Original Article

Genetic variants and clinical significance of pediatric acute lymphoblastic leukemia

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Background: Acute lymphoblastic leukemia (ALL), the most common childhood malignancy, is characterized by molecular aberrations. Recently, genetic profiling has been fully investigated on ALL; however, the interaction between its genetic alterations and clinical features is still unclear. Therefore, we investigated the effects of genetic variants on ALL phenotypes and clinical outcomes.

Methods: Targeted exome sequencing technology was used to detect molecular profiling of 140 Chinese pediatric patients with ALL. Correlation of genetic features and clinical outcomes was analyzed.

Results: T-cell ALL (T-ALL) patients had higher initial white blood cell (WBC) count (34.8×10^9/L), higher incidence of mediastinal mass (26.9%), more relapse (23.1%), and enriched *NOTCH1* (23.1%), *FBXW7* (23.1%) and *PHF6* (11.5%) mutations. Among the 18 recurrently mutated genes, *SETD2* and *TP53* mutations occurred more in female patients (P=0.041), *NOTCH1* and *SETD2* mutants were with higher initial WBC counts ($\geq50\times10^9$/L) (P=0.047 and P=0.041), *JAK1* mutants were with higher minimal residual disease (MRD) level both on day 19 and day 46 (day 19 MRD ≥1%, P=0.039; day 46 MRD ≥0.01%, P=0.031) after induction chemotherapy. Multivariate analysis revealed that initial WBC counts ($\geq50\times10^9$/L), *MLLr*, and *TP53* mutations were independent risk factors for 3-year relapse free survival (RFS) in ALL. Furthermore, *TP53* mutations, age (<1 year or ≥10 years), and *MLLr* were independently associated with adverse outcome in B-cell ALL (B-ALL).

Conclusions: *MLLr* and *TP53* mutations are powerful predictors for adverse outcome in pediatric B-ALL and ALL. Genetic profiling can contribute to the improvement of prognostication and management in ALL patients.

Keywords: Acute lymphoblastic leukemia (ALL); clinical significance; genetic variants; next-generation sequencing (NGS); pediatric

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Introduction

Acute lymphoblastic leukemia (ALL), the most common childhood tumor, results in the malignant transformation of lymphoid progenitor cells, with more than 80% originating from B-cell progenitors (1). Childhood ALL develops more frequently in boys than in girls (male: female ratio, 55% to 45%) with the peak incidence occurring at 2 to 5 years of age (2). With intensified chemotherapy, remarkable

progress has been made in the treatment, and the five-year overall survival (OS) rate can reach 85% to 90% in pediatric ALL (2,3). However, relapse occurs in approximately 20% of children and is associated with a high rate of treatment failure and death, particularly when occurring in the first 18 months of therapy. It remains the leading cause of cancer-related death in children and young adults (4-6).

Cytogenetic alterations and molecular abnormalities are frequent, and several molecular markers have been identified to stratify risk and predict prognosis, as they play key roles in ALL pathogenesis. A few genetic alterations have been shown to have clinical significance and different mutation distributions have been revealed; for example, rare germline mutations in the genes *PAX5* (7) and *ETV6* (8) were found to be linked to familial leukemia. *PHF6* mutants had higher mutation prevalence in males (32.0% *vs.* 2.5%) in T-cell ALL (T-ALL) (9); Ras mutations (*KRAS*, *NRAS*, *FLT3*, *PTPN11*, *NF1*) are recurrent in pediatric B-cell ALL (B-ALL) and relapsed ALL patients, and their mutations may lead to prednisolone resistance (10,11); *TP53* mutations mostly occur in low hypodiploid (chromosome <44) and are associated with relapse (12,13); *SETD2* mutations often exist in relapsed ALL patients that are resistant to DNA-damaging chemotherapy agents (e.g., cytarabine, 6-TG, doxorubicin) and with a poor long-term survival (14-16); *CREBBP* mutations in the histone acetyltransferase (HAT) domain confer glucocorticoid resistance (13,17,18); *NT5C2* mutations confer resistance to 6-mercaptopurine and 6-thioguanine (19,20); *PRPS1* mutations are associated with thiopurine resistance (21).

However, the relevance of genetic alterations on disease phenotypes and clinical outcomes is largely unknown. Thus, understanding the genetic variants and clinical characteristics combined with evaluating the therapeutic effect and prognosis, may help us to explore the clinical significance and molecular pathogenesis. This may even improve the prognostic prediction for patients and help inform the selection of specific therapies. Also, with the advance of next-generation sequencing (NGS) technologies, simultaneous sequencing of multiple cancer-related genes through multiplex assay panels has become a more time and cost-efficient genetic testing strategy than single gene testing.

In this study, we intended to investigate the possible associations between genetic alterations and clinical phenotypes in Chinese pediatric patients with ALL, focusing on the influence of gene mutations on clinical significance and outcome.

Methods

Ethical compliance

Informed consent was obtained in accordance with the Declaration of Helsinki and approved by the Institutional Review Board of Children's Hospital of Fudan University (No. [2015]005), Shanghai, China.

Patients and samples

We evaluated a total of 140 Chinese pediatric patients with ALL enrolled consecutively, who had been diagnosed and treated in the children's hospital of Fudan University in China between January of 2015 and December of 2017. The diagnosis was based on the World Health Organization's classification and patients were treated using the CCCG-ALL-2015 protocol, which was modified from St. Jude Children's Research Hospital Total-XV protocol for newly diagnosed patients with ALL (Chinese protocol). Morphological, immunophenotyped and cytogenetic analyses were performed at the time of diagnosis. Bone marrow (BM) biopsy provided conclusive proof of ALL, typically with ≥20% of blast cells being leukemic lymphoblasts. Immunophenotypic determination of lineage commitment and developmental stage by flow cytometry are essential for correct diagnosis of ALL, while minimal residual disease (MRD) is also monitored by flow cytometry at day 19 and day 46. Cytogenetic analysis can be stratified according to ploidy, number of sets of chromosomes in the cell, and specific genetic abnormalities, such as translocations. The transcripts of *BCR-ABL1*, *ETV6-RUNX1*, *TCF3-PBX1*, and *SIL-TAL1* fusion genes, along with *MLL* rearrangement (*MLLr*), were detected with a reverse transcriptase polymerase chain reaction (RT-PCR) or fluorescence in situ hybridization (FISH), as previously described (22).

Retrospective evaluation included an assessment of underlying disease, clinical manifestations, laboratory findings, treatment, and outcomes. Laboratory findings included peripheral blood examination, serum ferritin (SF), lactate dehydrogenase (LDH), BM morphology, flow cytometry, cerebrospinal fluid examination (CSF); imaging tests for testicular invasion and lymph nodes included B ultrasound examination and computerized tomography (CT).

BM samples were collected at the time of diagnosis and matched with remission samples or fingernails as germline controls. Genomic DNA was extracted from cell pellets

using the DNAeasy Blood and Tissue Kit (Qiagen, USA). DNA was quantified using a Qubit Fluorometer (Life Technologies, USA), and DNA integrity was assessed by agarose gel electrophoresis.

Targeted capture sequencing and mutation analysis

Mutation analysis was performed by deep sequencing of 950 targeted exons genes related to cancer with sufficient reads coverage using a probe sequence capture array of Roche (http://www.nimblegen.com/products/seqcap/ez/v2/index.html) to enrich the exonic DNA (Joy Orient, China). The samples were sequenced on an Illumina Hiseq2500, and two parallel reactions were performed for each sample. After sequencing, BclToFastq (Illumina) was used to process the raw image files for base calling, and low-quality variations (quality score ≥20, Q20) were filtered. Cleaned reads were aligned to NCBI human reference genome (hg19) using Bowties2 (version 2.3.1), Samtools (version 1.1) and GATK (version 3.1.1) were used to analyze the single nucleotide variants (SNVs) and insertion or deletion in the sequence. Variants were queried against publicly available datasets such as 1,000 Genomes, NHLBI GO Exome Sequencing Project (ESP), and Exome Aggregation Consortium (ExAC) to filter out common polymorphisms [minor allele frequency (MAF) >0.01]. Synonymous changes and single nucleotide polymorphisms (SNPs) that MAF determined to be higher than 5% were removed (http://www.ncbi.nlm.nih.gov/projects/SNP). Nonsynonymous changes and small indels were filtered using SIFT software (version 1.03), Polyphen2 (version 2.2.2), PROVEAN (version 1.1.3), and MutationTaster 2. Variants associated with no-functional or truncating-proteins were classified as deleterious mutations. Deleterious mutations included stop-gain mutations, frameshift mutations, and splice site mutations. To identify candidate driver mutations, we filtered events, and the filtering criteria were a minimum coverage ≥10, minimum tumor variant frequency ≥0.10, normal variant frequency ≤0.05; any two prediction algorithms predicted to be deleterious or identified as recurrent in COSMIC were considered as candidate driver genes. Variants presented only in tumor samples were classified as somatic mutations.

In this study, we analyzed the association between clinical phenotypes and significant somatic mutations in 18 genes, these mutated genes occurred in more than 3 patients and were only limited to sequence analysis.

Statistical analysis

SPSS 24.0 (SPSS, Chicago, IL, USA) was used for statistical analysis. Comparisons of the categorical variables and continuous parameters were determined by Pearson's Chi-square test or Fisher's exact test. The Kaplan-Meier method was used to calculate the estimates of survival probability, which were compared by the log-rank test. Cox proportional hazards regression was used to analyze the possible factors of a recurrence by using a backward-selection stepwise modeling process. Relapse free survival (RFS) was defined as the time from a complete remission to relapse, or when follow-up was terminated. Two-sided P<0.05 was considered statistically significant.

Results

Comparisons of clinical characteristics between B-ALL and T-ALL

Among the 140 pediatric ALL patients enrolled in the analysis, 81.4% (n=114) were B-cell ALL (B-ALL) and 18.6% (n=26) were T-ALL. They included 86 males and 54 females, and the mean diagnosis age was 4.9 (range, 0.3–13.8 years). When compared with B-ALL patients, we found that newly diagnosed T-ALL patients had higher initial white blood cell (WBC) counts (34.8×10^9/L *vs.* 7.6×10^9/L, P=0.046), higher hemoglobin level (median 103 *vs.* 74.7 g/L, P=0.02), higher incidence of mediastinal mass (26.9% *vs.* 1.8%, P<0.001), higher LDH level (LDH ≥448 IU/mL, 86.4% *vs.* 49.6%, P=0.001) and easily occurring relapse (23.1% *vs.* 7.0%, P=0.036). There were no differences in gender, central nervous system (CNS) leukemia, testicular invasion at diagnosis, and treatment response of day 19 or day 46. Clinical characteristics of ALL patients at diagnosis are described in *Table 1*.

Recurrent deleterious mutations in pediatric ALL

Most of the ALL patients (n=138, 98.6%) harbored somatic mutations, and a majority, 72.9% (n=102) of ALL patients, carried more than one deleterious mutation. Even though T-ALL patients had higher mutational numbers in both coding mutations (average 6 *vs.* 8, P=0.267) and driver mutations (average 1 *vs.* 2, P=0.179), there was no significant difference between them (*Figure 1A*,*B*).

In all mutations, we found 18 deleterious mutations occurred in more than three ALL patients, and recurrently mutated genes with a mutation prevalence over 5%

Table 1 Comparison of clinical characteristics and outcomes between B-ALL and T-ALL patients

Variable	B-ALL	T-ALL	P value
	N (%)/median (range)	N (%)/median (range)	
Total	114 (81.4)	26 (18.6)	
Age (years)	4.3 (0.3–9.7)	5.5 (1.1–13.8)	0.112
WBC ($\times10^9$/L)	7.6 (1.1–445.1)	34.8 (0.5–381.2)	0.046
PLT ($\times10^9$/L)	60.1 (7–441)	70 (15–443)	0.308
Hb (g/L)	74.7 (13–298)	103 (31–309)	0.02
Gender			0.365
Male	68 (59.6)	18 (69.2)	
Female	46 (40.4)	8 (30.8)	
Cytogenetics			–
HeH	30 (26.3)	0	
Low hyperdiploid	7 (6.1)	0	
Hypodiploidy	2 (1.8)	0	
Gene fusions			–
ETV6-RUNX1	31 (27.1)	0	
BCR-ABL1	8 (7.0)	1 (3.8)	
TCF3-PBX1	3 (2.6)	0	
SIL-TAL1	0	5 (19.2)	
MLLr	3 (2.6)	1 (3.8)	
EVI1	5 (4.4)	1 (3.8)	
CNSL	1 (0.9)	1 (3.8)	0.338
Testicular invasion	3 (2.6)	1 (3.8)	0.565
Mediastinal mass	2 (1.8)	7 (26.9)	<0.001
SF			0.232
<500 ng/mL	74 (69.2)	18 (81.8)	
≥500 ng/mL	33 (30.8)	4 (18.2)	
LDH			0.001
<448 IU/mL	57 (50.4)	3 (13.6)	
≥448 IU/mL	56 (49.6)	19 (86.4)	
Day 19 BM			0.587
<5%	107 (93.9)	23 (88.5)	
≥5%	7 (6.1)	3 (11.5)	
Day 19 MRD			0.068
<0.01%	72 (63.7)	11 (44.0)	
≥0.01%	41 (36.3)	14 (56.0)	

Table 1 (*continued*)

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Variable	B-ALL	T-ALL	P value
	N (%)/median (range)	N (%)/median (range)	
Day 46 BM			0.161
<5%	110 (98.2)	24 (92.3)	
≥5%	2 (1.8)	2 (7.7)	
Day 46 MRD			0.242
<0.01%	102 (91.1)	21 (80.8)	
≥0.01%	10 (8.9)	5 (19.2)	
Risk group			–
LR	77 (67.5)	0	
IR	37 (32.5)	22 (84.6)	
HR	0	4 (15.4)	
Relapse	8 (7.0)	6 (23.1)	0.036

There was a statistically significant difference between the groups (P<0.05). HeH, (51–67 chromosomes), low hyperdiploidy (47–50 chromosomes), hypodiploidy (<44 chromosomes). B-ALL, B-cell ALL; T-ALL, T-cell ALL; WBC, white blood cells; PLT, platelet; Hb, hemoglobin; HeH, high hyperdiploidy; CNSL, central nervous system leukemia; SF, serum ferritin; LDH, lactate dehydrogenase; BM, bone marrow; MRD, minimal residual disease; LR, low risk; IR, intermediate risk; HR, high risk.

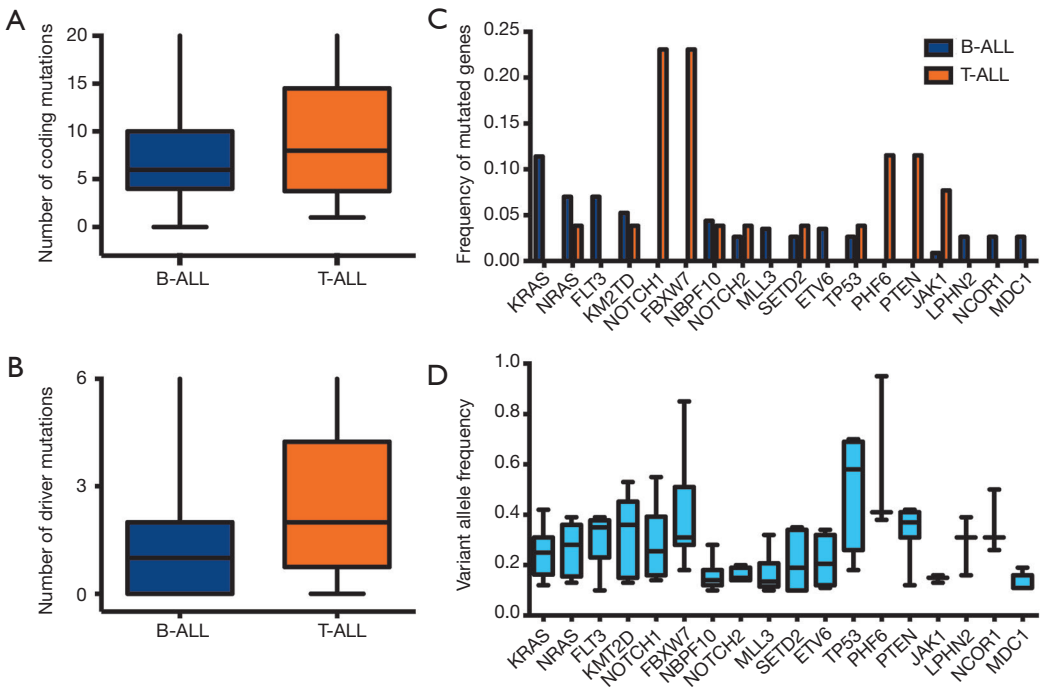

Figure 1 Recurrently somatic mutations in pediatric ALL patients. Boxplots shows the number of coding mutations (A) and driver mutations (B) in ALL patients; (C) comparison of frequencies of driver mutations between B-ALL and T-ALL patients. Recurrently mutated genes (n=18) presented more than 3 cases are shown; (D) boxplots of variant allele frequencies of recurrent mutations (n=18) presented in ALL patients. ALL, acute lymphoblastic leukemia; B-ALL, B-cell ALL; T-ALL, T-cell ALL.

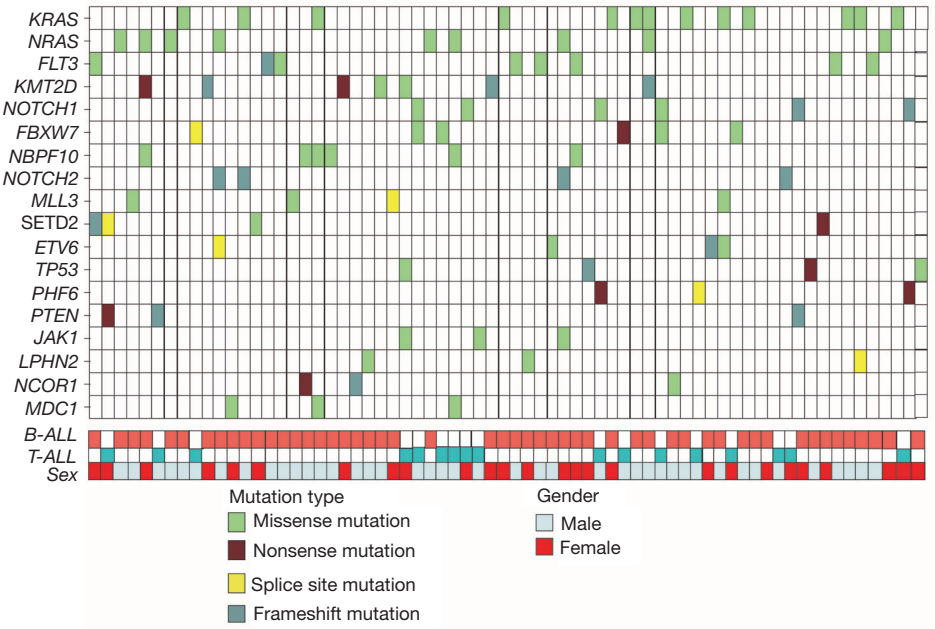

Figure 2 Recurrently mutated genes (n=18) in pediatric ALL patients. The heatmap diagram shows the recurrent mutations of ALL patients, and those genes occurring more than 3 times are shown. In the top panel, each row represents a gene, and each color box indicates a type of mutation. ALL, acute lymphoblastic leukemia.

included *KRAS* (9.3%), *NRAS* (6.4%), *FLT3* (5.7%), and *KMT2D* (5.0%) in childhood ALL. Genetic profiling was substantially different between B-ALL and T-ALL, including *KRAS* (11.4%), *NRAS* (7.0%), *FLT3* (7.0%), and *KMT2D* (5.3%) which were frequently mutated in B-ALL. Meanwhile, *NOTCH1* (23.1%), *FBXW7* (23.1%), *PHF6* (11.5%), and *PTEN* (11.5%) were enriched in T-ALL (*Figure 1C*). Among these mutations, *JAK1* mutations showed a low allelic burden and were considered more frequently to be from a subclone than a clone, suggesting that these mutations were more likely to be late events than founder alterations (*Figure 1D*, *Table S1*). However, some mutations co-existed in the same patients; for example, *NRAS/KRAS* occurred in one B-ALL patient, while *NOTCH1/FBXW7* and *NOTCH1/ PHF6* existed in two T-ALL patients (*Figure 2*).

Associations of genetic features with clinical characteristics

The association between genetic variations and clinical characteristics in 140 ALL patients were analyzed. In our study, there were no significant differences between clinical features and the number of somatic mutations. Furthermore, gene fusion-positive patients often co-existed with gene mutations (*Table S2*). Considering that clinical significance for deleterious mutations may exist, we evaluated the effects of individual alterations on the clinical features and treatment responses. Within the limits presented by the small number of subjects analyzed, we found a high rate were positive for *KRAS* mutations in our cohort, corresponding to an 11.4% incidence in B-ALL; however, no clinical correlation was found in this subgroup of patients. Interestingly, among other recurrently mutant genes, we found that *SETD2* and *TP53* mutations were more frequent in females (7.4%, P=0.041), and *SETD2* mutants were older than *SET2D* wild patients (5.5 *vs.* 4.5 years, P=0.041). However, *TP53* mutants were not characterized by an older median age as previously published by Stengel *et al.* (23). Meanwhile, *NOTCH1* or *SETD2* mutants were often found with higher initial WBC counts ($\geq 50\times10^9$/L, P=0.047 and P=0.044 respectively) for newly diagnosed ALL patients, but *ETV6* or *JAK1* mutants had lower primary BM blast cells. It seems that the number of initial WBC in peripheral blood is not associated with the number of primitive blast cells in the BM.

Furthermore, when comparing genetic alterations and treatment responses, it was revealed that *PTEN* mutants with higher BM blast cells at day 19 (20% *vs.*

Table 2 HR for RFS according to the presence of each genetic alterations and clinical features in ALL

Variable	Univariate analysis		Multivariate analysis	
	HR (95% CI)	P value	HR (95% CI)	P value
Whole group (n=140)				
KRAS mutations	0.04 (0–195.68)	0.465		
NRAS mutations	0.95 (0.124–7.29)	0.091		
FLT3 mutations	1.21 (0.158–9.26)	0.854		
NOTCH mutations	6.36 (1.40–28.91)	0.017		
TP53 mutations	7.39 (1.63–33.63)	0.010	20.37 (3.60–115.16)	0.001
WBC (≥50×10^9/L)	4.48 (1.55–12.92)	0.006	6.25 (1.88–20.83)	0.003
MLLr	38.20 (5.37–271.58)	<0.001	40.39 (5.56–293.70)	<0.001
T-ALL	4.42 (1.53–12.77)	0.006		
IR/HR	8.52 (1.91–38.08)	0.005		
Age (<1 y, ≥10 y)	3.67 (1.02–13.22)	0.047		
Gender (male)	2.31 (0.80–6.65)	0.122		
ETV6-RUNX1	0.26 (0.03–1.96)	0.190		
SIL-TAL1	1.95 (0.26–14.93)	0.542		
B-ALL (n=114)				
WBC (≥50×10^9/L)	4.54 (1.13–18.19)	0.033		
Age (<1 y, ≥10 y)	7.38 (1.76–30.96)	0.006	8.61 (1.02–72.43)	0.048
TP53 mutations	19.82 (3.82–102.80)	<0.001	13.38 (1.34–133.95)	0.027
MLLr	40.18 (6.36–253.75)	<0.001	171.35 (16.36–1,794.75)	<0.001
IR/HR	6.45 (1.30–31.97)	0.022		
Gender (male)	0.21 (0.04–1.06)	0.059		

There was a statistically significant difference between the groups (P<0.05). The prognostic impact on RFS was evaluated by univariate and multivariate Cox regression analyses. The prognostic impact on RFS was evaluated by the log-rank test. HR, hazard ratio; RFS, relapse free survival; CI, confidence interval; WBC, white blood cells; T-ALL, T-cell ALL; y, year; IR, intermediate risk; HR, high risk.

0.8%, P=0.013) and *JAK1* mutants had higher MRD level on both day 19 and day 46 (day 19 MRD ≥1%, P=0.039; day 46 MRD ≥0.01%, P=0.031) (*Tables S3-S8*). No other correlation with clinical features, such as gender, age, initial WBC counts, the percentage of blasts at diagnosis, and treatment outcomes, emerged from this analysis. The clinical significance of 18 mutated genes is summarized in *Table 2*, *S3-S8*.

Associations of genetic features with clinical outcomes

At the deadline of December 31st, 2017, in all 14 patients (*Table S9*), a relapse occurred, including 4 cases of early relapse (within more than 18 months from the first remission but less than 6 months after chemotherapy finished) and 10 cases of very early relapse (within less than 18 months from the first remission). In the treatment program, it was divided into low-risk and medium-high-risk protocol. Therefore, we further analyzed the relationship between individual alterations, clinical characteristics and RFS. Indeed, the frequency of relapse in patients mutated for *TP53* (50%) and *NOTCH1* (33.3%) was higher than other mutations. We identified six *NOTCH1* mutations, including 4 novel missenses mutations (L1678P, A375G, R1598P and I1616N) and 2 frameshift mutations (Q1455 Lfs*25, V2443Gfs*35) in primary diagnosed ALL patients,

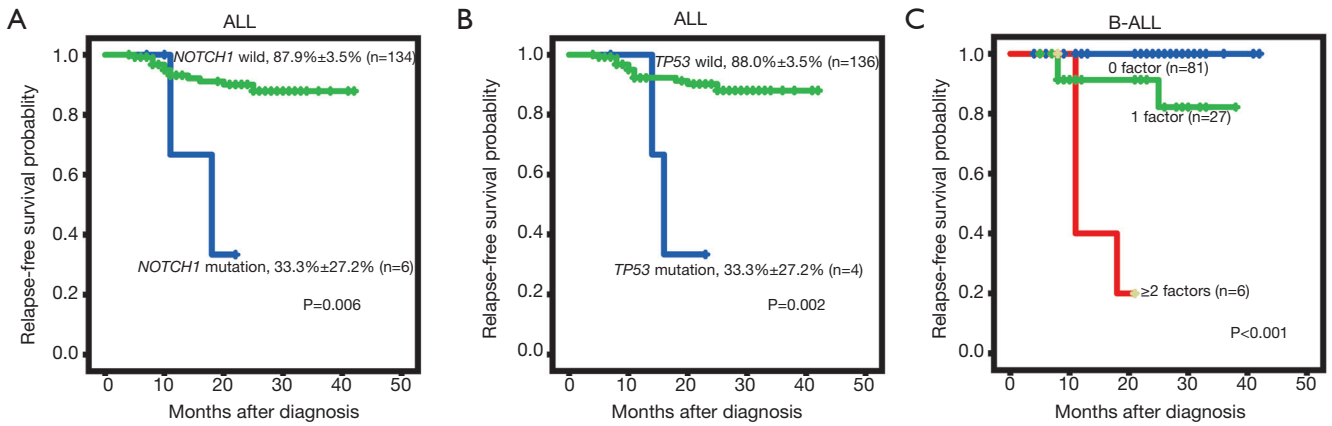

Figure 3 Prognostic impact of the genetic alterations in ALL patients. Kaplan-Meier survival curves of RFS of *NOTCH1* mutations (A) and *TP53* mutations (B) in ALL; (C) Kaplan-Meier survival curves of RFS of the accumulative numbers of driver mutations in B-ALL. The prognostic impact on RFS was evaluated by log-rank test; P<0.05 was considered a statistically significant difference. ALL, acute lymphoblastic leukemia; B-ALL, B-cell ALL; RFS, relapse free survival.

two pediatric patients with L1678P or A375G relapsed (*Tables S1*,*S9*) and with a shorter 3-year RFS rate 33.3% (P=0.006) (*Figure 3A*). Similarly, 4 patients carrying a *TP53* alteration entered clinical remission after induction therapy, but 2 patients with H179Mfs*68 or R273H suffered an early relapse, and the 3-year RFS rate was 33.3% (*Figure 3B*).

In a univariate analysis, somatic mutations involving *NOTCH1* and *TP53* were significantly associated with a poor outcome. In addition, several clinical prognostic factors were evaluated, and we found that WBC counts (≥50×10^9/L), *MLLr*, T-ALL, diagnosed age (<1 year or ≥10 years) as well as risk stratification (intermediate-high risk) were also significant predictors of an inferior survival (*Table 2*). In particular, *MLLr* was strongly predictive, with an odds ratio of 38.20 (5.37–271.58). Finally, we evaluated the relative effects of different mutations and clinical features together using Cox proportional hazards modeling with stepwise variable selection, incorporating initial WBC counts (≥50×10^9/L), *MLLr*, T-ALL, age, *NOTCH1*, *TP53* mutations and intermediate-high risk as covariates. We found that higher initial WBC counts (≥50×10^9/L), *TP53* mutations, and *MLLr* were independently associated with a shorter RFS, of which MLLr was the most significant predictor of clinical outcome of ALL patients with an odds ratio of 40.39 (5.56–293.70), suggesting a major role of these alterations in the progression of ALL (*Table 2*). Importantly, the effects of genetic alterations strongly depended on disease subtype: *NOTCH1* mutations mainly occurred in T-ALL, and *TP53* mutations were in B-ALL. Therefore, in the subsequent analyses, we stratified patients into B-ALL and T-ALL subtypes, incorporating subtype-specific clinical prognostic factors.

For B-ALL, *TP53* mutations, together with age (<1 year or ≥10 years) and *MLLr* were independently associated with an adverse outcome, but high WBC counts (≥50×10^9/L) and intermediate-high risk were risk factor. Based on the number of these relevant risk factors they had, B-ALL patients were classified into three categories showing significantly different 1-year RFS rates (P<0.001): 100% for those with no risk factor, 91.3% for those with 1 risk factor, and 20% for those with ≥2 risk factors (*Figure 3C*). Thus, the evaluation of the molecular status of *TP53* mutations, patient age, initial WBC counts, as well as *MLLr* would be informative in prognostication of B-ALL (*Table 2*).

However, because the number of T-ALL patients enrolled in this study was limited, and itself as an independent risk factor for recurrence in our cohort, no association was identified between T-ALL and genetic alterations.

Discussion

By analyzing clinical characteristics and genotyping data, we intended to demonstrate the clinical effects of genetic alterations, and looked to understand the significance of genetic profiling for prognostication in pediatric ALL.

The mutation profiling which occurred in our pediatric ALL was comparable to that reported in previous studies (3-6), whereas the incidence of *NOTCH1* mutation was

lower than the incidence reported in some studies from western populations and Chinese populations (5,24). Due to the detection of sequence mutations in ALL being insufficient, large deletion, amplification, rearrangement, and translocation should be warranted in the future.

Among the recurrent alterations, *TP53* mutations and *MLLr* were a powerful predictor for an adverse outcome in B-ALL and pediatric ALL. The poor prognosis of *MLLr* is well-recognized, and the basis for risk stratification in chemotherapy regimens, small molecule inhibitor pinometostat, has entered phase 1 clinical trials in both adult and pediatric *MLLr* leukemia, with the expectation that it will improve the prognosis of patients with *MLLr* in the future (25). *TP53* mutations mostly presented in the low hypodiploid subtype of ALL, approximately 50% of which were germline in nature, and were independently associated with a short survival (12,13,23,26). In our cohort, 4 pathogenic mutations all occurred in the *TP53* DNA-binding domain, which was likely to result in the ablation of the p53-mediated DNA damage response, thus forming a general resistance to antileukemia agents (27). Children with *TP53* variants were at a higher risk of second cancers, with a 5-year cumulative incidence of 25.1% and *TP53* mutation had independent prognostic value (28). Also, we found that *TP53* mutations were common in females (P=0.041), and other research showed that *TP53* mutation incidence increased with age (23,26). ALL patients carrying *TP53* mutations entered clinical remission after induction therapy, but most of them suffered a very early relapse (less than 18 months from the first remission) resulting in a shorter RFS. These data show that the presence of mutated *TP53* itself did not produce a primary resistance to the induction chemotherapy, but rather lead to a greater susceptibility to relapse, as previously reported (29).

SETD2 mutations easily occurred in female patients, which were with old age and higher initial WBC counts, but it was not predicted to be a prognostic factor. However, *SETD2* mutations had functional involvement in relapsed ALL patients, whose loss lead to resistance to DNA-damaging chemotherapy agents (cytarabine, 6-TG, doxorubicin), caused chemotherapy resistance, and increased the mutation rate at the site of diminished H3K36me3, with poor long-term survival (14-16). As reported, *SETD2* alterations were frequently associated with *MLLr* (22%), *ETV6-RUNX1* (13%), and T-cell lymphoma (30,31), which is an essential interactor in the initiation and maintenance of *MLLr* leukemia (32); however, in our research, just one case co-existed with *ETV6-RUNX1*, and only one case occurred in T-ALL; thus, more data is needed to confirm this result.

JAK1 mutations were associated with higher day 19 and day 46 MRD level, even in T-ALL patients, with dramatically higher MRD level on day 19 (more than 10%) and a sustained MRD level of more than 0.01% on day 46, but *JAK1* mutations showed low allelic burden and were considered more frequently to be from subclones. *JAK1* mutations and rearrangement-activated JAKs were seen in Ph-like B-ALL, and in the *CRLF2* rearrangements, had a poor outcome (33). The *JAK1/JAK2* inhibitor, ruxolitinib, was approved for myeloproliferative neoplasm (MPN) patients (34), and preclinical activity was recently reported in models of childhood T-ALL (35), with HSP90 inhibition PU-H71 (36) showing preclinical efficacy in *JAK1/JAK2* models of ALL. It is promising that the usage of these targeted drugs can decrease MRD level, intensify chemotherapy effect, increase induction remission, and improve the clinical outcome of these patients with *JAK* mutation.

In our cohort, there were two cases with *NOTCH1* mutation who suffered a very early relapse (less than 18 months from the first remission) and showed a significant influence in RFS of ALL (P=0.006). *NOTCH1* mutation was predicted to be a risk factor for early relapse in ALL patients. However, *NOTCH1* mutations just occurred in T-ALL patients and did not have a definite relationship with T-ALL outcome. Several types of research revealed that pediatric patients with mutated *NOTCH1* tended to show improved OS and EFS compared to those with wild-type *NOTCH1* (24,37,38); however, other studies showed a poorer survival or no impact on T-ALL outcome (39). Therefore, using *NOTCH1* mutations as an early relapse risk factor is still controversial, and requires further validation in larger prospective studies or T-ALL specific subtype.

Conspicuously, a molecular profile of subtypes, such as WBC ($\geq 50\times10^9$/L), age (<1 year, ≥10 years), *TP53* mutations, and MLLr, significantly predicted poor prognosis in B-ALL. Combination of these risk factor would enable us to identify a subset of patients who would benefit from more intensive treatment, such as combined chemotherapy, targeted therapy, and allogeneic hematopoietic stem cell transplantation (HSCT). These findings suggest that somatic mutations (e.g., *TP53*) combined clinical features help predict treatment outcomes and could improve the prognosis in B-ALL patients.

Conclusions

There are some limitations to the present study. The number of patients enrolled in the present study was relatively low; the follow-up time was too short, which may contribute to the discrepancies in the findings between our study and previous research. Also, due to the limitations of our technology, only detecting sequence mutations in ALL was insufficient, and large intragenic deletion, amplification, and translocation is warranted in the future. Thus, the prevalence of deleterious mutations among these genes may be underestimated.

In conclusion, using NGS to complete molecular profiling could potentially improve the prediction of prognosis in ALL patients and better guide therapy options, such as early intervention with combined chemotherapy and allogeneic HST, immune therapy or targeted therapy.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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Supplementary

Table S1 Recurrently mutated genes in the diagnostic ALL patients

Gene	NM_#	Location	Nucleotide change	Protein change	Mutation type	VAF	rs
KRAS (N=13)	NM_033360	12p12.1	c.436(exon4)G>A	p.A146T	Missense	0.150	rs121913527
			c.38(exon2)G>A	p.G13D	Missense	0.130	rs112445441
			c.38(exon2)G>A	p.G13D	Missense	0.360	rs112445441
			c.183(exon3)A>T	p.Q61H	Missense	0.250	rs17851045
			c.35(exon2)G>A	p.G12D	Missense	0.200	rs121913529
			c.173(exon3)C>T	p.T58I	Missense	0.420	rs104894364
			c.34(exon2)G>T	p.G12C	Missense	0.120	rs121913530
			c.436(exon4)G>A	p.A146T	Missense	0.310	rs121913527
			c.436(exon4)G>C	p.A146P	Missense	0.250	
			c.35(exon2)G>A	p.G12D	Missense	0.300	rs121913529
			c.57(exon2)G>T	p.L19F	Missense	0.250	rs121913538
			c.35(exon2)G>T	p.G12V	Missense	0.310	rs121913529
			c.351(exon4)A>T	p.K117N	Missense	0.320	
NRAS (N=9)	NM_002524	1p13.2	c.35(exon2)G>A	p.G12D	Missense	0.360	rs121913237
			c.34(exon2)G>A	p.G12S	Missense	0.360	rs121913250
			c.37(exon2)G>A	p.G13S	Missense	0.340	rs121434595
			c.38(exon2)G>A	p.G13D	Missense	0.130	rs121434596
			c.35(exon2)G>T	p.G12V	Missense	0.390	rs121913237
			c.181(exon3)C>A	*p.Q61K*	Missense	0.170	rs121913254
			c.35(exon2)G>A	p.G12D	Missense	0.280	rs121913237
			c.35(exon2)G>A	p.G12D	Missense	0.160	rs121913237
			c.38(exon2)G>A	p.G13D	Missense	0.150	rs121434596
FLT3 (N=8)	NM_004119	13q12	c.2533(exon20)A>G	p.R845G	Missense	0.230	
			c.1769(exon14)delT	p.F590Sfs*21	Frameshift	0.380	
			c.1775(exon14)T>A	p.V592D	Missense	0.330	
			c.2504(exon20)A>C	p.D835A	Missense	0.230	
			c.1775(exon14)T>C	p.V592A	Missense	0.130	
			c.2525(exon20)A>C	p.Y842S	Missense	0.270	
			c.1765(exon14)T>G	p.Y589D	Missense	0.390	
			c.1774(exon14)G>T	p.V592F	Missense	0.250	
KMT2D (N=7)	NM_003482	12q13.12	c.1273(exon10)C>T	p.Q425X,5113	Nonsense	0.530	
			c.14605(exon47)delC	p.L4869Xfs*1	Frameshift	0.180	
			c.7411(exon31)C>T	p.R2471X,3067	Nonsense	0.470	rs1057518571
			c.15118(exon48)G>T	p.D5040Y	Nonsense	0.320	
			c.7471(exon31)G>T	p.A2491S	Nonsense	0.140	
			c.5282(exon22)_c.5283(exon22)insAG	p.K1761Kfs*25	Frameshift	0.130	
			c.3074(exon11)_c.3075(exon11)insTGGGA	p.S1025Sfs*33	Frameshift	0.400	
NOTCH1 (N=6)	NM_017617	9q34.3	c.5033(exon27)T>C	*p.L1678P*	Missense	0.420	
			c.1124(exon7)C>G	*p.A375G*	Missense	0.310	
			c.4793(exon26)G>C	p.R1598P	Missense	0.550	
			c.4847(exon26)T>A	p.I1616N	Missense	0.240	
			c.4363(exon25)_c.4364(exon25)insTTGT	p.Q1455Lfs*25	Frameshift	0.160	
			c.7327(exon34)_c.7328(exon34)insGG	p.V2443Gfs*35	Frameshift	0.270	
FBXW7 (N=6)	NM_033632	4q31.3	c.727-1(IVS4)G>A		Splice site	0.360	
			c.1196(exon8)A>G	*p.D399G*	Missense	0.850	
			c.1436(exon10)G>A	p.R479Q	Missense	0.290	rs866987936
			c.1435(exon10)C>T	p.R479X,229	Nonsense	0.280	
			c.1394(exon9)G>A	p.R465H	Missense	0.560	
			c.1513(exon10)C>T	p.R505C	Missense	0.280	rs149680468
NBPF10 (N=6)	NM_001039703	1q21.1	c.580(exon5)G>A	p.A194T	Missense	0.120	rs12124464
			c.580(exon5)G>A	*p.A194T*	Missense	0.140	rs12124464
			c.5157(exon40)C>A	p.N1719K	Missense	0.280	rs372069112
			c.580(exon5)G>A	p.A194T	Missense	0.120	rs12124464
			c.580(exon5)G>A	*p.A194T*	Missense	0.100	rs12124464
			c.580(exon5)G>A	p.A194T	Missense	0.180	rs12124464
NOTCH2 (N=4)	NM_024408	1p13-p11	c.17(exon1)_c.18(exon1)delCC	p.P6Rfs*27	Frameshift	0.200	
			c.17(exon1)_c.18(exon1)delCC	p.P6Rfs*27	Frameshift	0.160	
			c.17(exon1)_c.18(exon1)delCC	p.P6Rfs*27	Frameshift	0.140	
			c.17(exon1)_c.18(exon1)delCC	p.P6Rfs*27	Frameshift	0.140	
MLL3 (N=4)	NM_170606	7q36.1	c.3340(exon21)T>C	p.C1114R	Missense	0.320	rs200559566
			c.2591(exon15)A>G	p.E864G	Missense	0.100	rs4024420
			c.1013-2(IVS7)A>G		Splice site	0.130	rs751158858
			c.1042(exon8)G>A	p.D348N	Missense	0.120	rs201834857
SETD2 (N=4)	NM_014159	3p21.31	c.493(exon3)delT	*p.S165Lfs*12*	Frameshift	0.190	
			c.4715(exon5)_c.4715+1(IVS5) insTTTTATGAT		Splice site	0.330	
			c.5015(exon8)G>A	p.G1672E	Missense	0.340	
			c.7573(exon21)A>T	p.K2525X,40	Nonsense	0.100	
ETV6 (N=4)	NM_001987	12p13	c.163+1(IVS2)G>A		Splice site	0.150	
			c.641(exon5)C>T	p.P214L	Missense	0.340	rs724159947
			c.722(exon5)_c.723(exon5)insACCC	p.E241Efs*4	Frameshift	0.110	
			c.641(exon5)C>T	p.P214L	Missense	0.260	rs724159947
TP53 (n=4)	NM_001126114	17p13.1	c.613(exon6)T>G	p.Y205D	Missense	0.180	
			c.535(exon5)delC	*p.H179Mfs*68*	Frameshift	0.700	
			c.497(exon5)C>G	p.S166X,176	Nonsense	0.660	
			c.818(exon8)G>A	*p.R273H*	Missense	0.500	rs28934576
PHF6 (n=3)	NM_032458	Xq26.3	c.673(exon7)C>T	p.R225X,141	Nonsense	0.950	
			c.582(exon6)_c.585+3(IVS6)delCTATGTA		Splice site	0.410	
			c.820(exon8)C>T	p.R274X,92	Nonsense	0.380	
PTEN (n=3)	NM_000314	10q23.3	c.388(exon5)C>T	p.R130X,274	Nonsense	0.420	
			c.956(exon8)delC	*p.T319Ifs*2*	Frameshift	0.380	
			c.49(exon1)delC	p.Q17Kfs*7	Frameshift	0.120	
JAK1 (n=3)	NM_002227	1p32.3-p31.3	c.2108(exon15)G>T	p.S703I	Missense	0.160	
			c.1810(exon13)G>T	p.D604Y	Missense	0.150	
			c.2729(exon20)T>C	p.L910P	Missense	0.130	
LPHN2 (n=3)	NM_012302	1p31.1	c.2500(exon13)C>G	p.L834V	Missense	0.160	
			c.1366(exon6)C>T	p.P456S	Missense	0.310	rs112681863
			c.3232-1(IVS17)G>T		Splice site	0.390	
NCOR1 (n=3)	NM_006311	17p11.2	c.1048(exon10)C>T	*p.R350X,2091*	Nonsense	0.500	
			c.919(exon10)_c.920(exon10)insA	p.I307Nfs*6	Frameshift	0.310	
			c.551(exon5)G>T	p.S184I	Missense	0.260	
MDC1 (n=3)	NM_014641	6p21.3	c.4831(exon10)C>A	p.P1611T	Missense	0.110	
			c.4831(exon10)C>A	p.P1611T	Missense	0.190	
			c.4831(exon10)C>A	*p.P1611T*	Missense	0.130	

Recurrently mutated genes (n=18) presented more than 3 cases were shown; italic-labeled genes in 5th column indicate that the patient had relapsed. ALL, acute lymphoblastic leukemia; VAF, variant allele fraction; del, deletion; ins, insertion.

Table S2 Comparation of clinical characteristics and treatment outcomes between mutation positive and negative ALL patients

Variable	Mutation		P value
	Positive (n=102)	Negative (n=38)	
Sex			0.518
Male	61	25	
Female	41	13	
Age			0.732
<1 y, ≥10 y	9	2	
1–10 y	93	36	
WBC			0.061
$<50\times10^9$/L	73	33	
$\geq50\times10^9$/L	29	5	
Immunology			0.605
B-ALL	82	32	
T-ALL	20	6	
Gene fusion			**0.004**
Yes	65	14	
No	37	24	
SF			0.069
<500 ng/mL	73	19	
≥500 ng/mL	23	13	
LDH			0.24
<448 IU/mL	47	13	
≥448 IU/mL	52	23	
Day 5			0.854
PB <1,000/mL	81	31	
PB ≥1,000/mL	20	7	
Day 19			0.587
BM <5%	94	36	
BM ≥5%	8	2	
Day 19 MRD			0.248
<0.01%	58	25	
0.01–1%	28	11	
≥1%	15	2	
Day 46			0.105
BM <5%	96	38	
BM ≥5%	4	0	
Day 46 MRD			0.601
<0.01%	90	33	
≥0.01%	10	5	
Risk group			0.088
LR	55	22	
IR	46	13	
HR	1	3	
3-RFS	83.5%±4.7%	93.7%±4.3%	0.267

There was a statistically significant difference between the groups (P<0.05). y, year; WBC, white blood cells; B-ALL, B-cell ALL; T-ALL, T-cell ALL; SF, serum ferritin; LDH, lactate dehydrogenase; PB, peripheral blood; BM, bone marrow; MRD, minimal residual disease; LR, Low risk; IR, intermediate risk; HR, high risk; RFS, relapse free survival.

Table S3 Gene variants with gender

Gene variants	Male (%)	Female (%)	P value
KRAS mutation-type	10/86 (11.6)	3/54 (5.6)	0.399
Wild-type	76/86 (88.4)	51/54 (94.4)	
NRAS mutation-type	7/86 (8.1)	2/54 (3.7)	0.492
Wild-type	79/86 (91.9)	52/54 (96.3)	
FLT3 mutation-type	5/86 (5.8)	3/54 (5.6)	1.000
Wild-type	81/86 (94.2)	51/54 (94.4)	
KMT2D mutation-type	2/86 (2.3)	5/54 (9.3)	0.152
Wild-type	84/86 (97.7)	49/54 (90.7)	
NOTCH1 mutation-type	3/86 (3.5)	3/54 (5.6)	0.874
Wild-type	83/86 (96.5)	51/54 (94.4)	
FBXW7 mutation-type	5/86 (5.8)	1/54 (1.9)	0.485
Wild-type	81/86 (94.2)	53/54 (98.1)	
NBPF10 mutation-type	4/86 (4.7)	2/54 (3.7)	1.000
Wild-type	82/86 (95.3)	52/54 (96.3)	
NOTCH2 mutation-type	3/86 (3.5)	1/54 (1.9)	0.964
Wild-type	83/86 (96.5)	53/54 (98.1)	
MLL3 mutation-type	3/86 (3.5)	1/54 (1.9)	0.964
Wild-type	83/86 (96.5)	53/54 (98.1)	
SETD2 mutation-type	0/86 (0)	4/54 (7.4)	**0.041**
Wild-type	86/86 (100.0)	50/54 (92.6)	
ETV6 mutation-type	3/86 (3.5)	1/54 (1.9)	0.964
Wild-type	83/86 (96.5)	53/54 (98.1)	
TP53 mutation-type	0/86 (0)	4/54 (7.4)	**0.041**
Wild-type	86/86 (100.0)	50/54 (92.6)	
PHF6 mutation-type	2/86 (2.3)	1/54 (1.9)	1.000
Wild-type	84/86 (97.7)	53/54 (98.1)	
PTEN mutation-type	2/86 (2.3)	1/54 (1.9)	1.000
Wild-type	84/86 (97.7)	53/54 (98.1)	
JAK1 mutation-type	1/86 (1.2)	2/54 (3.7)	0.681
Wild-type	85/86 (98.8)	52/54 (96.3)	
LPHN2 mutation-type	2/86 (2.3)	1/54 (1.9)	1.000
Wild-type	84/86 (97.7)	53/54 (98.1)	
NCOR1 mutation-type	3/86 (3.5)	0/54 (0)	0.569
Wild-type	83/86 (96.5)	54/54 (100.0)	
MDC1 mutation-type	2/86 (2.3)	1/54 (1.9)	1.000
Wild-type	84/86 (97.7)	53/54 (98.1)	

There was a statistically significant difference between the groups ($P<0.05$). Recurrently mutated genes (n=18) were examined, and only significant alterations in univariate analyses are shown.

Table S4 Gene variants with age

Gene variants	Age (year)	P value
KRAS mutation-type	3.6 (1.2–6.0)	0.09
Wild-type	5.0 (2.2–7.9)	
NRAS mutation-type	4.7 (2.4–67.9)	0.801
Wild-type	4.9 (2.0–7.8)	
FLT3 mutation-type	4.0 (3.6–4.4)	0.069
Wild-type	5.0 (2.2–7.6)	
KMT2D mutation-type	4.9 (2.9–6.8)	0.97
Wild-type	4.9 (2.0–7.8)	
NOTCH1 mutation-type	5.8 (0.9–10.6)	0.445
Wild-type	4.9 (2.0–7.7)	
FBXW7 mutation-type	7.0 (3.0–10.8)	0.071
Wild-type	4.8 (2.0–7.6)	
NBPF10 mutation-type	5.2 (1.9–8.4)	0.802
Wild-type	4.9 (2.0–7.7)	
NOTCH2 mutation-type	4.2 (2.0–6.4)	0.629
Wild-type	4.9 (2.0–7.8)	
MLL3 mutation-type	4.0 (2.0–6.0)	0.538
Wild-type	4.9 (2.0–7.8)	
SETD2 mutation-type	5.5 (4.0–10.5)	0.041
Wild-type	4.5 (0.3–9.6)	
ETV6 mutation-type	2.7 (2.2–3.2)	0.101
Wild-type	5.0 (2.0–7.8)	
TP53 mutation-type	7.5 (3.5–11.5)	0.06
Wild-type	4.8 (2.1–7.6)	
PHF6 mutation-type	6.6 (5.7–7.4)	0.295
Wild-type	4.9 (2.0–7.7)	
PTEN mutation-type	5.8 (4.7–6.8)	0.606
Wild-type	4.9 (2.0–7.7)	
JAK1 mutation-type	6.0 (2.8–9.2)	0.492
Wild-type	4.9 (2.0–7.7)	
LPHN2 mutation-type	6.0 (5.4–6.6)	0.503
Wild-type	4.9 (2.0–7.7)	
NCOR1 mutation-type	6.8 (4.2–9.5)	0.237
Wild-type	4.9 (2.0–7.7)	
MDC1 mutation-type	6.0 (4.3–7.7)	0.492
Wild-type	4.9 (2.0–7.7)	

There was a statistically significant difference between the groups ($P<0.05$). Recurrently mutated genes (n=18) were examined, and only significant alterations in univariate analyses are shown.

Table S5 Gene variants with initial WBC counts

Gene variants	WBC <50×10^9/L (%)	WBC ≥50×10^9/L (%)	P value
KRAS mutation-type	9/106 (8.5)	4/34 (11.8)	0.816
Wild-type	97/106 (91.5)	30/34 (88.2)	
NRAS mutation-type	8/106 (7.5)	1/34 (2.9)	0.582
Wild-type	98/106 (92.5)	33/34 (97.1)	
FLT3 mutation-type	7/106 (6.6)	1/34 (2.9)	0.707
Wild-type	99/106 (93.4)	33/34 (97.1)	
KMT2D mutation-type	5/106 (4.7)	2/34 (5.9)	0.574
Wild-type	101/106 (95.3)	32/34 (94.1)	
NOTCH1 mutation-type	2/106 (1.9)	4/34 (11.8)	0.047
Wild-type	104/106 (98.1)	30/34 (88.2)	
FBXW7 mutation-type	3/106 (2.8)	3/34 (8.8)	0.310
Wild-type	103/106 (97.2)	31/34 (91.2)	
NBPF10 mutation-type	4/106 (3.8)	2/34 (5.9)	0.967
Wild-type	102/106 (96.2)	32/34 (94.1)	
NOTCH2 mutation-type	4/106 (3.8)	0/34 (0)	0.572
Wild-type	102/106 (96.2)	34/34 (100.0)	
MLL3 mutation-type	3/106 (2.8)	1/34 (2.9)	1.000
Wild-type	103/106 (97.2)	33/34 (97.1)	
SETD2 mutation-type	1/106 (0.9)	3/34 (8.8)	0.044
Wild-type	105/106 (99.1)	31/34 (91.2)	
ETV6 mutation-type	3/106 (2.8)	1/34 (2.9)	1.000
Wild-type	103/106 (97.2)	33/34 (97.1)	
TP53 mutation-type	4/106 (3.8)	0/34 (0)	0.572
Wild-type	102/106 (96.2)	34/34 (100.0)	
PHF6 mutation-type	1/106 (0.9)	2/34 (5.9)	0.146
Wild-type	105/106 (99.1)	32/34 (94.1)	
PTEN mutation-type	1/106 (0.9)	2/34 (5.9)	0.146
Wild-type	105/106 (99.1)	32/34 (94.1)	
JAK1 mutation-type	3/106 (2.8)	0/34 (0)	1.000
Wild-type	103/106 (97.2)	34/34 (100.0)	
LPHN2 mutation-type	3/106 (2.8)	0/34 (0)	1.000
Wild-type	103/106 (97.2)	34/34 (100.0)	
NCOR1 mutation-type	2/106 (1.9)	1/34 (2.9)	0.569
Wild-type	104/106 (98.1)	33/34 (97.1)	
MDC1 mutation-type	3/106 (2.8)	0/34 (0)	1.000
Wild-type	103/106 (97.2)	34/34 (100.0)	

There was a statistically significant difference between the groups (P<0.05). Recurrently mutated genes (n=18) were examined, and only significant alterations in univariate analyses are shown. WBC, white blood cell.

Table S6 Gene variants with BM blast cells

Gene variants	BM blats cells (%)	P value
KRAS mutation-type	85.0 (22.5–98.5)	0.925
Wild-type	84.5 (28.5–99.5)	
NRAS mutation-type	89.5 (78.0–98.5)	0.404
Wild-type	84.5 (22.5–99.5)	
FLT3 mutation-type	89.5 (38.0–99.5)	0.423
Wild-type	84.0 (22.5–99.5)	
KMT2D mutation-type	95.0 (31.5–99.5)	0.547
Wild-type	85.0 (22.5–99.5)	
NOTCH1 mutation-type	87.0 (52.5–95.5)	0.783
Wild-type	84.5 (22.5–99.5)	
FBXW7 mutation-type	88.0 (64.5–96.0)	0.886
Wild-type	84.5 (22.5–99.5)	
NBPF10 mutation-type	95.0 (78.0–98.0)	0.255
Wild-type	84.0 (22.5–99.5)	
NOTCH2 mutation-type	82.5 (59.0–99.5)	0.311
Wild-type	85.0 (22.5–99.5)	
MLL3 mutation-type	82.0 (22.5–98.5)	0.12
Wild-type	92.0 (28.5–99.5)	
SETD2 mutation-type	85.5 (46.0–90.0)	0.369
Wild-type	84.5 (22.5–99.5)	
ETV6 mutation-type	82.5 (22.5–85.0)	0.049
Wild-type	94.0 (28.5–99.5)	
TP53 mutation-type	56.5 (28.5–98.0)	0.133
Wild-type	85.0 (22.5–99.5)	
PHF6 mutation-type	88.5 (73.0–95.5)	0.914
Wild-type	84.5 (22.5–99.5)	
PTEN mutation-type	86.5 (83.0–99.5)	0.876
Wild-type	84.5 (22.5–99.5)	
JAK1 mutation-type	47.5 (31.5–81.0)	0.001
Wild-type	92.0 (22.5–99.5)	
LPHN2 mutation-type	84.0 (34.5–98.5)	0.218
Wild-type	85.0 (22.5–99.5)	
NCOR1 mutation-type	97.0 (92.5–98.5)	0.569
Wild-type	84.5 (22.5–99.5)	
MDC1 mutation-type	78.0 (48.5–96.5)	0.303
Wild-type	85.0 (22.5–99.5)	

There was a statistically significant difference between the groups ($P<0.05$). Recurrently mutated genes (n=18) were examined, and only significant alterations in univariate analyses are shown. BM, bone marrow.

Table S7 Gene variants with BM blast cells level on day 19 and day 46

Gene variants	Day 19 BM level			Day 46 BM level		
	BM <5% (%)	BM ≥5% (%)	P value	BM <5% (%)	BM ≥5% (%)	P value
KRAS mutation-type	11/130 (8.5)	2/10 (20.0)	0.234	13/134 (9.7)	0/6 (0.0)	1.000
Wild-type	119/130 (91.5)	8/10 (80.0)		121/134 (90.3)	6/6 (100.0)	
NRAS mutation-type	7/130 (5.4)	2/10 (20.0)	0.126	9/134 (6.7)	0/6 (0.0)	1.000
Wild-type	123/130 (94.6)	8/10 (80.0)		125/134 (93.3)	6/6 (100.0)	
FLT3 mutation-type	8/130 (6.2)	0/10 (0.0)	1.000	8/134 (6.0)	0/6 (0.0)	1.000
Wild-type	122/130 (93.8)	10/10 (100.0)		126/134 (94.0)	6/6 (100.0)	
KMT2D mutation-type	7/130 (5.4)	0/10 (0.0)	1.000	7/134 (5.2)	0/6 (0.0)	1.000
Wild-type	123/130 (94.6)	10/10 (100.0)		127/134 (94.8)	6/6 (100.0)	
NOTCH1 mutation-type	5/130 (3.8)	1/10 (10.0)	0.364	6/134 (4.5)	0/6 (0.0)	1.000
Wild-type	125/130 (96.2)	9/10 (90.0)		128/134 (95.5)	6/6 (100.0)	
FBXW7 mutation-type	6/130 (4.6)	0/10 (0.0)	1.000	6/134 (4.5)	0/6 (0.0)	1.000
Wild-type	124/130 (95.4)	10/10 (100.0)		128/134 (95.5)	6/6 (100.0)	
NBPF10 mutation-type	6/130 (4.6)	0/10 (0.0)	1.000	6/134 (4.5)	0/6 (0.0)	1.000
Wild-type	124/130 (95.4)	10/10 (100.0)		128/134 (95.5)	6/6 (100.0)	
NOTCH2 mutation-type	4/130 (3.1)	0/10 (0.0)	1.000	4/134 (3.0)	0/6 (0.0)	1.000
Wild-type	126/130 (96.9)	10/10 (100.0)		130/134 (97.0)	6/6 (100.0)	
MLL3 mutation-type	4/130 (3.1)	0/10 (0.0)	1.000	4/134 (3.0)	0/6 (0.0)	1.000
Wild-type	126/130 (96.9)	10/10 (100.0)		130/134 (97.0)	6/6 (100.0)	
SETD2 mutation-type	3/130 (2.3)	1/10 (10.0)	0.259	3/134 (2.2)	1/6 (16.7)	0.162
Wild-type	127/130 (97.7)	9/10 (90.0)		131/134 (97.8)	5/6 (83.3)	
ETV6 mutation-type	4/130 (3.1)	0/10 (0.0)	1.000	4/134 (3.0)	0/6 (0.0)	1.000
Wild-type	126/130 (96.9)	10/10 (100.0)		130/134 (97)	6/6 (100.0)	
TP53 mutation-type	4/130 (3.1)	0/10 (0.0)	1.000	4/134 (3.0)	0/6 (0.0)	1.000
Wild-type	126/130 (96.9)	10/10 (100.0)		130/134 (97)	6/6 (100.0)	
PHF6 mutation-type	3/130 (2.3)	0/10 (0.0)	1.000	3/134 (2.2)	0/6 (0.0)	1.000
Wild-type	127/130 (97.7)	10/10 (100.0)		131/134 (97.8)	6/6 (100.0)	
PTEN mutation-type	1/130 (0.8)	2/10 (20.0)	0.013	2/134 (1.5)	1/6 (16.7)	0.124
Wild-type	129/130 (99.2)	8/10 (80.0)		132/134 (98.5)	5/6 (83.3)	
JAK1 mutation-type	3/130 (2.3)	0/10 (0.0)	1.000	3/134 (2.2)	0/6 (0.0)	1.000
Wild-type	127/130 (97.7)	10/10 (100.0)		131/134 (97.8)	6/6 (100.0)	
LPHN2 mutation-type	3/130 (2.3)	0/10 (0.0)	1.000	3/134 (2.2)	0/6 (0.0)	1.000
Wild-type	127/130 (97.7)	10/10 (100.0)		131/134 (97.8)	6/6 (100.0)	
NCOR1 mutation-type	3/130 (2.3)	0/10 (0.0)	1.000	3/134 (2.2)	0/6 (0.0)	1.000
Wild-type	127/130 (97.7)	10/10 (100.0)		131/134 (97.8)	6/6 (100.0)	
MDC1 mutation-type	3/130 (2.3)	0/10 (0.0)	1.000	3/134 (2.2)	0/6 (0.0)	1.000
Wild-type	127/130 (97.7)	10/10 (100.0)		131/134 (97.8)	6/6 (100.0)	

There was a statistically significant difference between the groups (P<0.05). Recurrently mutated genes (n=18) were examined, and only significant alterations in univariate analyses are shown. BM, bone marrow.

Table S8 Gene variants with MRD level on day 19 and day 46

Gene variants	Day 19 MRD level			Day 46 MRD level		
	MRD <1% (%)	MRD ≥1% (%)	P value	MRD <0.01% (%)	MRD ≥0.01% (%)	P value
KRAS mutation-type	5/83 (6.0)	8/56 (14.3)	0.101	10/123 (8.1)	3/15 (20.0)	0.309
Wild-type	78/83 (94.0)	48/56 (85.7)		113/123 (91.9)	12/15 (80.0)	
NRAS mutation-type	3/83 (3.6)	6/56 (10.7)	0.188	8/123 (6.5)	1/15 (6.7)	1.000
Wild-type	80/83 (96.4)	50/56 (89.3)		115/123 (93.5)	14/15 (93.3)	
FLT3 mutation-type	4/83 (4.8)	4/56 (7.1)	0.837	7/123 (5.7)	1/15 (6.7)	1.000
Wild-type	79/83 (95.2)	52/56 (92.9)		116/123 (94.3)	14/15 (93.3)	
KMT2D mutation-type	3/83 (3.6)	4/56 (7.1)	0.591	6/123 (4.9)	1/15 (6.7)	1.000
Wild-type	80/83 (96.4)	52/56 (92.9)		117/123 (95.1)	14/15 (93.3)	
NOTCH1 mutation-type	2/83 (2.4)	4/56 (7.1)	0.357	6/123 (4.9)	0/15 (0.0)	1.000
Wild-type	81/83 (97.6)	52/56 (92.9)		117/123 (95.1)	15/15 (100.0)	
FBXW7 mutation-type	6/83 (7.2)	0/56 (0.0)	0.103	6/123 (4.9)	0/15 (0.0)	1.000
Wild-type	77/83 (92.8)	56/56 (100.0)		117/123 (95.1)	15/15 (100.0)	
NBPF10 mutation-type	3/83 (3.6)	3/56 (5.4)	0.944	5/123 (4.1)	1/15 (6.7)	0.505
Wild-type	80/83 (96.4)	53/56 (94.6)		118/123 (95.9)	14/15 (93.3)	
NOTCH2 mutation-type	3/83 (3.6)	1/56 (1.8)	0.908	4/123 (3.3)	0/15 (0.0)	1.000
Wild-type	80/83 (96.4)	55/56 (98.2)		119/123 (96.7)	15/15 (100.0)	
MLL3 mutation-type	4/83 (4.8)	0/56 (0.0)	0.250	4/123 (3.3)	0/15 (0.0)	1.000
Wild-type	79/83 (95.2)	56/56 (100.0)		119/123 (96.7)	15/15 (100.0)	
SETD2 mutation-type	0/83 (0.0)	3/56 (5.4)	0.124	3/123 (2.4)	1/15 (6.7)	0.372
Wild-type	83/83 (100.0)	53/56 (94.6)		120/123 (97.6)	14/15 (93.3)	
ETV6 mutation-type	3/83 (3.6)	1/56 (1.8)	0.908	3/123 (2.4)	1/15 (6.7)	0.372
Wild-type	80/83 (96.4)	55/56 (98.2)		120/123 (97.6)	14/15 (93.3)	
TP53 mutation-type	1/83 (1.2)	3/56 (5.4)	0.358	3/123 (2.4)	1/15 (6.7)	0.372
Wild-type	82/83 (98.8)	53/56 (94.6)		120/123 (97.6)	14/15 (93.3)	
PHF6 mutation-type	1/83 (1.2)	2/56 (3.6)	0.729	3/123 (2.4)	0/15 (0.0)	1.000
Wild-type	82/83 (98.8)	54/56 (96.4)		120/123 (97.6)	15/15 (100.0)	
PTEN mutation-type	1/83 (1.2)	1/56 (1.8)	1.000	3/123 (2.4)	0/15 (0.0)	1.000
Wild-type	82/83 (98.8)	55/56 (98.2)		120/123 (97.6)	15/15 (100.0)	
JAK1 mutation-type	1/122 (0.8)	2/17 (11.8)	0.039	1/123 (0.8)	2/15 (13.3)	0.031
Wild-type	121/122(99.2)	15/17 (88.2)		122/123 (99.2)	13/15 (86.7)	
LPHN2 mutation-type	3/83 (3.6)	0/56 (0.0)	0.399	3/123 (2.4)	0/15 (0.0)	1.000
Wild-type	80/83 (96.4)	56/56 (100.0)		120/123 (97.6)	15/15 (100.0)	
NCOR1 mutation-type	3/83 (3.6)	0/56 (0.0)	0.399	3/123 (2.4)	0/15 (0.0)	1.000
Wild-type	80/83 (96.4)	56/56 (100.0)		120/123 (97.6)	15/15 (100.0)	
MDC1 mutation-type	2/83 (2.4)	1/56 (1.8)	1.000	2/123 (1.6)	1/15 (6.7)	0.294
Wild-type	81/83 (97.6)	55/56 (98.2)		121/123 (98.4)	14/15 (93.3)	

There was a statistically significant difference between the groups (P<0.05). Recurrently mutated genes (n=18) were examined, and only significant alterations in univariate analyses are shown. MRD, minimal residual disease.

Table S9 Clinical and genetic characteristics of 14 relapsed ALL patients

NO.	Sex/age (years)	WBC ($\times 10^9$/L)	Immunology	Gene fusion	Somatic mutation	Protein change	Relapse free survival (M)/relapse site
1	Female/10.5	70	B-ALL	N	*SETD2*	p.S165Lfs*12	25/BM
2	Male/5.5	49	T-ALL	*SIL-TAL1*	*PTEN*	p.T319Ifs*2	20/CNS
3	Male/3.3	46	B-ALL	*ETV6-RUNX1*	*SUZ12*	c.1875-1(IVS15)G>A	11/BM
4	Female/0.8	20	B-ALL	N	N		11/BM
5	Male/9.9	41	B-ALL	N	*NBPF10*	p.A194T	8/BM
					NCOR1	p.R350X,2091	
6	Male/8.1	124	T-ALL	N	*NOTCH1*	p.L1678P	18/CNS
					FBXW7	p.D399G	
7	Male/5.5	22	T-ALL	N	*NRAS*	p.Q61K	10/BM
					NBPF10	p.A194T	
					MDC1	p.P1611T	
8	Female/1.1	37	T-ALL	N	*NOTCH1*	p.A375G	11/BM
9	Female/11.8	28	B-ALL	N	*TP53*	p.H179Mfs*68	18/BM
10	Female/1.9	25	B-ALL	*MLLr*	N		8/BM
11	Female/6.1	51	B-ALL	*MLLr*	N		11/BM
12	Male/5.5	70	T-ALL	N	N		10/BM
13	Female/8.3	75	T-ALL	N	N		5/BM
14	Female/6.6	80	B-ALL	N	*TP53*	p.R273H	14/BM

N means negative or not included in recurrently mutated genes (n=18). ALL, acute lymphoblastic leukemia; WBC, white blood cells; M, months; B-ALL, B-cell ALL; T-ALL, T-cell ALL; BM, bone marrow; CNS, central nervous system.