried out at 94ºC for 15 minutes, followed by 45 cycles consisting of 20 seconds at 94ºC, 30 seconds at 56ºC, and 1 minute at 72ºC, and a final extension at 72ºC for 3 minutes.

2.4. SAP processing

For removing the remnant dNTPs, 1.53 µL of deionized water, 0.17 µL of SAP buffer, and 0.3 U shrimp alkaline phosphatase (Sequenom) were added to the PCR reaction system after PCR amplification. This reaction was incubated at 37ºC for 40 minutes, then followed by incubation at 85ºC for 5 minutes to inactivate the enzyme.

2.5. iPLEX single base extension reaction

The single base extension reaction system was 2 µL including deionized water, 0.755 µL; 10× iPLEX buffer, 0.2 µL; iPLEX termination mix, 0.2 µL; iPLEX enzyme (Sequenom), 0.041 µL; single base extension primer (sequences showed in Table 1), 0.804 µL. The extension reaction was performed at 94ºC for 30 seconds, followed by 40 cycles consisting of 5 seconds at 94ºC, 5 cycles of 5 seconds at 52ºC and 5 seconds at 80ºC, and 3 minutes at 72ºC.

2.6. Resin desalination and mass spectrometry

The single base extension reactant was centrifuged at 1000 rpm for 1 minute and resuspended in 25 µL deionized water. The resuspension solution was prepared for genotyping after desalination with cation exchange resin (Sequenom). MassARRAY Nanodispenser (Sequenom) was used to add the samples to be genotyped to a 384 well spectrCHIP (Sequenom). The genotyping reaction was performed on the matrix-assisted laser desorption ionization time-of-flight mass spectrometer, and the results were analyzed by using software MassARRAY Typer version 3.4 (Sequenom).

2.7. Statistical analysis

Goodness-of-fit χ² test was used to determine the Hardy–Weinberg equilibrium for each SNP. For analyzing statistical differences between patients and controls, χ² test was used in allele frequencies and genotype distributions of the SNPs. In addition, the odds ratios (OR) and 95% CI were obtained by using Logistic regression analysis. p < 0.05 was considered statistically significant.

3. RESULTS

In the current study, the pediatric ALL patients were aged from 5 months to 15-years-old with the average age of 5.86 ± 3.59 years at diagnosis. The proportions of age group 0-1 year, 1-3 years, 3-6 years, 6-9 years, 9-12 years, and above 12 years were 2.6%, 22.1%, 36.8%, 17.9%, 13.7%, and 6.9%, respectively.

Table 1

<table>
<thead>
<tr>
<th>SNP</th>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7089424</td>
<td>Forward</td>
<td>5’-ACGTTGGATGCGTGGTTGCTATATTC-3’</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-ACGTTGGATGCGTGGTTGCTATATTC-3’</td>
</tr>
<tr>
<td></td>
<td>Single base ext.</td>
<td>5’-CTCAAGAAAGAAAGAAAGAAAGAA-3’</td>
</tr>
<tr>
<td>rs10994982</td>
<td>Forward</td>
<td>5’-ACGTTGGATGCGTGGTTGCTATATTC-3’</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>5’-ACGTTGGATGCGTGGTTGCTATATTC-3’</td>
</tr>
<tr>
<td></td>
<td>Single base ext.</td>
<td>5’-AATGGATGCGTGGTTGCTATATTC-3’</td>
</tr>
</tbody>
</table>

In this study were shown in Table 2.

No significant differences were found in term of age and gender between patients and controls (Table 2). The detailed information about the clinical risk classification, immunophenotype, subtypes of cytogenetics and molecular biology of ALL patients in this study were shown in Table 2.

The genotyping results of ARID5B rs7089424 and rs10994982 were represented in Figure 1. The two SNPs were tested by Hardy–Weinberg equilibrium in ALL patients and controls, and no significant differences were found in either rs7089424 (p = 0.29) or rs10994982 (p = 0.32). As showed in Table 3, there were significant differences in the risk allele frequencies of ARID5B rs7089424 and rs10994982 between B-ALL patients and controls (rs7089424, G allele: p = 0.001, OR = 1.59, 95% CI = 1.21-2.09; rs10994982, A allele: p = 0.000, OR = 1.67, 95% CI = 1.27-2.19). The genotype distributions of ARID5B rs7089424 and rs10994982 were also statistically significant.
statistically different in B-ALL patients compared with controls (rs7089424, p = 0.004; rs10994982, p = 0.001). However, no differences in the risk allele frequencies and genotype distributions of ARID5B rs7089424 and rs10994982 between T-lineage ALL (T-ALL) patients and controls were found (Supplementary Table 1).

After further analyzing the relevance of ARID5B rs7089424 and rs10994982 genotypes to clinical risk classification of ALL, we found that GG genotype of ARID5B rs7089424 was strikingly correlated with the medium-risk and low-risk groups of B-ALL patients (medium risk: OR = 3.10, 95% CI = 1.47-6.44; low risk: OR = 2.98, 95% CI = 1.34-6.66), while GT genotype of this SNP had no relationship to B-ALL clinical risk classification. Similarly, AA genotype of ARID5B rs10994982 was also significantly correlated with the medium-risk and low-risk groups of B-ALL (medium risk: OR = 2.64, 95% CI = 1.24-5.60; low risk: OR = 3.55, 95% CI = 1.50-8.40) but no correlation was found between rs10994982 AG genotype and clinical risk classification of B-ALL (Table 4). For T-ALL patients, no correlation was found between genotypes of ARID5B rs7089424 or rs10994982 and clinical risk groups (Supplementary Table 2).

We finally compared the genotype distributions of ARID5B rs7089424 and rs10994982 among the dominant subtypes of cytogenetics and molecular biology in B-ALL patients, and found GG and GT genotypes of ARID5B rs7089424 and AA genotype of rs10994982 seemed to be responsible for the hyperdiploid subtype susceptibility of childhood B-ALL (rs7089424, GG genotype: OR = 5.74, 95% CI = 1.88-17.54; rs7089424, GT genotype: OR = 4.39, 95% CI = 1.62-11.86; rs10994982, AA genotype: OR = 4.92, 95% CI = 1.58-15.28) (Table 5).

4. DISCUSSION

In this case–control study, we confirmed the association of ARID5B rs7089424 and rs10994982 with B-ALL susceptibility in a Chinese pediatric population. The G-allele carriers of ARID5B rs7089424 and A-allele carriers of ARID5B rs10994982 had a higher B-ALL incidence than the T-allele carriers of rs7089424 and G-allele carriers of rs10994982, respectively. Therefore, the G allele of ARID5B rs7089424 and A allele of ARID5B rs10994982 seemed to be potential predictive markers for developing childhood B-ALL in Chinese population.

As most previous studies, the correlation analysis between the genotypes of ARID5B SNPs and ALL immunophenotypes in this study confirmed ARID5B rs7089424 and rs10994982 were associated with the risk of developing B-ALL. The mechanisms possibly involved the recombination activating gene 1 (RAG1), which plays a role in early B-cell differentiation. Two rounds of DNA recombination necessary for precursor B-cell development in bone marrow depended on the activation of RAG-1 and RAG-2 endonucleases. Moreover, the expression of ARID5B had been reported to be associated with RAG1 expression in bone marrow, providing a possible explanation about how ARID5B participated in the pathogenesis of B-ALL.

The conclusions on the association of ARID5B rs7089424 and rs10994982 with B-ALL were not consistent in several studies based on Asian population. The results of the case–control study carried out in Yemeni children agreed with ours, revealing the two SNPs of ARID5B were the risk factors for ALL. A GWAS conducted in Korean population merely found an association between ARID5B rs7089424 and B-cell precursor ALL was found in a study conducted in Thai population. Similarly, the genotype distribution of ARID5B rs7089424 was reported showing no significant difference between Chinese ALL cases and controls. The reasons for this discrepancy probably included the size of research sample, the selection criteria of study objects, the techniques for detecting polymorphisms, and the statistical methods of genotyping results.
**Table 3**

Allele frequencies and genotype distributions of ARID5B rs7089424 and rs10994982 in B-ALL patients and controls

| Genotype | Patients | | Controls | | χ² | p | OR | 95% CI |
|----------|----------|---|----------|---|---|---|---|---|---|
| rs7089424 | n | % | n | % | | | | | |
| T > G | | | | | | | | | |
| TT | 46 | 26.3 | 107 | 39.6 | | 10.88 | 0.004 | | |
| GT | 86 | 49.1 | 122 | 45.2 | | 1.64 | 1.05-2.55 | | |
| GG | 43 | 24.6 | 41 | 15.2 | | 2.44 | 1.41-4.23 | | |
| G allele | 172 | 49.1 | 204 | 37.8 | | 11.21 | 0.001 | 1.59 | 1.21-2.09 |
| rs10994982 | n | % | n | % | | | | | |
| G > A | | | | | | | | | |
| GG | 30 | 17.1 | 71 | 26.3 | | 1.0 | | | |
| AG | 75 | 42.9 | 134 | 49.6 | | 1.33 | 0.79-2.21 | | |
| AA | 70 | 40.0 | 65 | 24.1 | | 2.55 | 1.48-4.39 | | |
| A allele | 215 | 61.4 | 264 | 48.9 | | 13.51 | 0.000 | 1.67 | 1.27-2.19 |

OR = odds ratio.

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**Fig. 1** Genotyping results of ARID5B rs7089424 and rs10994982. A, TT genotype of rs7089424; B, GT genotype of rs7089424; C, GG genotype of rs7089424; D, GG genotype of rs10994982; E, AG genotype of rs10994982; F, AA genotype of rs10994982.
Table 4
Relationships between genotypes of ARID5B rs7089424 and rs10994982 and clinical risk classification of B-ALL

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>%</th>
<th>High risk</th>
<th>Medium risk</th>
<th>Low risk</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs7089424</td>
<td></td>
<td></td>
<td>n</td>
<td>n</td>
<td>n</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>107</td>
<td>39.6</td>
<td>15</td>
<td>17</td>
<td>14</td>
<td>1.0</td>
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<tr>
<td>GT</td>
<td>122</td>
<td>45.2</td>
<td>21</td>
<td>35</td>
<td>30</td>
<td>1.23(0.60-2.50)</td>
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<td>GG</td>
<td>41</td>
<td>15.2</td>
<td>7</td>
<td>20</td>
<td>16</td>
<td>1.22(0.46-3.20)</td>
</tr>
<tr>
<td>rs10994982</td>
<td></td>
<td></td>
<td>n</td>
<td>n</td>
<td>n</td>
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</tr>
<tr>
<td>GG</td>
<td>71</td>
<td>26.3</td>
<td>10</td>
<td>12</td>
<td>8</td>
<td>1.0</td>
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<tr>
<td>AG</td>
<td>134</td>
<td>49.6</td>
<td>18</td>
<td>31</td>
<td>26</td>
<td>0.95(0.42-2.18)</td>
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<tr>
<td>AA</td>
<td>65</td>
<td>24.1</td>
<td>15</td>
<td>29</td>
<td>26</td>
<td>1.64(0.67-3.90)</td>
</tr>
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</table>

B-ALL = B-lineage acute lymphoblastic leukemia; OR = odds ratio.

Table 5
Relationships between genotypes of ARID5B rs7089424 and rs10994982 and the dominant subtypes of B-ALL cytogenetics and molecular biology

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>%</th>
<th>Hyperdiploid</th>
<th>TEL/AML1</th>
<th>Others</th>
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<td>rs7089424</td>
<td></td>
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<tr>
<td>TT</td>
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<td>39.6</td>
<td>5</td>
<td>16</td>
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<tr>
<td>GT</td>
<td>122</td>
<td>45.2</td>
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<td>16</td>
<td>4.39(1.62-11.86)</td>
</tr>
<tr>
<td>GG</td>
<td>41</td>
<td>15.2</td>
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<td>4</td>
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<td>5.74</td>
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<tr>
<td>GG</td>
<td>71</td>
<td>26.3</td>
<td>4</td>
<td>10</td>
<td>10</td>
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<tr>
<td>AG</td>
<td>134</td>
<td>49.6</td>
<td>19</td>
<td>14</td>
<td>14</td>
<td>2.52(0.83-7.68)</td>
</tr>
<tr>
<td>AA</td>
<td>65</td>
<td>24.1</td>
<td>18</td>
<td>11</td>
<td>16</td>
<td>4.02(1.58-10.28)</td>
</tr>
</tbody>
</table>

B-ALL = B-lineage acute lymphoblastic leukemia; OR, odds ratio.

On subtype analysis, we found GG genotype of ARID5B rs7089424 and AA genotype of ARID5B rs10994982 were significantly correlated with the medium-risk and low-risk subgroups of B-ALL, suggesting homozygous mutation of the two SNPs might have bigger chances to develop into medium or low risk B-ALL.

By further analysis on the subtypes of B-ALL in this study, GG and GT genotype of ARID5B rs7089424 and AA genotype of ARID5B rs10994982 distinguished B-hyperdiploid ALL from other subtypes. The partial results were in line with those of previous studies, which verified the association between ARID5B rs10994982 and B-hyperdiploid ALL. Furthermore, there was no previous study focusing on the role of ARID5B rs7089424 in the risk of developing B-hyperdiploid ALL. In consideration of B-hyperdiploid ALL having a better response to methotrexate chemotherapy than other ALL subtypes, ARID5B SNPs might be helpful to instruct treatment and estimate prognosis of ALL besides predicting the risk of this disease.

In conclusion, the results of the current study suggest ARID5B rs7089424 and rs10994982 might serve as genetic susceptibility markers for B-ALL in Chinese pediatric population. Moreover, ARID5B rs7089424 and rs10994982 are associated with the risk of B-hyperdiploid ALL, which has a better therapeutic response than other ALL subtypes.

APPENDIX A. SUPPLEMENTARY DATA
Supplementary data related to this article can be found at http://links.lww.com/JCMA/A16

REFERENCES


