Prognostic significance of copy number alterations detected by multi-link probe amplification of multiple genes in adult acute lymphoblastic leukemia

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Abstract. The multiplex ligation-dependent probe amplification (MLPA) method was used to detect the copy number alterations (CNAs) of IKAROS family zinc finger 1 (*IKZF1*), paired box 5 (*PAX5*), ETS variant 6 (*ETV6*), RB transcriptional corepressor 1 (*RB1*), BTG anti-proliferation factor 1 (*BTG1*), early B-cell factor 1 (*EBF1*), cyclin dependent kinase inhibitor 2A/2B (*CDKN2A/2B*) and cytokine receptor like factor 2 (*CRLF2*) genes in 87 adults with acute lymphoblastic leukemia (ALL) in China. The effects of CNAs on prognosis were analyzed. Gene deletions were detected in 58/87 (66.7%) ALL patients. The most common deletions were observed in the following genes: *IKZF1* (40.6%), *CDKN2A* (31.9%), *CDKN2B* (29%), *PAX5* (21.7%), *RB1* (14.5%) and *BTG1* (10.1%). B cell-ALL (B-ALL) patients with *CDKN2A/2B* deletions exhibited poor 2-year overall survival (OS; P=0.055) and relapse-free survival (RFS; P=0.054) rates. *CDKN2A/2B* deletions were associated with poor 2-year OS (P=0.045) and RFS (P=0.071) rates in Philadelphia chromosome positive (Ph^+) B-ALL patients, as well as in the high risk (HR) B-ALL group (P=0.037 and P=0.047, respectively). Patients with *PAX5* deletions displayed poor 2-year OS (P=0.004) and RFS (P=0.016) rates in Philadelphia chromosome negative (Ph^-) B-ALL patients. Patients with ≥3 gene deletions exhibited a poorer prognosis than other patients (OS, P=0.001; RFS, 0.002).

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Introduction

Acute lymphoblastic leukemia (ALL) is the most common type of leukemia in children, with a low morbidity in adults (1). The efficacy of treatment is significantly increased by optimization of chemotherapy, improved treatment conditions and risk stratification (2). Factors, including age, white blood cell count, genetic characteristics and treatment response, determine the prognosis of adults with ALL (3). The genetic characteristics encompass genomic mutations and gene variations, and genomic analysis proposes a novel perspective on the pathogenesis and prognosis of ALL (4). The association between gene copy number variations (CNVs) and prognosis in adults with ALL has been investigated, but remains inconclusive.

Multi-link probe amplification (MLPA) was initially reported by Schwab *et al* (5) and Schouten *et al* (6). This method permits detection of multiple minor CNVs in the human genome and differences in the relative copy number of the target sequences. The method is commonly used to analyze the multiple gene polymorphisms underlying the disease, particularly for the analysis of large samples.

The present study used MLPA to analyze the gene CNVs in 87 adults with ALL treated between July 2009 and March 2015 at the Institute of Hematology and Blood Diseases Hospital (Tianjin, China). The aim of the present study was to determine the association between gene CNVs and the prognosis of a Chinese population of adults with ALL.

Materials and methods

Patients and samples. A total of 87 adult patients with ALL that were diagnosed and treated at the Leukemia department, Institute of Hematology and Blood Diseases Hospital between July 2009 and March 2015 were enrolled in the present study. The inclusion criteria was patients who were diagnosed with ALL aged >14 years. Individuals who had received treatment in other hospitals or were unable to afford regular chemotherapy were excluded. All the patients enrolled in the present study provided written informed consent and the study was

approved by Ethics Committee of the Institute of Hematology and Blood Diseases Hospital (Tianjin, China). The diagnosis was based on the morphology, immunophenotype, and molecular and cytogenetic analysis. The median follow-up time was 12.12 months (range, 1.25-63 months) and the rate of loss to follow-up was 5.7% (5/87). The patients were treated with regimens prescribed by ChiCTR-TRC-00000397 as described in Zhao *et al* (7), Bone marrow (BM) mononuclear cells (MNC) were collected prior to the induction of treatment and a QIAamp DNA Blood Mini kit (cat. no. 51104; Qiagen GmbH, Hilden, Germany) was used for DNA extraction, according to the manufacturer's protocols. TRIzol™ (Life Technologies; Thermo Fisher Scientific, Inc., Waltham, MA, USA) was used to extract RNA, RNA was also extracted from the MNCs of 50 patients (MNCs $<10^6$, as dictated by the TRIzol protocol) and was synthesized into cDNA, as previously described (8). Nested reverse transcription polymerase chain reaction (RT-PCR) was performed, as previously described (PCR Master mix; Takara Biotechnology Co., Ltd., Dalian, China) (8).

The present study investigated 87 adults with ALL, including 54 males and 33 females, with a median age of 19 years (range, 14-61 years). Of these patients, 69 presented with B-ALL and 18 with T-ALL. Among the patients with B-ALL, 29 patients exhibited abnormal t(9;22)/BCR-ABL1, which is also described as Ph positive chromosome (Ph^+ ALL) and 40 exhibited the Ph negative chromosome (Ph^- ALL).

Subgroups included 53 patients in the high-risk group (HR) and 16 in the low-risk group (SR). The T-ALL group included 15 cases of HR and 3 cases of SR. The prognosis was based on the guidelines by Gökbuget and Hoelzer (9). The age of the SR group was ≤35 years and the white blood cell count was $<30x10^9$/l; and TEL-AML1, HOX11, NOTCH1, 9p and polyploidy were observed. In contrast, the HR group included patients aged ≥35 years with a white blood cell count of $>30x10^9$/l in B-ALL ($>100x10^9$/l in T-ALL), diagnosed with pro B-ALL and exhibiting a complex and hypodiploid karyotype. Furthermore, DNA of 10 healthy people were extracted as normal control, including 6 males and 4 females (age range, 22-45 years). The samples from volunteers were collected from January to April 2015 at the Institute of Hematology and Blood Diseases Hospital.

Analysis of copy number alterations (CNAs). The SALSA MLPA P335 ALL-IKZF1 kit (MRC Holland, Amsterdam, the Netherlands) was applied to detect the gene CNAs, according to the manufacturer's protocol. This kit was able to detect the deletions of IKAROS family zinc finger 1 (*IKZF1*), purinergic receptor P2Y8 (*P2RY8*), zinc finger protein, Y-linked (*ZFY*), Janus kinase 2 (*JAK2*), paired box 5 (*PAX5*), ETS variant 6 (*ETV6*), RB transcriptional corepressor 1 (*RB1*), BTG anti-proliferation factor 1 (*BTG1*), early B-cell factor 1 (*EBF1*), cyclin dependent kinase inhibitor 2A/2B (*CDKN2A/2B*), cytokine receptor like factor 2 (*CRLF2*), interleukin 3 receptor subunit α (*IL3RA*), colony-stimulating factor 2 receptor α subunit (*CSF2RA*) and short stature homeobox (*SHOX*) genes. Electrophoresis (pop7 polymer used as supplied) and quantification of fluorescein amidite-labeled amp (4 nmol/ml) icons were performed on an ABI-3730 genetic analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc.), 80˚C, 2 min. The resulting peak intensities were normalized to the manufacturer's control probes and the DNA from the normal control was used as a reference.

Statistical analysis. All statistical analyses were performed using SPSS 21.0 software (IBM Corp., Armonk, NY, USA). The data are presented as median ± quartile. Relapse-free survival (RFS; defined as the time between diagnosis and relapse) and overall survival (OS; defined as the time between diagnosis and mortality or last follow-up) were analyzed using the Kaplan-Meier method and the differences between multiple groups were analyzed using the log-rank test. Cox proportional hazards regression models were used to assess the prognostic relevance of different factors. Other comparisons were performed using the X2, Fisher exact, as appropriate. $P<0.05$ was considered to indicate a statistically significant difference.

Results

MLPA

Analysis of gene deletions. Gene deletions were detected in 58/87 (66.7%) cases of ALL. The common deletions included those in the *IKZF1* 32.2% (28/87), *CDKN2A* 35.6% (31/87), *CDKN2B* 29.9% (26/87), *PAX5* 18.4% (16/87) and *RB1* 13.8% (12/87) genes. Deletions in the genes, *EBF1* (8/87, 9.2%), *BTG1* (8/87, 9.2%) and *ETV6* (7/87, 8%), while deletions in the genes, *IL3RA-1*, *JAK2*, *CSF2RA-1* and *P2RY8,* accounted for <5%, and no gene deletions were observed in 29 patients (33.3%; Fig. 1A).

In the B-ALL group, gene deletions were detected in 45/69 patients (65.2%). The commonly deleted genes were *IKZF1* (28/69, 40.6%), *CDKN2A* (22/69, 31.9%), *CDKN2B* (20/69, 29%), *PAX5* (15/69, 21.7%), *RB1* (10/69, 14.5%), *BTG1* (7/69, 10.1%), *EBF1* (8/69, 9.2%) and *ETV6* (7/69, 8%). No gene deletions were observed in 24 patients (34.8%; Fig. 1B). Among those with gene deletions, one single gene deletion was observed in 16 patients (16/45, 35.6%), two were observed in 11 patients (11/45, 24.4%) and ≥3 were observed in 18 patients (18/45, 40%). The loss of *CDKN2A* and/or *CDKN2B* (*CDKN2A/2B)* was reported in 25 patients (25/45, 55.6%). The loss of *IKZF1* and *CDKN2A/2B* was observed in 11 patients (11/45, 22.4%). Simultaneous deletions of *IKZF1* and other genes were reported in 23 patients (23/28, 82.1%). A total of 14 (14/15, 93.3%) cases of *PAX5* deletions and 16 (17/25, 64%) of *CDKN2A/2B* deletions were accompanied by the deletion of other genes. All patients with *BTG1* and *RB1* deletions exhibited deletions in other genes. At the end of the follow-up period, 22 cases of recurrence were observed in patients with B-ALL. Of these, 15 exhibited gene deletions, including 9 (9/28, 32.1%) with the loss of *IKZF1*, 9 (9/25, 36%) with the loss of *CDKN2A/2B* and 5 (5/15, 33.3%) with the deletion of ≥3 genes.

Of the patients with T-ALL, 13/18 (72.2%) harbored the following deletions: *CDKN2A* (9/18, 50%), *CDKN2B* (6/18, 33.3%), *ETV6* (4/18, 22.2%), *RB1* (2/18, 11.1%), *EBF1* (2/18, 11.1%), *PAX5* (1/18, 5.6%) and *BTG1* (1/18, 5.6%; Fig. 1B). Of the 13 patients with gene deletions, 8 (8/18, 44.4%) exhibited 1 gene deletion, 4 (4/18, 22.2%) exhibited 2 and 1 (1/18, 5.6%) exhibited ≥3. Three cases (3/18, 16.7%) were identified with

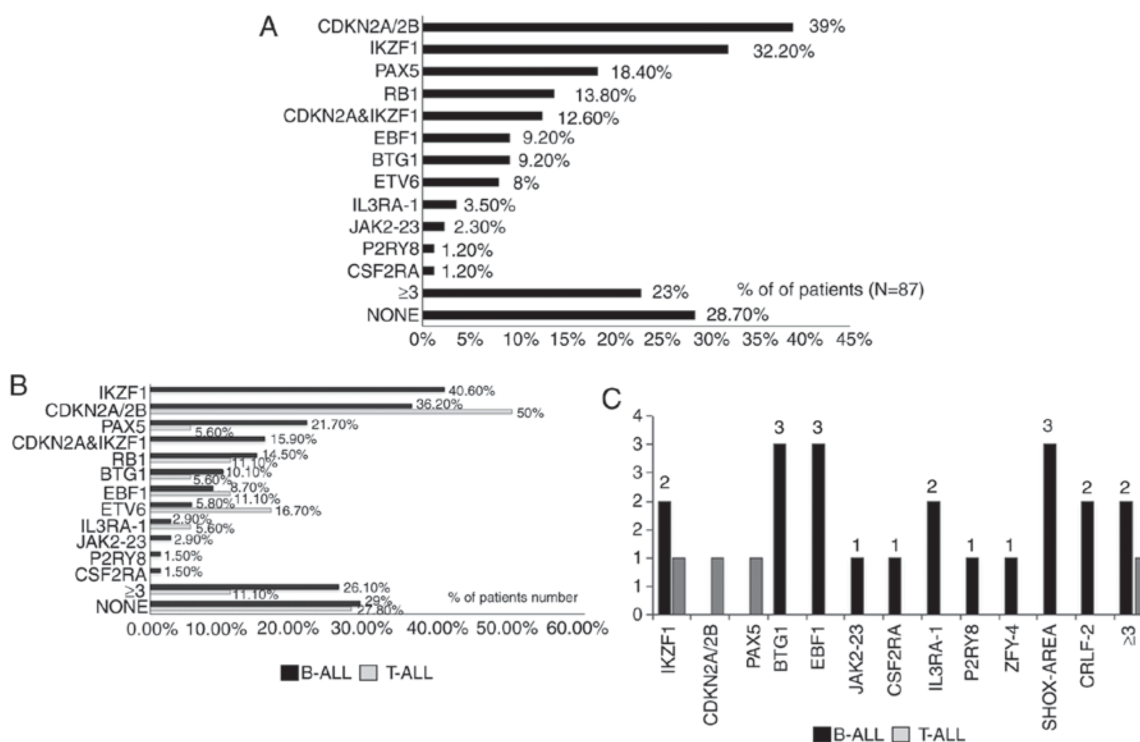

Figure 1. Frequencies of copy number alterations. (A) Gene deletions in the entire cohort (n=87). (B) Gene deletions in patients with B-ALL or T-ALL. (C) Gene amplification in the whole cohort; B-ALL, B-cell acute lymphoblastic leukemia; T-ALL, T-cell acute lymphoblastic leukemia; IKZF1, IKAROS family zinc finger 1; CDKN2A/2B, cyclin dependent kinase inhibitor 2A/2B; C&I, CDKN2A/2B and IKZF1; RB1, RB transcriptional corepressor 1; ETV6, ETS variant 6; PAX5, paired box 5; BTG1, BTG anti-proliferation factor 1; EBF1, early B-cell factor 1; JAK2, Janus kinase 2; CSF2RA, colony-stimulating factor 2 receptor α subunit; IL3RA-1, interleukin 3 receptor subunit α; P2RY8, purinergic receptor P2Y8.

the co-deletion of *CDKN2A/2B* and other genes. Two (2/18, 11.1%) patients with *ETV6* deletions exhibited concurrent deletions of other genes. All the T-ALL patients with *BTG1* and *RB1* deletions also exhibited other deletions, as observed in the patients with B-ALL. A total of 15 cases of T-ALL displayed recurrence, including 11 patients with gene deletions, of which 6 (6/9, 66.7%) exhibited *CDKN2A/2B* deletion.

IKZF1 gene deletion analysis. IKZF1 gene deletion was identified in 28/87 patients (32.2%). The *IKZF1* gene deletion is significantly more common in Ph^+ patients compared with Ph^- B-ALL patients (21/29, 72.4% vs. 7/40, 17.5%; P<0.01). The patients in the HR group exhibited a deletion of the *IKZF1* gene more frequently than those in the SR group (27/53, 50.9% vs. 1/16, 6.3%. P=0.001). The deletion of *CDKN2A/2B* and *IKZF1* together in patients with Ph^+ B-ALL was more frequently observed than in those with Ph^- B-ALL (9/29, 31% vs. 3/40, 7.5%; P=0.021). The frequencies of *CDKN2A/2B* and *IKZF1* deletions were higher in the HR group than in the SR group (20.8 vs. 0%; P=0.056; Table I).

A total of 12 cases (12/28, 42.9%) revealed the deletion of exons 4-7, which were the most common deletions, and deletions of exons 1-8 were observed in 2 cases (2/28, 7.1%). Only single cases exhibited a deletion of exons 1 or 6.

A total of 50 cases, including 38 cases of B-ALL and 12 cases of T-ALL, were analyzed by nested RT-PCR for *IKZF1* deletion. A total of 32 patients with *IKZF1* deletion (64%; 20 patients with IK6 subtype), including 28 patients with B-ALL (73.7%; 16 patients with Ph^+) and 4 patients with T-ALL (33.3%), were evaluated by PCR. However, MLPA indicated that only 16/38 patients with B-ALL exhibited the deletion of *IKZF1* (8 cases of IK6 subtype), while none of the 12 patients with T-ALL presented with an *IKZF1* deletion. Therefore, the sensitivity of the two methods was different.

Analysis of other gene deletions. RB1 deletions in the Ph^+ group of patients with B-ALL were more frequent than in the Ph^- group (8/29, 31% vs. 1/40, 2.5%; P=0.001). However, single-gene defects in *RB1* were not observed. More than three gene deletions were commonly observed in the Ph^+ group of patients with B-ALL compared with the Ph^- group (13/29, 44.8% vs. 5/40, 12.5%; P=0.004). This phenomenon was more common in the SR group than in the HR group (16/53, 30.2% vs. 2/16, 12.5%; P=0.003). Furthermore, no significant differences were observed in the distribution of other gene deletions across different groups.

Analysis of gene amplification. Amplification of 12 genes was detected in 15 patients (15/87, 17.2%). The common amplifications were noted for *SHOX-AREA* (3/15, 20%), *BTG1* (3/15, 20%) and *EBF1* (3/15, 20%) genes. A total of 4/15 patients harbored only the gene amplification and the remaining 11 patients displayed concurrent gene deletions. A single gene amplification was reported in 12 cases and >2 amplifications were observed in 3 patients. The gene amplifications were identified in 14/15 cases of B-ALL, 1 case of T-ALL, 1 case in the SR group and 14 cases in the HR group (6.25 vs. 26.4%). The specific gene amplifications are presented in Fig. 1C.

Effects of gene deletion on survival

Prognostic significance of IKZF1 deletion. In B-ALL, the 2-year OS and RFS rates in patients with *IKZF1* deletions were slightly worse than in those without, although

Table I. Frequencies of gene deletions in different groups of B-cell acute lymphoblastic leukemia patients.

	Ph Chromosome			Risk stratification		
Deleted gene	Ph^+ (n=29)	Ph^- (n=40)	P-value	HR (n=53)	SR (n=16)	P-value
IKZF1	21 (72.4%)	7 (17.5%)	0.00	27 (50.9%)	1 (6.3%)	0.001
CDKN2A/2B	10 (34.5%)	15 (37.5%)	1	21 (39.6%)	4 (25%)	0.379
C&I	8 (27.6%)	3 (7.5%)	0.021	11 (20.8%)	0	0.056
RB1	9 (31%)	1 (2.5%)	0.001	9 (17%)	1 (6.3%)	0.433
ETV6	2 (6.9%)	2 (5%)	1	3 (5.7%)	1 (6.3%)	1
PAX5	7 (24.1%)	8 (20%)	0.771	11 (20.8%)	4 (25%)	0.736
BTG1	5 (17.2%)	2 (5%)	0.122	6 (11.3%)	1 (6.3%)	1
EBF1	5 (17.2%)	1 (2.5%)	0.122	5 (9.4%)	1 (6.3%)	1
JAK2	2 (6.9%)	0	0.173	2 (3.8%)	0	1
CSF2RA	1 (3.4%)	0	0.42	1 (1.9%)	0	1
IL3RA-1	2 (6.9%)	0	0.173	2 (3.8%)	0	1
P2RY8	1 (3.4%)	0	0.42	1 (1.9%)	0	1
No deletion	5 (17.2%)	19 (47.5%)	0.011	15 (28.3%)	10 (62.5%)	0.018
≥3 deletions	13 (44.8%)	5 (12.5%)	0.004	16 (30.2%)	2 (12.5%)	0.003

Ph, Philadelphia chromosome; HR, high-risk; SR, low-risk; IKZF1, IKAROS family zinc finger 1; CDKN2A/2B, cyclin dependent kinase inhibitor 2A/2B; C&I, CDKN2A/2B and IKZF1; RB1, RB transcriptional corepressor 1; ETV6, ETS variant 6; PAX5, paired box 5; BTG1, BTG anti-proliferation factor 1; EBF1, early B-cell factor 1; JAK2, Janus kinase 2; CSF2RA, colony-stimulating factor 2 receptor a subunit; IL3RA-1, interleukin 3 receptor subunit a; P2RY8, purinergic receptor P2RY8. Treatment: Patients with Ph^-: Introduction therapy: VDCLP; early stage consolidation therapy: CAM; HD-MTX; late stage consolidation therapy: MA; VDLD; COATD; HD-MTX; TA; maintenance therapy: MM; MOACD (take maintenance therapy every 6 months). Patients with Ph^+: Patients take chemotherapy regimens like patients with Ph^-, and they also take tyrosine kinase inhibitor (TKI) simultaneously.

no significant difference was observed (OS, 60.8 vs. 51.2%, P=0.247; RFS, 51.3 vs. 35.5%, P=0.169). Furthermore, no significant differences in the 2-year OS (57.5 vs. 47.6%; P=0.256) and RFS (55.6 vs. 34.3%; P=0.209) rates were observed between patients with *IKZF1* deletion and those without in the Ph^- B-ALL group. Additionally, no significant differences in the 2-year OS (50 vs. 52%, P=0.284) and RFS (50 vs. 42.4%, P=0.256) rates were observed between the 21 patients with *IKZF1* deletion and those without in the Ph^+ B-ALL group.

Prognostic analysis of gene deletions. Among the patients with B-ALL, the 2-year OS (61.8 vs. 30.2%; P=0.055) and RFS (53.3 vs. 29.3%; P=0.054) rates in patients with *CDKN2A/2B* deletions were slightly worse than in those without these deletions (Fig. 2). In addition, no significant differences in the 2-year OS (55.2 vs. 34.7%; P=0.296) and RFS (51.7 vs. 31.2%; P=0.275) rates were observed between patients with and without the *CDKN2A/2B* deletions in the Ph^- B-ALL group. The 2-year OS and RFS rates in patients with *CDKN2A/2B* deletions were worse than in the other patients (60.6 vs. 18.7%, P=0.045) and (63.2 vs. 31.1%, P=0.071) in the Ph^+ B-ALL group (Fig. 3). No significant differences were observed in the 2-year OS (72.2 vs. 50%; P=0.544) and RFS (70.7 vs. 50%; P=0.726) rates of patients with *CDKN2A/2B* deletion in the SR group. The HR group carrying the *CDKN2A/2B* deletion exhibited poor 2-year OS (58.4 vs. 24.7%, P=0.037) and RFS (50.7 vs. 25%, P=0.047) rates compared with the other patients (Fig. 4).

Prognostic analysis of PAX5 deletion. In the present study, 16 patients carried the *PAX5* gene deletion. Among the patients with B-ALL, no significant difference in the 2-year OS (76.7 vs. 54%; P=0.432) and RFS (54.3 vs. 45%; P=0.44) rates was observed between those with and without *PAX5* deletions. The Ph^- B-ALL patients with *PAX5* deletions exhibited poor 2-year OS (90.1 vs. 19%; P=0.004) and RFS (83.7 vs. 21.4%; P=0.016) rates compared with those without this deletion. No significant difference was observed in the OS (59.1 vs. 53.6%; P=0.749) and RFS (63.7 vs. 57.1%; P=0.785) rates of Ph^+ B-ALL patients with *PAX5* deletion with respect to those in the Ph^+ B-ALL group (Fig. 5).

Prognosis of patients with ≥3 gene deletions. No notable differences were observed in the 2-year OS (56.7 vs. 49.4%; P=0.738) and RFS (60.1 vs. 49.7%; P=0.455) rates of B-ALL patients with ≥3 gene deletions (18 cases) compared with the B-ALL patients who harbored <3 gene deletions. Patients with ≥3 gene deletions exhibited poor 2-year OS (0 vs. 85.3%; P=0.001) and RFS (0 vs. 79.2%; P=0.002) rates compared with those in the Ph^- B-ALL group. No marked differences were observed in the 2-year OS (50.5 vs. 56.1%, P=0.88) and RFS (61.5 vs. 50.5%, P=0.873) rates of patients carrying ≥3 gene deletions compared with those in the Ph^+ group (Fig. 6).

Multifactor analysis of Cox multiple regression. The OS and RFS rates of different patient groups were analyzed using Cox regression based upon multiple factors. The white blood cell count for OS and RFS demonstrated independent prognostic significance in patients with B-ALL [P=0.056 and hazard ratio (HR)=1.004, and P=0.011 and HR=1.005, respectively]. In the Ph^- group of patients with B-ALL,

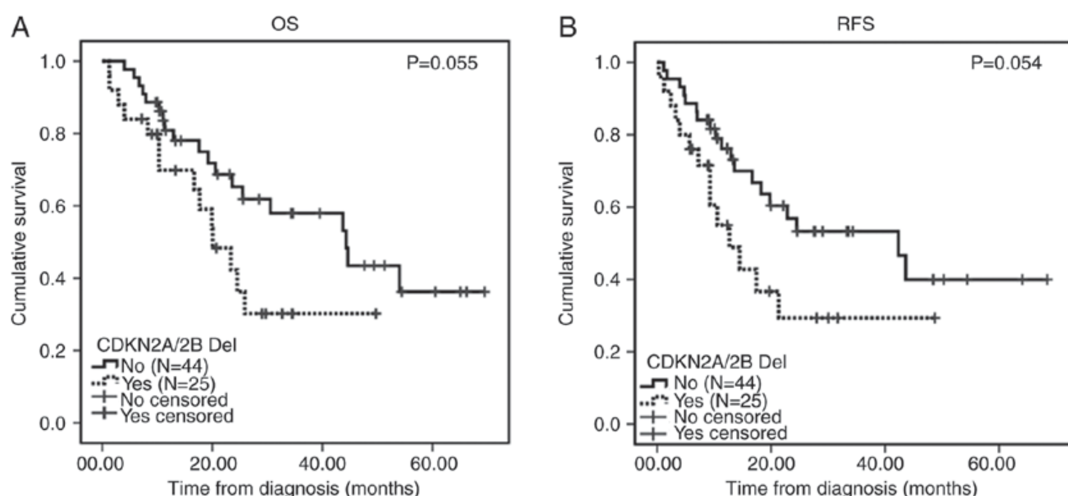

Figure 2. Survival based on *CDKN2A/B* deletions in patients with B-cell acute lymphoblastic leukemia. (A) OS in patients with and without the deletion of *CDKN2A/2B*. (B) RFS in patients with and without the deletion of *CDKN2A/2B*. *CDKN2A/2B*, cyclin dependent kinase inhibitor 2A/2B; Del, deletions; OS, overall survival; RFS, relapse-free survival.

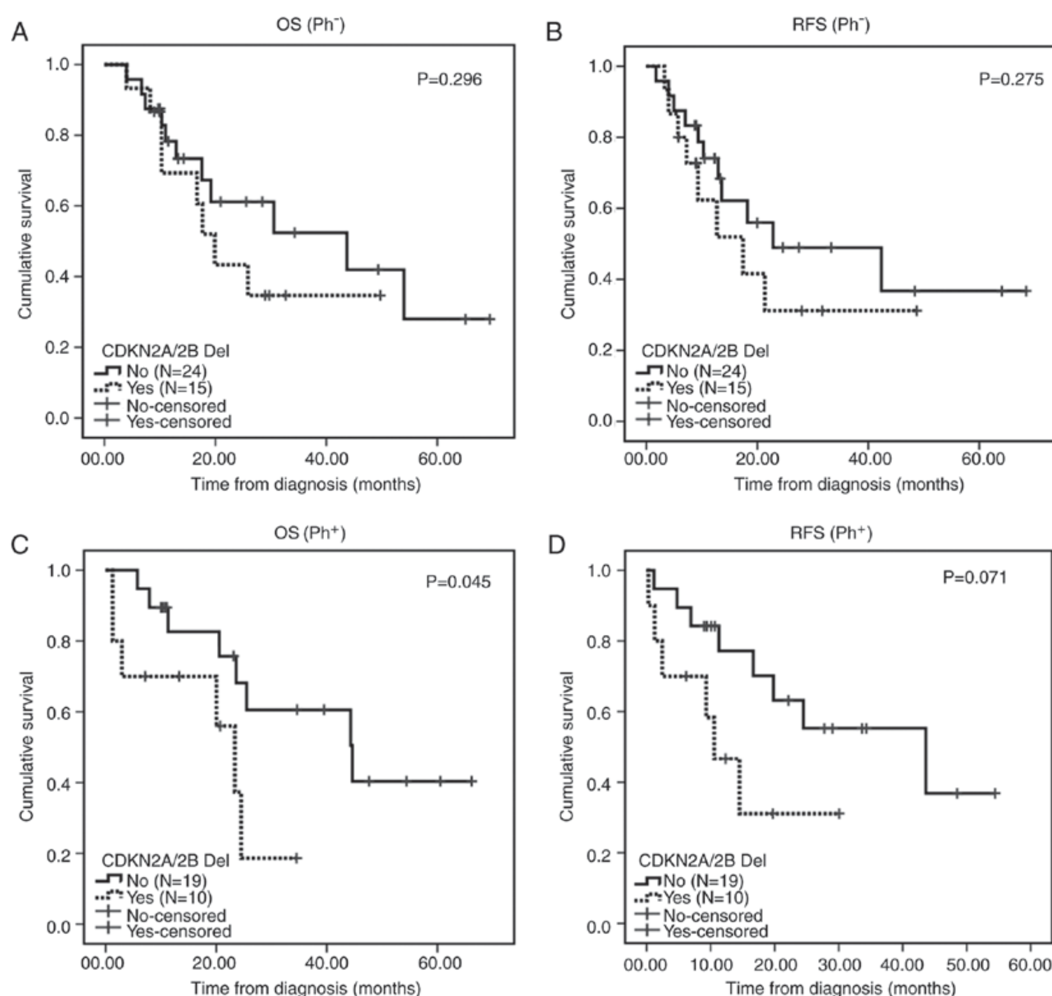

Figure 3. OS and RFS in B-ALL patients with and without *CDKN2A/2B* gene deletions. (A) OS and (B) RFS in Ph⁻ and (C) OS and (D) RFS in Ph⁺ B-ALL patients with and without *CDKN2A/2B* gene deletions. OS, overall survival; RFS, relapse-free survival; Ph, Philadelphia chromosome; B-ALL, B-cell acute lymphoblastic leukemia; CDKN2A/2B, cyclin dependent kinase inhibitor 2A/2B; Del, deletions.

the white blood cell count (OS, P=0.003, HR=1.007; RFS, P=0.001, HR=1.007) and ≥3 gene deletions were independent prognostic factors (OS, P=0.007, HR=12.4; RFS, P=0.06, HR=10.301). The *CDKN2A/2B* gene deletions (OS, P=0.056, HR=3.0) demonstrated independent prognostic significance in the Ph^+ B-ALL group. The white blood cell count (RFS,

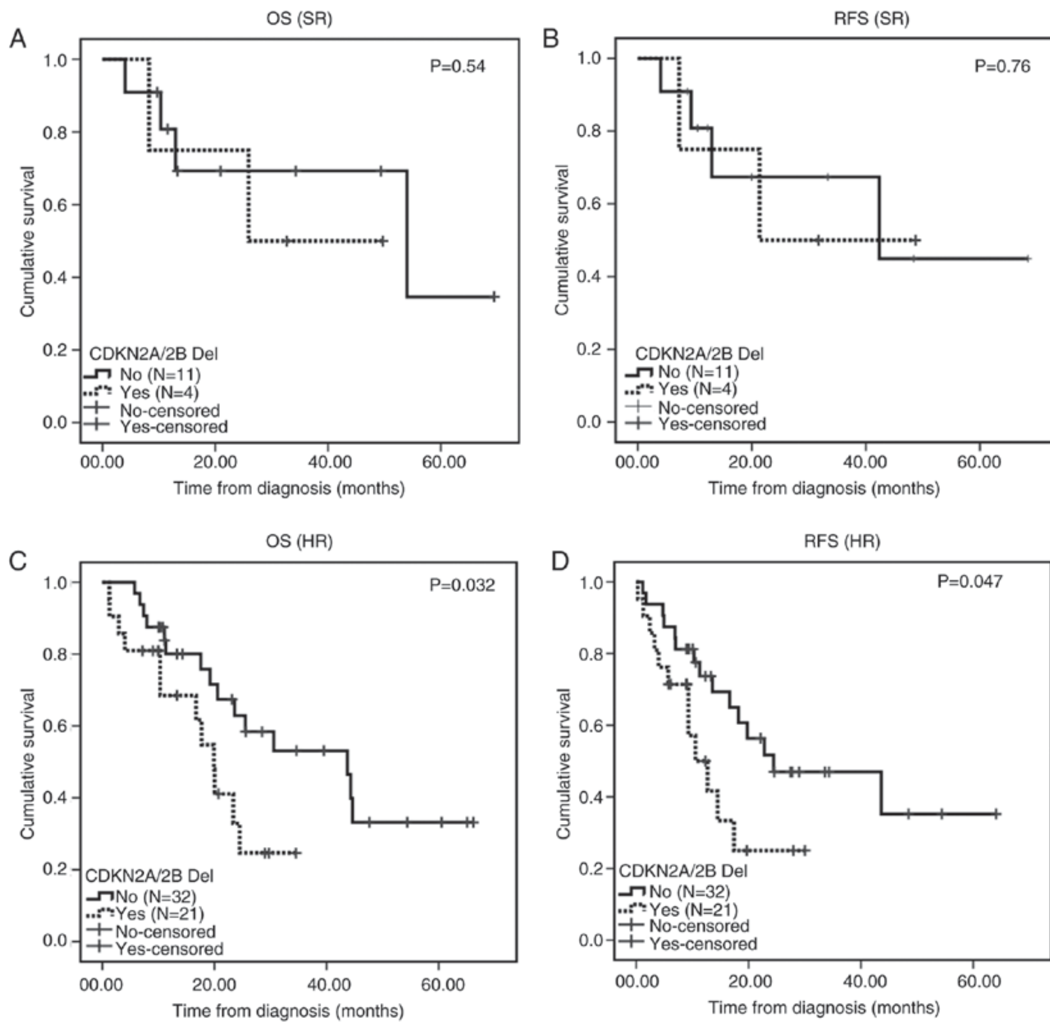

Figure 4. OS and RFS in the SR and HR groups with and without *CDKN2A/2B* gene deletions. (A) OS and (B) RFS in the SR group, and (C) OS and (D) RFS in the HR group with and without *CDKN2A/2B* gene deletions. OS, overall survival; RFS, relapse-free survival; SR, low-risk; HR, high risk; CDKN2A/2B, cyclin dependent kinase inhibitor 2A/2B; Del, deletions.

P=0.004, HR=1.005) and *CDKN2A/2B* gene deletions (OS, P=0.059, HR=2.322) demonstrated independent prognostic significance in the HR group. The *PAX5* gene deletion (OS, P=0.049, HR=2.322; RFS, P=0.056, HR=104.7) demonstrated independent prognostic significance in the SR group of patients with B-ALL.

Due to the limited number of T-ALL patients, and limited number of patients exhibiting gene amplification, no significant differences were observed in the survival analysis between different groups.

Discussion

MLPA was used previously to detect the ALL gene copy number in children (10), which revealed that the commonly deleted genes included *CDKN2A/B* (41%), *PAX5* (35%), *ETV6* (26%), *RB1* (5.1%), *BTG1* (4.3%) and *EBF1* (1.7%). The *IKZF1* deletions accounted for 16, and 26% of the patients with *IKZF1* deletions were categorized as the IK6 subtype (4-7 exons deletion). A similar method of detection was observed in 1,644 cases among British children with ALL in 2014 (11). *CDKN2A/2B* and *ETV6* are the commonly (20-25%) deleted genes in children with ALL; and *IKZF1* and *PAX5* gene deletions occurred simultaneously in 15% of patients. The proportion of other gene deletions was <10%. In ~43% patients, no gene deletions were detected and patients with ≥3 types of gene deletions were observed in 10% of all patients. MLPA facilitated the screening of 204 children with ALL relapse (12). The common gene deletions included *CDKN2B* (37.7%), *CDKN2A* (37.3%), *IKZF1* (33.3%), *PAX5* (26.5%) and *ETV6* (25%). The proportion of *IKZF1* gene deletions in these cases of ALL relapse was ~2-fold that reported previously in children newly diagnosed with ALL (33 vs. 14-19%). The common gene deletions identified in 142 cases of adolescent and adult ALL were *CDKN2A/2B* (42%), *IKZF1* (35%), *PAX5* (34%), *RB1* (15%), *BTG1* (10%), *EBF1* (11%) and *ETV6* (7%) (12). The majority of the patients with *IKZF1* and *CDKN2A/2B* deletions also harbored other deletions. The proportion of *IKZF1* deletions in Ph^+ patients was higher, and the age and white blood cell count of patients with *IKZF1* deletion were significantly higher than that of those without this deletion.

The results of the present study demonstrated that 58/87 (66.7%) patients with ALL harbored a gene deletion. The genes that were frequently deleted included *IKZF1* (40.6%), *CDKN2A* (31.9%), *CDKN2B* (29%), *PAX5* (21.7%), *RB1* (14.5%), *BTG1* (10.1%), *EBF1* (9.2%) and *ETV6* (7/69, 8%).

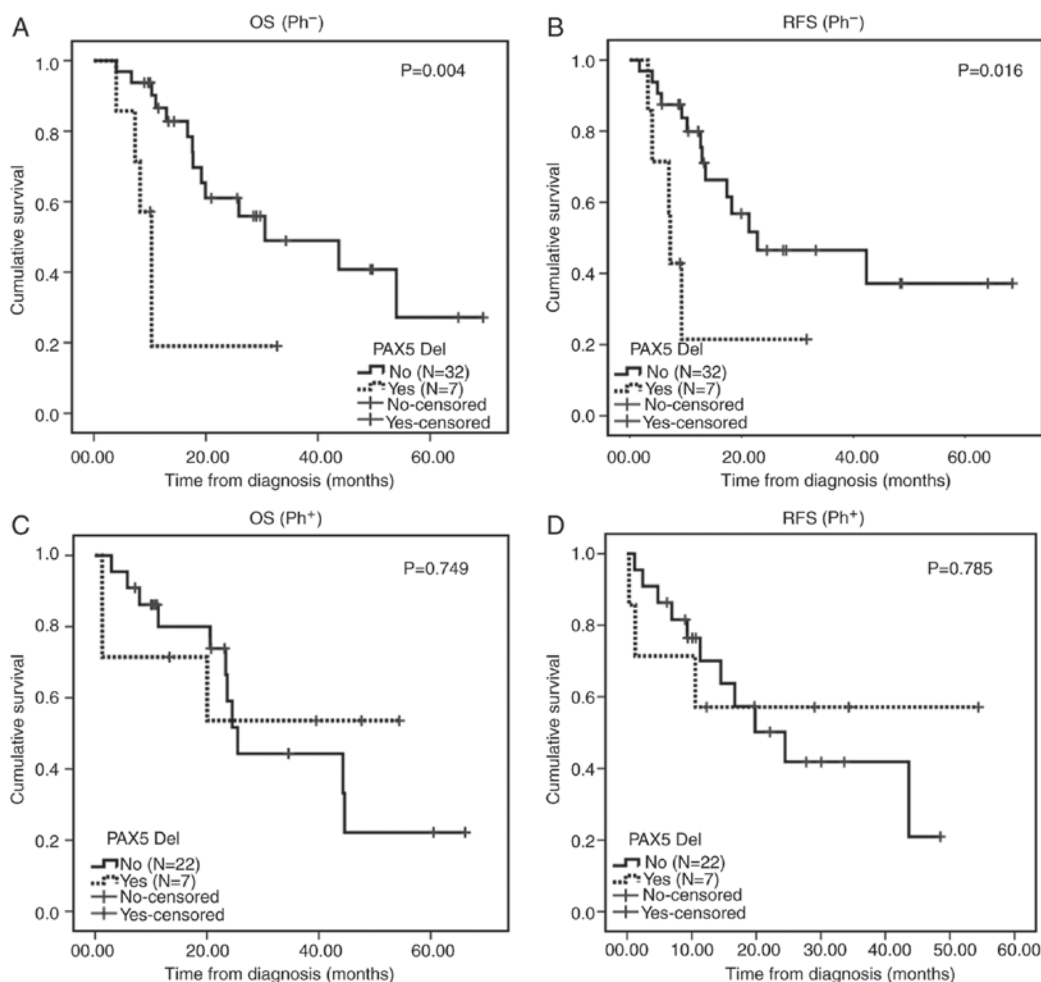

Figure 5. OS and RFS in B-ALL patients with and without *PAX5* gene deletions. (A) OS and (B) RFS in Ph⁻, and (C) OS and (D) RFS in Ph⁺ B-ALL patients with and without *PAX5* gene deletion. OS, overall survival; RFS, relapse-free survival; Ph, Philadelphia chromosome; Pax5, Paired Box 5; Del, deletions.

The 24 patients without gene deletions comprised 34.8% of the cohort. A total of 25 patients (55.6%) carried deletions in *CDKN2B* and/or *CDKN2A* genes. A concurrent deletion of *IKZF1* and *CDKN2A/2B* genes was observed in 11 patients (22.4%). Furthermore, in 16 patients (35.6%), only 1 gene was deleted, while 11 (24.4%) patients carried two gene deletions. More than 3 types of gene deletion were detected in a total of 18 cases (40%). Concurrent mutations, including 82.1% with a deletion of *IKZF1* and other genes, and 93.3% with a deletion of *PAX5* and other genes, were detected. In addition, *IKZF1* gene deletions were more common in the Ph⁺ group of patients with B-ALL than in the Ph⁻ group (72.4% vs. 17.5%), and were more frequent in the HR group than in the SR group (50.9% vs. 6.3%), which was in agreement with previous international adolescent and adult ALL studies (13). A total of 42.9% (12/28) patients with *IKZF1* deletions exhibited the IK6 subtype, a ratio that was higher than that reported in previous studies regarding pediatric ALL (14).

As demonstrated in Table II, the gene CNVs differed between adults and children with ALL. The ratio of *ETV6* deletions in children with ALL was higher, and the ratios of *IKZF1*, *CDKN2A/2B* and *EBF1* gene deletions were significantly lower than those in the adults. The prevalence of multiple gene deletions was lower in children than in adults. In the present study, *IKZF1* gene deletions were predominant, followed by CDKN2A/2B deletions. Ribera *et al* (15) reported that German adolescents and adult patients with ALL exhibited prevalent deletions of *CDKN2A/2B*.

Ofverholm *et al* (10) revealed that deletion of the *IKZF1* gene in childhood ALL was associated with poor OS and event-free survival (EFS) while no significant differences in OS and EFS were observed in children with *CDKN2A/2B* deficiency. Moorman *et al* (11) combined the incidence of CNA with risk stratification and revealed that the deletion ratios of *CDKN2A/2B*, *PAX5* and *IKZF1* in patients with a poor OS and EFS accounted for 70, 45 and 45%, respectively. The proportion of patients with a better prognosis was 1, 5, and 0%, respectively. Ribera *et al* (15) reported that the 5-year cumulative incidence rate (CIR) was higher and that the OS was poorer in patients with *IKZF1* deletion than in those without the deletion. Additionally, *CDKN2A/2B* deficiency in patients with B-ALL was associated with a poor OS. The OS of patients with B-ALL, particularly those with Ph⁻ ALL carrying ≥3 gene deletions was poor with an increased CIR. Adult Ph⁺ ALL patients carrying *CDKN2A/2B* deletions also exhibited a poor disease-free survival (DFS) (16). The present study evaluated the 2-year OS and RFS in different groups of patients. The deletions of *CDKN2A/2B* in B-ALL patients were associated with a poorer prognosis compared with that of other patients without *CDKN2A/2B* deletions. Patients with

Table II. Gene copy number variation in ALL patients across different study groups.

Patients	No.	Gene Del (%)									(Refs.)
		IKZF1	CDKN2A/2B	PAX5	RB1	BTG1	EBF1	ETV6	≥3 Del	No Del	
UK, Pediatric ALL	1,644	27	11	17	2	6	1	23	10	43	(11)
Sweden, Pediatric ALL	116	41	16	3.5	5.1	4.1	7.1	26	-	-	(10)
Germany, Relapse Pediatric ALL	204	37.7	33.3	26.5	6.4	9.3	37.7	25	-	-	(12)
Germany, Young and Adult B-ALL	142	42	35	34	15	10	11	7	48.6	18	(13)
Adult ALL	87	35.6	32.2	18.4	13.8	9.2	9.2	8	21.8	33.3	The present study
Adult B-ALL	69	31.9	40.6%	21.7	14.5	10.1	9.2	8	40	34.8	

ALL, acute lymphoblastic leukemia; Del, deletions; IKZF1, IKAROS family zinc finger 1; CDKN2A/2B, cyclin dependent kinase inhibitor 2A/2B; PAX5, paired box 5; RB1, RB transcriptional corepressor 1; BTG1, BTG anti-proliferation factor 1; EBF1, early B-cell factor 1; ETV6, ETS variant 6.

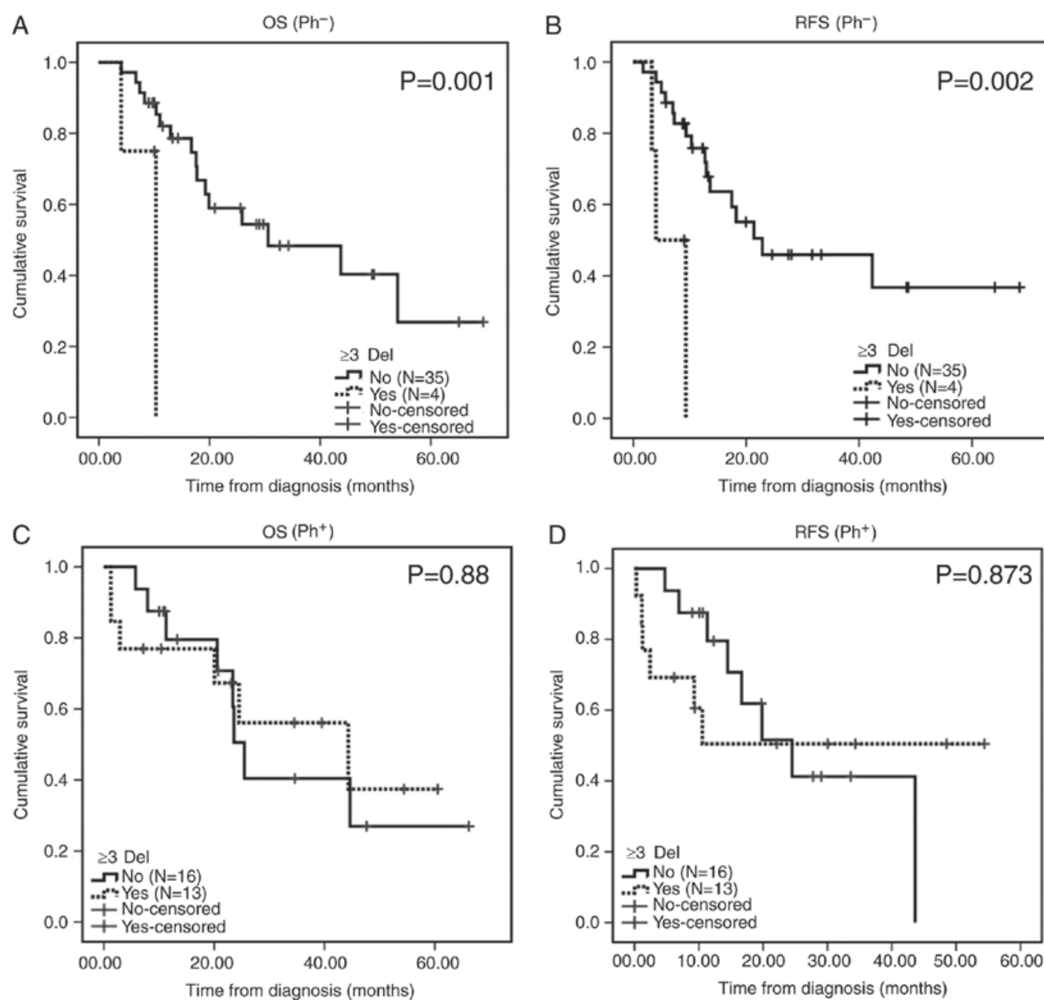

Figure 6. OS and RFS in B-ALL patients with and without three or more gene deletions. (A) OS and (B) RFS in Ph$^-$, and (C) OS and (D) RFS in Ph$^+$ B-ALL patients with and without three or more gene deletions. OS, overall survival; RFS, relapse-free survival; B-ALL, B-cell acute lymphoblastic leukemia; Ph, Philadelphia chromosome; Del, deletions.

CDKN2A/2B deletions and those with concurrent *IKZF1* and *CDKN2A/2B* deletions exhibited a poorer prognosis than the patients in the Ph$^+$ ALL group. Compared with the patients without *PAX5* deletions, those with *PAX5* deletions exhibited a

poorer prognosis in the SR group of patients with B-ALL and those with *CDKN2A/2B* deletion exhibited a poorer prognosis in the HR group of B-ALL patients than patients who did not harbor CDKN2A/2B deletion. Patients with *PAX5* deletions and ≥3 gene deletions exhibited a poorer prognosis than the patients in the Ph^- group. As mentioned earlier, patients with *IKZF1* gene deletions did not exhibit a poor prognosis in the present study, which may be attributed to the small sample size and short follow-up duration. In addition, it was revealed that MLPA was less sensitive than PCR in analyzing the *IKZF1* gene deletions, which may result in an increased number of false negative cases and may influence the prognostic significance of such observations (8).

To conclude, 66.7% of adult patients with ALL in a Chinese population exhibited variations in gene copy number. The types and proportions of gene variation were consistent with the results reported in the literature for adult ALL and it was concluded that certain gene copy number variations may be used to predict the prognosis of ALL.

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Competing interests

The authors declare that they have no competing interests.

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