IKZF1 Deletion in Acute Lymphoblastic Leukemia: A Single-Center Experience in Thailand

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Abstract

Genetic deletions of *IKZF1* have been extensively studied and linked to unfavorable prognosis in B-cell precursor acute lymphoblastic leukemia (BCP-ALL). However, the distribution and prognostic significance of restricted *IKZF1* deletion in BCP-ALL is unclear. This study aimed to analyze the distribution, clinical impact, and overall survival of *IKZF1* deletions in both adult and childhood BCP-ALL. Sixty-two genomic DNA samples isolated from patients with BCP-ALL were analyzed using the MLPA method. Gene deletions were detected in 64.5% of cases. The deletion of *CDKN2A* was the most frequent genetic alteration (50.0%) in the tested samples, followed by the deletion of *IKZF1* (47.5%) and deletion of *CDKN2B* (45.0%). Gene duplications were detected in 32.3% of cases. The duplications of *CSF2RA* and *IL3RA* were the most frequent genetic alteration (75.0%) in the tested samples, followed by duplications of *SHOX-AREA-Down*, *CRLF2*, *P2RY8* (70.0%) and duplications of *PAX5* (30.0%). 42.1% detected deletion of *IKZF1* with t(9;22) and *BCR::ABL1* p190 positive. In addition, the impact of *IKZF1* deletions had shorter overall survival (OS) compared to those without *IKZF1* deletions (p = 0.047), and patients with both *IKZF1* and *BCR::ABL1* p190 deletions had significantly poorer outcomes, with shorter eight-year overall survival (OS) (p = 0.0234). In summary, we revealed the association between *IKZF1* deletions and survival outcomes of patients with BCP-ALL. Our data demonstrated that patients with *IKZF1* deletion have shorter OS than those without *IKZF1* deletion. Furthermore, the combination of *BCR::ABL1* positivity and *IKZF1* deletion, showing a poorer prognosis, as indicated by shorter OS, compared to patients without *BCR::ABL1* or *IKZF1* detection.

Keywords: *IKZF1* deletion, B-cell precursor acute lymphoblastic leukemia (BCP-ALL), multiplex ligation-dependent probe amplification (MLPA), *BCR::ABL1* p190 positive

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การขาดหายไปของชิ้นส่วนยีน *IKZF1* ในผู้ป่วยมะเร็งเม็ดเลือดขาวชนิดเฉียบพลันสายลิมฟอยด์: ประสบการณ์จากศูนย์การศึกษาเดียวในประเทศไทย

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บทคัดย่อ

การขาดหายไปของชิ้นส่วนยีน *IKZF1* (*IKZF1* deletion) ได้มีการศึกษาอย่างกว้างขวางและพบว่ามีความสัมพันธ์กับการพยากรณ์โรคที่ไม่ดีในผู้ป่วยโรคมะเร็งเม็ดเลือดขาวชนิดเฉียบพลันสายลิมฟอยด์ ชนิดบีเซลล์ (BCP-ALL) อย่างไรก็ตาม การกระจายตัวและการพยากรณ์โรคของ BCP-ALL ที่มี *IKZF1* deletion ยังไม่ชัดเจน การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อวิเคราะห์การกระจายตัว ความสัมพันธ์ทางคลินิกและอัตราการรอดชีวิตของผู้ป่วยที่ตรวจพบ *IKZF1* deletion ในกลุ่มผู้ป่วย BCP-ALL ทั้งในเด็กและผู้ใหญ่ จากการศึกษาใช้สารพันธุกรรมดีเอ็นเอจากผู้ป่วย BCP-ALL จำนวน 62 ราย นำมาทดสอบด้วยเทคนิค multiplex ligation-dependent probe amplification (MLPA) พบว่า ความผิดปกติของสารพันธุกรรมชนิด deletion ตรวจพบได้ร้อยละ 64.5 โดยพบ *CDKN2A* deletion มากที่สุด (50.0%) รองลงมาคือ *IKZF1* deletion (47.5%) และ *CDKN2B* deletion (45.0%) ความผิดปกติของสารพันธุกรรมชนิด duplication ตรวจพบได้ร้อยละ 32.3 โดยพบ *CSF2RA* และ *IL3RA* duplications มากที่สุด (75.0%) รองลงมาคือ *SHOX-AREA-Down*, *CRLF2*, *P2RY8* duplications (70.0%) และ *PAX5* duplication (30.0%) ผู้ป่วยที่มีการตรวจพบ *IKZF1* deletion ร่วมกับการตรวจพบ t(9;22) และยีนลูกผสม *BCR*::*ABL1* ชนิด p190 มีจำนวนร้อยละ 42.1 นอกจากนี้อัตราการรอดชีวิตของผู้ป่วยที่ตรวจพบ *IKZF1* deletion สั้นกว่าผู้ป่วยที่ไม่มี *IKZF1* deletion (p = 0.047) และผู้ป่วยที่มี *IKZF1* deletion ร่วมกับมียีนลูกผสม *BCR*::*ABL1* ชนิด p190 มีอัตราการรอดชีวิตที่สั้นอย่างมีนัยสำคัญร่วมกับอัตราการรอดชีวิตที่สั้นกว่า 8 ปี (p = 0.0234) การศึกษาครั้งนี้พบว่า การตรวจพบ *IKZF1* deletion มีความสัมพันธ์กับอัตราการรอดชีวิตของผู้ป่วย BCP-ALL โดยพบว่าผู้ป่วยที่มี *IKZF1* deletion สัมพันธ์กับอัตราการรอดชีวิตสั้นลงเมื่อเทียบกับผู้ป่วยที่ไม่มี *IKZF1* deletion ดังนั้นการตรวจพบยีนลูกผสม *BCR*::*ABL1* ร่วมกับการตรวจพบ *IKZF1* deletion ส่งผลต่อการพยากรณ์โรคที่ไม่ดีและอัตราการรอดชีวิตสั้นลงเมื่อเทียบกับผู้ป่วยที่ไม่มียีนลูกผสม *BCR*::*ABL1* หรือผู้ป่วยที่ไม่มี *IKZF1* deletion

คำสำคัญ: การขาดหายไปของชิ้นส่วนยีน *IKZF1* โรคมะเร็งเม็ดเลือดขาวชนิดเฉียบพลันสายลิมฟอยด์ ชนิดบีเซลล์ multiplex ligation-dependent probe amplification (MLPA) การตรวจพบยีนลูกผสม *BCR*::*ABL1* ชนิด p190

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1. Introduction

Acute lymphoblastic leukemia (ALL) is the most common malignancy in children and adolescents, showing a wide range of clinical and genetic heterogeneity. ALL is marked by the accumulation of malignant immature lymphoid cells in organs and tissues like bone marrow, peripheral blood, liver, and the central nervous system. Based on cellular origin, ALL is classified into two subgroups: T-lineage ALL (T-ALL) and B-lineage ALL (B-ALL).[1] Despite advancements in therapeutic strategies worldwide, achieving cure rates exceeding 80% in childhood ALL,[2] about 20% of patients relapse after initial therapy.[3] Currently, risk stratification by assessing genetic alterations before therapy is crucial to identify patients who may respond well to treatments or have a high relapse risk, necessitating intensive care or allogeneic stem cell transplantation.[4]

The *IKZF1* gene on chromosome 7p12.2 encodes the IKAROS transcription factor, which is crucial for normal lymphopoiesis. Joshi *et al.* (2014) demonstrated that conditional inactivation of *IKZF1* restricted normal B-cell maturation at a pre-B cell stage, exhibiting a gene expression signature of cell proliferation and self-renewal. Additionally, transplantation of *IKZF1* mutant pre-B cells into an immunocompromised mouse could develop pre-B cell ALL, indicating the leukemogenic property of *IKZF1* in developing pre-B cell ALL.[5] Recurrent mutations of *IKZF1* (alone or with other genetic features) are frequently identified in pediatric and adult ALL cases.[6, 7] The whole gene and intragenic deletion of exon 2-7 are the most common forms of *IKZF1* alterations observed in B-cell precursor ALL (BCP-ALL).[8] Approximately 15% and 40-50% of *IKZF1* deletions were identified in childhood and adult BCP-ALL, respectively. Notably, *IKZF1* deletion is highly positive (70%) in patients with aggressive phenotypes, *BCR*::*ABL1* positive, and *BCR*::*ABL1*-like BCP-ALL.[6, 9] While the prognostic value of the *IKZF1* mutation among ALL patients remains controversial, mainly depending on study cohorts, *IKZF1* alteration in ALL with *BCR*::*ABL1*-positive and *BCR*::*ABL1*-like ALL is associated with poor outcomes in BCP-ALL.[1, 7, 10-11]

In Thailand, the distribution of *IKZF1* deletions and CNVs of other genes in BCP-ALL patients in Thailand have not been investigated. Due to the importance of these alterations in the prognosis and choosing the best treatment approach, determining their presence is of great importance. Therefore, this study aimed to analyze samples from Thai patients who had been diagnosed with BCP-ALL to examine the distribution, clinical impact, and overall survival of *IKZF1* deletions in adult and childhood BCP-ALL patients.

2. Materials and methods

2.1 Patients and samples

This study included 62 genomic DNA samples isolated from patients with BCP-ALL. Genomic DNA (gDNA) was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany). The quality and quantity of the isolated gDNA were assessed using a NanoDrop 2000 (Thermo Fisher Scientific, USA), following the manufacturer instruction. The DNA samples were stored at 2-8°C until MLPA analysis. This study was approved by the Local Ethics Committee on Human Rights related to research involving human subjects at Ramathibodi Hospital, Mahidol University (COA. MURA2021/951), and informed consent was waived due to the study's retrospective design. All procedures performed in this study adhered to the principles of the Declaration of Helsinki.

2.2 Cytogenetic study

A comprehensive cytogenetic study was conducted at the Human Genetic Laboratory, Department of Pathology, Ramathibodi Hospital, using the G-banding technique after short-term culture without mitogen activation. On-screen karyotyping was performed on 20-30 metaphases using IKAROS software (MetaSystems, Germany), and chromosomal abnormalities were documented according to the International System for Human Cytogenetic Nomenclature (ISCN, 2016).

2.3 Screening for 28 translocations

The HemaVision 28Q reverse transcription-quantitative polymerase chain reaction (RT-qPCR-KIT) (DNA Diagnostics, Risskov, Denmark) was utilized to screen for 28 chromosome translocations and fusion genes associated with recurrent leukemia in both AML and ALL.

2.4 MLPA analysis

For MLPA analysis, 60 nanograms of gDNA were analyzed using the SALSA MLPA P335-C1 ALL-*IKZF1* probe mix (MRC-Holland, Amsterdam, Netherlands). This probe mix included 57 probes targeting specific regions such as 5q33.3 (*EBF1*), 7p12.2 (*IKZF1*), 9p24.1 (*JAK2*), 9p21.3 (*CDKN2A* and *CDKN2B*), 9p13.2 (*PAX5*), 12p13.2 (*ETV6*), 12q21.33 (*BTG1* and its downstream region), 13q14.2 (*RB1*), and XP22PAR (*SHOX*, *CRLF2*, *CSF2RA*, *IL3RA*, and *P2RY8*). MLPA reactions, including internal quality and negative controls, were conducted per the manufacturer's instructions. The resulting PCR products were analyzed using an ABI 3500 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and Coffalyser.net software (MRC Holland, Amsterdam, Netherlands), following the manufacturer's guidelines.

2.5 Statistical analysis

Demographic and clinical characteristics of all participants, along with genetic alterations in the B-ALL panel genes, were assessed using the Chi-square or Fisher's exact test for categorical variables. Overall survival (OS) was defined as the time from diagnosis to the outcome (survival or death) in years. The impact of *IKZF1* and *BCR*::*ABL1* status on OS was estimated using the Kaplan-Meier method, with differences between survival curves compared using the log-rank test. Statistical analyses were performed using SPSS Statistics version 26 and GraphPad Prism version 10.0.2, with a *p*-value of less than 0.05 considered statistically significant.

3. Results

3.1 Demographic and clinical characteristics data

This report includes 62 samples from BCP-ALL patients, comprising 29 males (46.8%) and 33 females (53.2%), with a median age at onset of 11 years (ranging from 1 year to 63 years). Children (1-14 years) represented the largest age group (33, 53.2%), followed by AYA (15-39 years) (15, 24.1%) and older adults (> 40 years) (14, 22.6%). Most patients had a WBC count of < 50,000 cells/µl (46, 74.2%) and ≥ 50,000 cells/µL (8, 12.9%). Most karyotypes present abnormal karyotypes without t(9;22) (27, 43.5%), followed by normal karyotypes (18, 29.0%) and abnormal karyotypes with t(9;22) (11, 17.7%). Positive for *BCR*::*ABL1* p190 (9, 14.5%) and negative for *BCR*::*ABL1* p190 (47, 75.8%). *IKZF1* gene status was classified into two categories as *IKZF1* deletion group was found to be 30.6% (19/62), and no *IKZF1* deletion group was found at 69.4% (43/62). *IKZF1* deletion group comprising 5 males (26.3%) and 14 females (73.7%). AYA (15-39 years) and older adults (> 40 years) represented the largest age group (7, 36.8%) and children (1-14 years) (5, 26.3%). Most patients had a WBC count of < 50,000 cells/µL (13, 68.4%) and ≥ 50,000 cells/µL (4, 21.0%). Most karyotypes present abnormal karyotypes with t(9;22) (10, 52.6%), followed by abnormal karyotypes without t(9;22) (6, 31.6%) and normal

karyotype (2, 10.5%). Positive for *BCR*::*ABL1* p190 (9, 47.4%) and negative for *BCR*::*ABL1* p190 (8, 42.1%). There was no significant difference in gender and WBC count between samples with and without *IKZF1* deletion. However, age karyotypes and *BCR*::*ABL1* p190 were significant between samples with and without *IKZF1* deletion. Table 1 summarizes the clinical characteristics of all participants.

Table 1 Demographic and clinical characteristics of all participants.

Characteristic	N	*IKZF1* deletion	No *IKZF1* deletion	*p*-value
Sample size, n (%)	62 (100)	19 (30.6)	43 (69.4)	
Gender, n (%)				
Male	29 (46.8)	5 (26.3)	24 (55.8)	0.052
Female	33 (53.2)	14 (73.7)	19 (44.2)	
Age in year, median (range)	11 (1-63 years)			
Age in group, n (%)				
Children (1-14 years)	33 (53.2)	5 (26.3)	28 (65.1)	0.015
AYA (15-39 years)	15 (24.1)	7 (36.8)	8 (18.6)	
Older adults (≥ 40 years)	14 (22.6)	7 (36.8)	7 (16.3)	
WBC counts in group, n (%)				
< 50,000 cells/μl	46 (74.2)	13 (68.4)	33 (76.7)	0.445
≥ 50,000 cells/μl	8 (12.9)	4 (21.0)	4 (9.3)	
Not analyzed	8 (12.9)	2 (10.5)	6 (13.9)	
Karyotype, n (%)				
Normal	18 (29.0)	2 (10.5)	16 (37.2)	<0.001
Abnormal with t(9;22)	11 (17.7)	10 (52.6)	1 (2.3)	
Abnormal without t(9;22)	27 (43.5)	6 (31.6)	21 (48.8)	
Not analyzed	6 (9.7)	1 (5.3)	5 (11.6)	
BCR::*ABL1* p190, n (%)				
Positive	9 (14.5)	9 (47.4)	-	<0.001
Negative	47 (75.8)	8 (42.1)	39 (90.7)	
Not analyzed	6 (9.7)	2 (10.5)	4 (9.3)	

Abbreviations: AYA; adolescent and young adults

3.2 Analysis of gene deletions in BCP-ALL

In the BCP-ALL patients group, gene deletions were detected in 40/62 patients (64.5%). The common deletions included those in the *CDKN2A* 50.0% (20/40), *IKZF1* 47.5% (19/40), *CDKN2B* 45.0% (18/40), *PAX5* 42.5% (17/40), *ETV6* and *JAK2* 25.0% (10/40), *EBF1* and *RB1* 15.0% (6/40), *BTG1* and *BTG1-AREA-Down* 10.0% (4/40), *CRLF2*, *CSF2RA* and *IL3RA* 2.5% (1/40) and no gene deletions were observed in 22 patients 35.5% (22/62) (Fig. 1A). Among those with gene deletions, one single gene deletion was observed in 11 patients (11/40, 27.5%), two were observed in 10 patients (10/40, 25.0%), and ≥ 3 were observed in 19 patients (19/40, 47.5%). The loss of *CDKN2A* and *CDKN2B* was reported in 18 patients (18/40, 45.0%). The loss of *CDKN2A*, *CDKN2B* and *IKZF1* genes were observed in 7 patients (7/40, 17.5%). The loss of *CDKN2A*, *CDKN2B*, *IKZF1* and *PAX5* genes were observed in 6 patients (6/40, 15.0%). A total of 2 (2/40, 5.0%) cases

of only *IKZF1* gene deletions and *IKZF1* gene deletions with other genes were observed in 17 patients (17/40, 42.5%).

The *IKZF1* gene deletion in the BCP-ALL patients was detected in 19/40 patients (47.5%). The *IKZF1* gene deletion of > 5 exon was detected in 8/19 cases (42.1%) (patient number HGU001, HGU011, HGU017, HGU020, HGU031, HGU037, HGU051 and HGU059). Moreover, 1/19 cases (5.3%) (patient number HGU031) were detected exon 1-8. The *IKZF1* gene deletion is significantly more common in Philadelphia (Ph) positive patients and *BCR*::*ABL1* p190 positive. This study, we found 8/19 cases (42.1%) were detected deletion 7p12.2 (*IKZF1*) with t(9;22) and *BCR*::*ABL1* p190 positive results (patient number HGU011, HGU016, HGU017, HGU019, HGU020, HGU022, HGU037 and HGU052). In addition to deletion 7p12.2 (*IKZF1*) with t(9;22) results were detected 1/19 cases (5.3%) (patient number HGU053). Only a single case was detected deletion 7p12.2 (*IKZF1*) with *BCR*::*ABL1* p190 positive results (patient number HGU033) (1/19 case, 5.3%). Moreover, we found 1/19 case (5.3%) were detected deletion 7p12.2 (*IKZF1*) with t(9;22) and *BCR*::*ABL1* p210 positive results (patient number HGU051).

3.3 Analysis of gene duplications in BCP-ALL

In the BCP-ALL patients group, gene duplications were detected in 20/62 patients (32.3%). The common duplications included those in the *CSF2RA* and *IL3RA* 75.0% (15/20), *SHOX-AREA-Down*, *CRLF2* and *P2RY8* 70.0% (14/20), *PAX5* 30.0% (6/20), *BTG1* 20.0% (4/20), *ETV6* and *BTG1-AREA-Down* 10.0% (2/20), *IKZF1* and *JAK2* 5.0% (1/20) and no gene duplications were observed in 42 patients 67.7% (42/62). (Fig. 1B). Among those with gene duplications, one single gene duplication was observed in 3 patients (3/20, 15.0%), 2 were observed in 3 patients (3/20, 15.0%), and $\geq$ 3 were observed in 14 patients (14/20, 70.0%). The duplications of *SHOX-AREA-Down*, *CSF2RA*, *IL3RA*, *CRLF2* and *P2RY8* were observed in 13 patients (13/20, 65.0%). Moreover, we found 10/20 cases (50.0%) that detected duplication of *SHOX-AREA-Down*, *CSF2RA*, *IL3RA*, *CRLF2*, and *P2RY8* with other genes (Table 1).

3.4 The impact of *IKZF1* deletion and *BCR*::*ABL1* p190 positive on the OS of BCP-ALL

We further investigated the impact of *IKZF1* deletion and *BCR*::*ABL1* p190 positive on eight-year OS in BCP-ALL patients. In this cohort (62 BCP-ALL patients, aged 1 year to 63 years), those with *IKZF1* deletions had shorter OS compared to those without *IKZF1* deletions (p = 0.047) (Fig. 2A). Although the difference in OS between *IKZF1* deletions and no *IKZF1* deletion patients was minor, patients with both *IKZF1* and *BCR*::*ABL1* p190 deletions had significantly poorer outcomes, with shorter eight-year OS (p = 0.0234) (Fig. 2B). In summary, we reported the prevalence of common CNVs and the impact of *IKZF1* deletions on clinical outcomes in BCP-ALL patients. Deletion of *IKZF1* was associated with decreased OS, particularly in patients with both *BCR*::*ABL1* p190 positivity and *IKZF1* mutations.

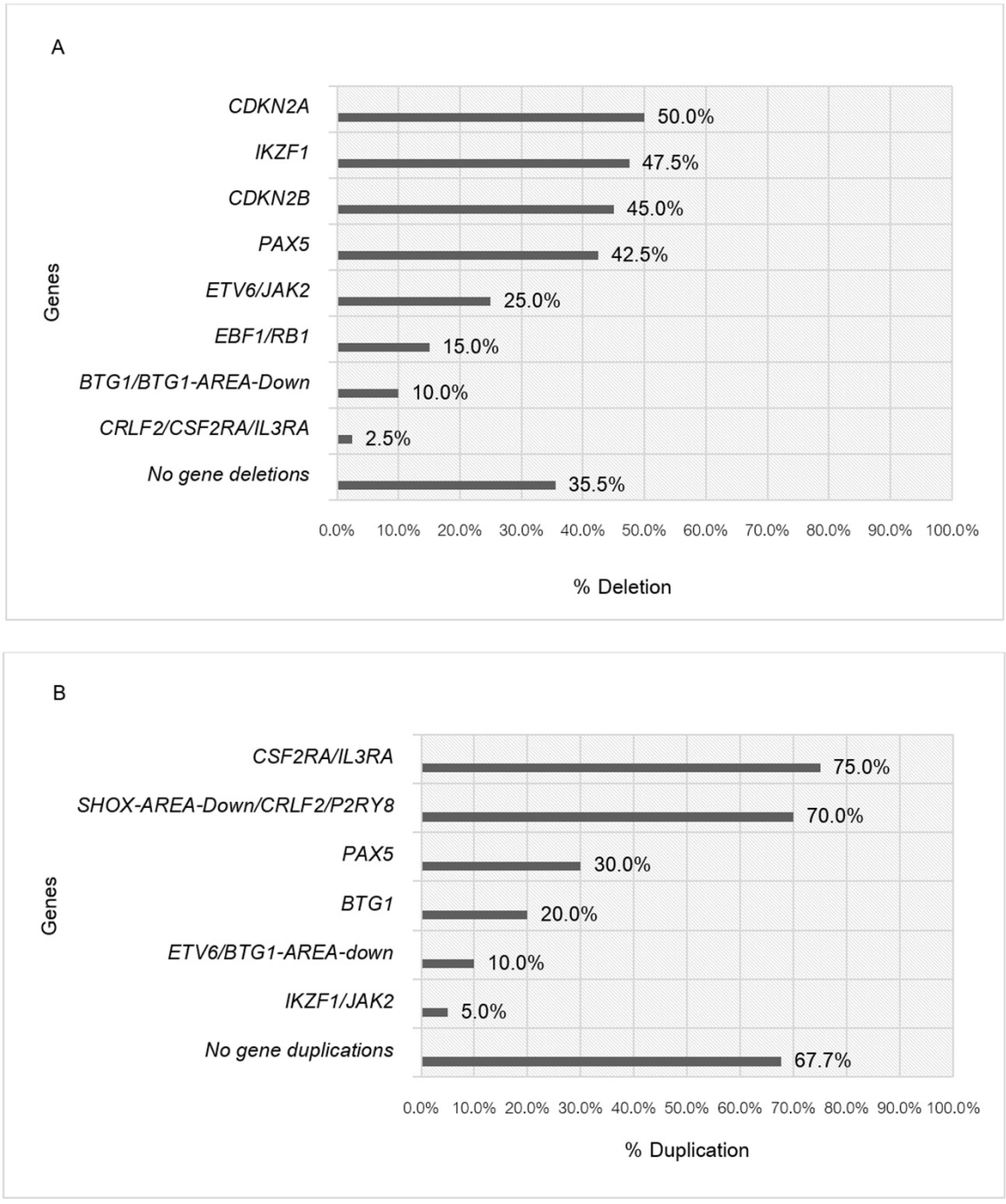

Fig. 1 Percentage of gene mutations in the entire cohort (N = 62). (A) Gene deletions (B) Gene duplications.

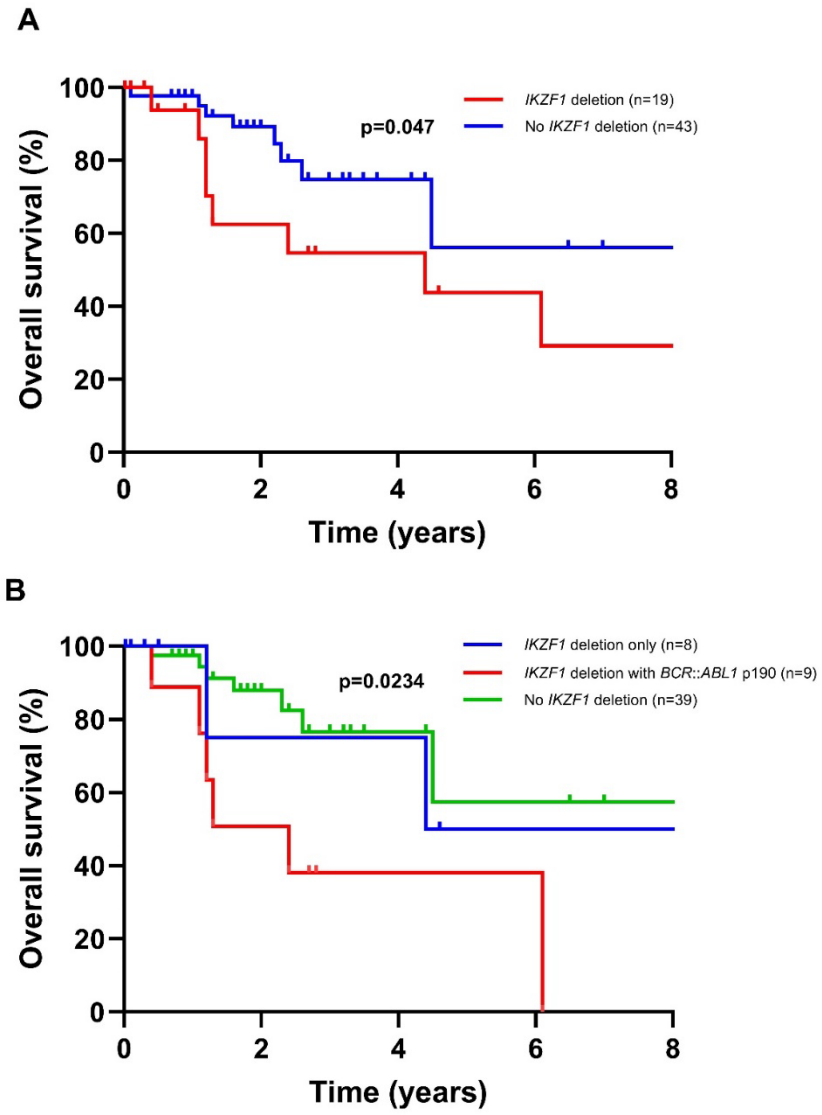

Fig. 2 Impact of *IKZF1* deletion status on OS in BCP-ALL patients. (A) 8-year OS comparison between patients with and without *IKZF1* deletion. (B) The 8-year OS comparison among patients with only *IKZF1* deletion, *IKZF1* deletion with *BCR*::*ABL1* p190, and no *IKZF1* deletion.

4. Discussions

BCP-ALL is a heterogeneous disease with diverse clinical representation and genetic backgrounds. In this study, we analyzed the mutational landscape of common CNVs, focusing on *IKZF1* deletion mutation pattern. We conducted MLPA analysis on 62 genomic DNA samples from BCP-ALL patients across various age and sex groups. Although the limited sample size may have affected the data, our findings revealed consistent distributions of *IKZF1* mutations and their prognostic impact on BCP-ALL with *IKZF1* mutation. Notably, the frequency of *IKZF1* deletion was approximately 15% in pediatric cases and 40% in adult BCP-ALL, based on the cohorts studied and the methods used for detection.[12] In our cohort, *IKZF1* deletion was observed in 15.2% of pediatric cases (children 1-14 years) (5 out of 33) and 48.3% of adult cases (AYA

15-39 years and older adults ≥ 40 years) (14 out of 29), aligning with previous studies.[13-17] Furthermore, half of the *IKZF1* deletion samples were positive for *BCR*::*ABL1*, a poor cytogenetic marker in ALL. However, the incidence of *IKZF1* deletion in *BCR*::*ABL1*-like ALL remains unclear, as we were unable to perform molecular characterization of *BCR*::*ABL1*-like ALL in this study.

In addition to the *IKZF1* deletion, this study also reports on the frequency of recurrent genetic alterations involved in ALL, including *CDKN2A/B*, and *PAX5*. The loss of *CDKN2A/B* is associated with diverse clinical outcomes in BCP-ALL and is recognized as a common driving mutation. Our findings align with previous reports, showing *CDKN2A* loss in 42.4% of pediatric cases (children 1-14 years) (14 out of 33), *CDKN2B* loss in 36.4% of pediatric cases (children 1-14 years) (12 out of 33),[6,18,19] *CDKN2A* loss in 20.7% of adult cases (AYA 15-39 years and older adults ≥ 40 years) (6 out of 29), *CDKN2B* loss in 20.7% of adult cases (AYA 15-39 years and older adults ≥ 40 years) (6 out of 29),[20] and *CDKN2A* loss in 50% and *CDKN2B* loss in 45% of overall BCP-ALL. Additionally, *PAX5* deletion, a transcription factor-encoding gene, has been linked to BCP-ALL development, with *PAX5* CNVs frequently co-occurring with *CDKN2A/B* and *IKZF1* deletions.[21] Prognostic outcomes associated with *PAX5* CNVs in BCP-ALL vary, often influenced by study population sizes and cohort designs. Additionally, *PAX5* deletion was not linked to OS and DSF in adult BCP-ALL with *BCR*::*ABL1* positivity.[22] However, evidence also suggests that *PAX5* deletion may shorten DFS in adult BCP-ALL.[23]

Over the past 15 years, extensive research has examined the impact of *IKZF1* mutations on the prognosis of BCP-ALL patients. *IKZF1* genetic deletions have been linked to high-risk phenotypes in pediatric ALL, with an increasing incidence observed among older patients.[12] This trend is consistent with our findings, where most samples analyzed were from adult BCP-ALL patients (AYA 15-39 years and older adults ≥ 40 years), many of whom exhibited high-risk clinical features, including elevated WBC counts and *BCR*::*ABL1* fusion, as detected by cytogenetic analysis and RT-PCR. In pediatric BCP-ALL (children 1-14 years), *IKZF1* deletions were present in approximately two-thirds of high-risk BCP-ALL cases with *BCR*::*ABL1* positivity and around 20% of *BCR*::*ABL1*-like cases.[12] Evidence indicates that childhood ALL patients with *BCR*::*ABL1* positivity and *IKZF1* deletion have poorer outcomes compared to those without *IKZF1* mutations. Additionally, patients with wild-type *IKZF1* respond more favorably to imatinib than those with *IKZF1* deletions.[24,25] Similarly, BCP-ALL patients with a *BCR*::*ABL1*-like signature and *IKZF1* deletion also have worse prognoses, as reported in several study cohorts.[26,27] In this study, across all age-defined subgroups of BCP-ALL, patients with *IKZF1* deletion alone had shorter OS compared to those without *IKZF1* deletion, with a *p*-value of 0.047. Furthermore, the combination of *BCR*::*ABL1* positivity and *IKZF1* deletion was predominantly found in adult BCP-ALL, showing a poorer prognosis, as indicated by shorter OS, compared to patients without *BCR*::*ABL1* or *IKZF1* detection.

5. Conclusion

We assessed the impact of *IKZF1* deletions on the clinical outcomes of BCP-ALL patients. Our MLPA analysis revealed that patients with either *IKZF1* deletion had shorter OS compared to those without *IKZF1* deletion, independent of other genetic alterations. Moreover, BCP-ALL patients positive for both *BCR*::*ABL1* and *IKZF1* deletion exhibited a worse prognosis than those negative for *BCR*::*ABL1*.

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