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# Genomic-wide Copy Number Variation Profile and a New IKZF1 Gene Variation in Chinese Adult Acute Lymphoblastic Leukemia

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## Abstract

**Purpose:** Copy Number Variation (CNV) and Loss of Heterozygosity (LOH) were investigated in adult Chinese patients with Acute Lymphoblastic Leukemia (ALL), and these patients were screened for adult ALL prognostic genes.

**Methods:** The CNV and LOH were detected with Affymetrix SNP 6.0 array. Using java and software R to construct the signal-net based on KEGG, enrichment the significant CNV genes associated with disease. Check the copy number variation of IKZF1 gene by FISH.

**Results:** In Ph+ B-ALL patients, the most frequent CNVs were gain at chr13q, loss at chr6 and recurrent LOH at chr17p and 1p33. In Ph- B-ALL patients, the most frequent CNVs were gain at chr7, loss at chr9p and recurrent LOH at chr15q. In T-ALL, the most frequent CNVs were gain at chr20 and 7q, loss at chr2p and recurrent LOH also at chr15q. These CNV regions included several candidate genes such as IKZF1, JAK2, CDKN2A/2B, ETV6 etc. EGFR and IKBKG are in the center position of the Signal-Net, which may play essential role in ALL. The copy number amplification of IKZF1 gene is confirmed by FISH.

**Discussion:** CNV and LOH identification in adult ALL patients can identify susceptible genes, guide diagnostic classification, and help with treatment selection.

**Keywords: Copy number variation; Genomic; Acute lymphoblastic leukemia; Adult**

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## Abbreviations

CNV: Copy Number Variation; LOH: Loss of Heterozygosity; ALL: Acute Lymphoblastic Leukemia; CR: Complete Remission; SNP: Single-Nucleotide Polymorphism; aCGH: Array Comparative Genomic Hybridization; GSEA: Gene Set Enrichment Analysis; BAC: Bacterial Artificial Chromosome

## Introduction

Acute Lymphoblastic Leukemia (ALL) is a clonal malignant disease that is highly heterogeneous and a complex polygenetic disorder, and it accounts for 15% to 25% of adult acute leukemia. Based on cytogenetic and molecular analyses, the adult ALL patients from different risk stratifications received therapies of various intensity, including the application of Tyrosine Kinase Inhibitors (TKIs) in Ph positive ALL patients, which has dramatically improved the prognosis for adult ALL patients [1,2]. However, the Overall Survival (OS) and Event Free Survival (EFS) of adult ALL patients are still worse than that of pediatric ALL patients. Some favorable prognostic predictors in pediatric ALL, such as t(12;21), are less frequent in adult ALL patients [3], while some common predictors in adult ALL, such as t(9;22), are rare in pediatric ALL patients [4]. Therefore, the cytogenetic and molecular biologic differences are likely to explain the different outcomes between adult and pediatric ALL patients. Additionally, the response to TKI treatment in adult ALL did not compare with that in BCR-ABL1 positive Chronic Myeloid Leukemia (CML), which indicates much more genetic factors may be involved in the pathogenic mechanism [5]. In recent years, multiple studies investigated the Copy Number Variations (CNVs) and Loss of Heterozygosity (LOH) in ALL using Single-Nucleotide Polymorphism (SNP) microarray analyses and high-resolution genome-wide array comparative genomic hybridization (aCGH) [6-11]. However, detailed genomic studies of

Chinese adult ALL patients are currently rare and incomplete. Hence, we performed genomic-wide copy number and LOH analyses using SNP microarray to determine molecular mechanisms involved in adult ALL in order to screen novel prognostic factors and therapeutic targets for this disease.

## Materials and Methods

### Patients and controls

Adult ALL patients (n=33) and healthy donors (n=5) were analyzed using an Affymetrix Genome-wide Human SNP 6.0 array. There were a total of 50 bone marrow samples, including 33 samples from newly diagnosed patients, 11 paired samples from patients in Complete Remission (CR), and 1 paired sample from a relapsed patient. The median age was 26 years (range 18 to 55 years). The median percentage of blast cells was 88% (range 69% to 98%). The characteristics of the above 33 patients are shown in Table 1. The diagnosis was made on the basis of morphology, immunology, cytogenetic, and molecular biology. The classification, treatment and therapeutic response was made according to the Chinese expert panel consensus on diagnosis and treatment of adult acute lymphoblastic leukemia, which was based on the WHO/EGIL standards and the GALGB 8811 treatment protocol [12]. Mononuclear bone marrow cells were separated using Ficoll-Hypaque density gradient centrifugation, and the samples were stored in liquid nitrogen. This study was approved by Institutional Review Boards (Ethical approval number: KT2015001-EC-1), and informed consent was obtained from each patient according to the revised declaration of Helsinki.

### SNP microarray analysis

Genomic DNA was extracted from mononuclear cells isolated from bone marrow samples using the DNA Blood Mini Kit (QIAGEN). DNA was quantified using the NanoDrop spectrophotometer, and quality was assessed using the NanoDrop and agarose gel electrophoresis. DNA samples were genotyped using Affymetrix Genome-Wide Human SNP 6.0 array micro arrays according to the manufacturer's instructions. DNA (250 ng) was digested using NspI and 250 ng with Styenzymes. Digested DNA was adaptor-ligated and PCR amplified using the Clontech Titanium TAQ DNA polymerase in 4PCRs for NspI and 3 for StyI. PCR products from each set of reactions were pooled, purified and fragmented. Fragmented PCR products were then labeled, denatured, and hybridized to the arrays. Arrays were then washed using Affymetrix fluidics stations and scanned using a Gene Chip Scanner 3000 7G. Array data were analyzed using Affymetrix genotyping console and chromosome analysis suit. All aberrations were calculated relative to normal donor bone marrow samples to reduce the noise of the raw copy number data.

### Bioinformatics

Gene Set Enrichment Analysis (GSEA) and pathway analysis (Ingenuity Systems) were employed to gain information about signal-networks [13]. The input datasets for each cohort comprised genes showing recurrent changes (≥ 2-times). Gene sets with a False Discovery Rate (FDR) q-value lower than 0.05 or p-value lower than 0.05, which is calculated using Fisher's exact test, were taken into consideration; all other parameters were set to default values. The significance of the association is determined based on the ratio of the number of genes from the test dataset that map to the pathway, divided by the total number of genes that map to the canonical pathway base in the KEGG (Kyoto Encyclopedia of Genes and Genomes) database.

### FISH

Mononuclear cells were treated with a hypotonic solution (0.075M KCL), fixed in methanol and then dropped onto glass slides. The slides were fixed using a warm solution of 2× saline sodium citrate, dehydrated in an alcohol series and then hybridized in situ with probes labeled by nick translation. Briefly, 2 μg of labeled probe was used for FISH experiments. Hybridization was performed at 37°C in 2× saline sodium citrate, 50% (vol/vol) formamide, 10% (wt/vol) dextran sulfate, 5 μg of COT1 DNA, and 3 μg of sonicated salmon sperm DNA in a volume of 10 μL. Post hybridization washing was performed at 55°C in 2 × SSPE (three times), followed by three washes in 100% ethanol.

Bacterial Artificial Chromosome (BAC) clones of RP11-663F2 and RP11-816F4 from the RPCI-11 de Jong BAC library (http://bacpac.chori.org/) were selected to cover the IKZF1 gene. The location of clones was based on the UCSC Genome Browser (http://genome.ucsc.edu/cgi-bin/hgGateway). The probe RP11-663F2 was directly labeled with PF415, and RP11-816F4 was labeled with Cy3. Chromosomes were identified using DAPI staining. Digital images were obtained using a ZEISS epifluorescence microscope equipped with a cooled CCD camera. PF-415 (Cascade Blue; Fermentas Life Sciences), Cy3 (Rhodamine Red; New England Nuclear), and DAPI (blue) fluorescence signals, which were detected using specific filters, were recorded separately as gray-scale images. Pseudo coloring and merging of images were performed using Axio Vision 4.8.2.

### Statistics

P values below 0.05 were considered statistically significant. Statistical analyses and image production were performed using SPSS19.0 and GraphPad Prism 5.

## Results and Discussion

### Copy number variations in newly diagnosed patients

Copy number variations of 33 newly diagnosed ALL patients were assessed using Affymetrix SNP6.0 arrays. The most frequently recurrent alterations of Ph positive (Ph+) B-ALL patients (n=7) were gains at 13q (7/7), 7(5/7), 18q (4/7); loss at 6(5/7), 1p36 (4/7), Xq22(2/7); and LOH at 17p(7/7), 1p33(7/7), 12q21(6/7). Overall, there were 2120 CNVs and 724 LOH. The most common alterations of 18 cases Ph negative (Ph-) B-ALL patients were gains at 7(10/18), 1q (8/18), 12p13 (6/18); loss at 9p21(9/18), X(8/18), 6q14-16(6/18); and LOH at 15q11-q13(16/18), 12q24(15/18), 17q21(14/18). Overall, there were 1274 CNVs and 1521 LOH. Also 8 newly diagnosed T-ALL patients were tested. The most frequent recurrent variations were gains at 20 (4/8), 7q (4/8), 6q22-q23 (3/8); loss at 2p21-p16 (6/8), 9p21 (5/8), X(4/8); and LOH at 15q(8/8), 9p(7/8), 1p32(5/8). There were a total of 917 CNVs and 554 LOH.

### Copy number variation in complete remission patients

We also analyzed 11 patients in Complete Remission (CR) with Affymetrix SNP6.0 arrays and compared them to un-treated ALL patients. The most frequent alterations in CR Ph+ B-ALL patients were gains at 7(5/5), 6p21 (3/5), 13q32-q34(3/5); loss at X(4/5), 10(4/5), 16q22(2/5); and LOH at 12q24.31(5/5), 18q11.2(4/5), 15q11-q12(4/5). Overall, there were 5 patients with 1918 CNVs and 466 LOH. The most frequent changes in CR Ph- B-ALL patients were gains at 8(2/6), 1q31-q32 (2/6), 22q11.22(2/6); loss at 5(5/6), X(2/6), 16q22(2/6); and LOH at 1q31-q32(4/6), 15q(3/6), 1q31.3(3/6). There were a total of 6 patients with 470 CNVs and 511 LOH.

**Table 1:** Characteristics of 33 patients detected by Affymetrix SNP6.0 array.

| Diagnosis | Gender (M: male; F: female) | Age (year) | Initial karyotype | CR karyotype* | Relapse karyotype |
|---|---|---|---|---|---|
| Common-B ALL | F | 26 | 46,XX [2] | | |
| Common-B ALL | F | 24 | 46,XX [5] | | |
| Common-B ALL | F | 20 | 46,XX [2] | | |
| Common-B ALL | F | 20 | 46,XX [3] | | |
| Common-B ALL | M | 26 | 47,XY,7p+ [2]/46,XY [16] | 46,XY [9] | |
| Common-B ALL | M | 30 | 47,XY,der(3),+8 [11] | 46,XY [20] | |
| Common-B ALL | F | 23 | 46,XX [10] | | |
| Common-B ALL | M | 41 | 46,XY [12] | | |
| Common-B ALL | M | 28 | 46,XY [20] | | |
| Common-B ALL | M | 19 | 46,XY [10] | | |
| Common-B ALL | M | 20 | no metaphase | | |
| P190+ ALL | M | 48 | 47,XY,+?/ 46,XY [9] | 46,XY [12] | |
| P190+ ALL | M | 18 | 47,XY,i(9q+),der(22)t(9;22)(q34;q11),-19 [1]/46,XY, t(9;22)(q34;q11) [7] | 46,XY [7] | |
| P190+ ALL | M | 55 | 46,XY [14] | | |
| P190+ ALL | M | 31 | 46,XY [9] | | |
| P190+ ALL | M | 26 | 46,XY, t(9;22)(q34;q11) [14]/ 46,XY [1] | | |
| P210+ ALL | M | 30 | 46,XY,t(9;22)(q34;q11) [9] | 46,XY [15] | 45,XY,i(9q+),der(22),t(9;22)(q34;q11),-19 [1]/46,XY,t(9;22)(q34;q11) [7] |
| P210+ ALL | M | 24 | 46,XY, t(9;22)(q34;q11) [20] | | |
| pre-B ALL | F | 36 | 46,XX, [1]/40-45,XX,-1,-2,-6,-8,-10,-11,-15,-17,18,-21 [cp5] | | |
| pre-B ALL | M | 30 | 46,XY, t(4;11)(q21;q23) [7] | | |
| pre-B ALL | F | 54 | 46,XX, t(1;9)(q23;p13) [9]/46,XX [5] | | |
| pre-B ALL | F | 21 | 50,XX,+X, t(1;9)(q23;p13), +4,+6,+18 [3]/46,XX [2] | | |
| pro-B ALL | M | 22 | 46,XY [2] | | |
| pro-B ALL | M | 40 | 46,XY, t(4;11)(q21;q23) [3]/46,XY [3] | | |
| pro-B ALL | F | 36 | 46,XX [15]/31-36,XX [5] | | |
| T-ALL | M | 35 | 46,XY [5] | | |
| T-ALL | M | 27 | 46,XY [10] | | |
| T-ALL | F | 24 | no metaphase | | |
| T-ALL | M | 20 | 47,XY,?7p+,+?9 [3] | | |
| T-ALL | M | 26 | no metaphase | | |
| T-ALL | M | 39 | 46,XY,3p-,t(11;14)(p13;q11) [2]/46,XY [2] | | |
| T-ALL | M | 39 | no metaphase | | |
| T-ALL | M | 26 | no metaphase | | |
| healthy donor 1 | F | 26 | 46,XX [9] | | |
| healthy donor 2 | F | 42 | 46,XX [10] | | |
| healthy donor 3 | M | 37 | 46,XY [18] | | |
| healthy donor 4 | F | 33 | 46,XX [6] | | |
| healthy donor 5 | M | 22 | 46,XY [12] | | |

*The karyotypes of patients in CR were not tested when their initial karyotype was normal

**Table 2:** CNV evolution of a P210+ B-ALL patient.

| | Initial diagnosis | Complete remission | Relapse |
|---|---|---|---|
| Chromosome karyotype | 46,XY,t(9;22)(q34;q11) | 46,XY | 45,XY,i(9q+),der(22), t(9;22)(q34;q11),-19/46,XY,t(9;22)(q34;q11) |
| Gains number and location (>10 Mbp) | 71 | 24 | 74 |
| | 12; 3q; 13q; 18; 7; 8 | 7; X; | 5; 1; 16; 8q; 17q |
| Loss number and location (>1 Mbp) | 20 | 60 | 24 |
| | 3; 1; 6q | 18q; 10q | 17p; 4q; 8p; 12q |
| LOH number and location (>10 Mbp) | 94 | 76 | 65 |
| | 6p; 5q; 3q; 16; 17p | 1p; 6p; 17p; 16 | 2; 12q; 8p; 17p; 16; 1 |

**Table 3:** The frequency of candidate genes in CNV regions.

| Gene | Location | Ph- B-ALL | Ph+ B-ALL | T-ALL |
|---|---|---|---|---|
| RB1 | chr13q14.2 | 56% | 71% | 75% |
| IKZF1 | chr7p12.2 | 50% | 57% | 0% |
| FHIT | chr3p14.2 | 50% | 57% | 25% |
| FLT3 | chr13q12 | 50% | 57% | 75% |
| CDKN2A | chr9p21 | 44% | 29% | 63% |
| CDKN2B | chr9p21 | 44% | 29% | 50% |
| PTEN | chr10q23.3 | 44% | 57% | 38% |
| ETV6(TEL) | chr12p13 | 39% | 71% | 50% |
| PHF6 | chrXq26.3 | 33% | 14% | 25% |
| JAK2 | chr9p24 | 33% | 29% | 63% |
| PAX5 | chr9p13 | 28% | 0% | 25% |
| DDC | chr18q21.3 | 28% | 57% | 13% |
| TCF12 | chr15q21 | 28% | 29% | 25% |
| MYC | chr8q24.21 | 22% | 43% | 13% |
| BCL2 | chr18q21.3 | 22% | 57% | 25% |
| JAK1 | chr1p32.3-p31.3 | 17% | 43% | 0% |
| PTPN2 | chr18p11.3-p11.2 | 17% | 29% | 0% |
| FOXO3 | chr6q21 | 11% | 57% | 38% |
| BAK1 | chr6p21.3 | 11% | 43% | 0% |
| CDKN1A | chr6p21.2 | 11% | 29% | 13% |
| BTLA | chr3q13.2 | 11% | 43% | 0% |
| RERE | chr1p36.23 | 11% | 43% | 0% |
| NF1 | chr17q11.2 | 11% | 14% | 13% |
| MLL | chr11q23 | 11% | 43% | 0% |
| WT1 | chr11p13 | 11% | 0% | 0% |
| NOTCH1 | chr9q34.3 | 6% | 0% | 13% |
| NR3C1 | chr5q31.3 | 6% | 14% | 0% |
| LEF1 | chr4q23-q25 | 6% | 0% | 25% |
| TP53 | chr17p13.1 | 6% | 29% | 25% |
| E2F4 | chr16q21-q22 | 6% | 0% | 0% |
| CREBBP | chr16p13.3 | 6% | 43% | 25% |

## CNV evolution of a Ph+ B-ALL patient

We followed a Ph+ (BCR/ABL P210+) B-ALL patient and determined the CNV changes at his initial diagnosis, during CR and during a relapse using Affymetrix SNP6.0 array. The CNVs evolved during the disease progression, but still had some similar segments, such as a gain at 8 and LOH at 16, 17p (Table 2).

**Table 4:** The frequency of candidate genes in LOH regions.

| Gene | Location | Ph- B-ALL | Ph+ B-ALL | T-ALL |
|---|---|---|---|---|
| E2F4 | chr16q21-q22 | 72% | 43% | 25% |
| JAK2 | chr9p24 | 44% | 14% | 63% |
| PTEN | chr10q23.3 | 39% | 29% | 13% |
| NF1 | chr17q11.2 | 28% | 29% | 25% |
| PAX5 | chr9p13 | 22% | 14% | 25% |
| FOXO3 | chr6q21 | 22% | 57% | 13% |
| BAK1 | chr6p21.3 | 22% | 43% | 0% |
| CDKN1A | chr6p21.2 | 22% | 29% | 0% |
| RERE | chr1p36.23 | 22% | 43% | 0% |
| TP53 | chr17p13.1 | 22% | 57% | 25% |
| FBXW7 | chr4q31.3 | 22% | 14% | 13% |
| ETV6(TEL) | chr12p13 | 17% | 14% | 25% |
| TCF12 | chr15q21 | 17% | 0% | 0% |
| WT1 | chr11p13 | 17% | 0% | 0% |
| NR3C1 | chr5q31.3 | 17% | 29% | 0% |
| IKZF1 | chr7p12.2 | 11% | 0% | 0% |
| FHIT | chr3p14.2 | 11% | 14% | 13% |
| DDC | chr18q21.3 | 11% | 0% | 0% |
| MYC | chr8q24.21 | 11% | 0% | 0% |
| JAK1 | chr1p32.3-p31.3 | 11% | 29% | 0% |
| BTLA | chr3q13.2 | 11% | 29% | 0% |
| MLL | chr11q23 | 11% | 29% | 0% |
| NOTCH1 | chr9q34.3 | 11% | 0% | 0% |
| LEF1 | chr4q23-q25 | 11% | 0% | 13% |
| CREBBP | chr16p13.3 | 11% | 14% | 25% |
| RB1 | chr13q14.2 | 6% | 29% | 0% |
| FLT3 | chr13q12 | 6% | 0% | 0% |
| BCL2 | chr18q21.3 | 6% | 14% | 0% |
| CDKN2A | chr9p21 | 0% | 14% | 13% |

## Gene and miRNA located in CNV and LOH regions of initial diagnosis ALL

The candidate genes located in CNV (≥ 1kbp) or LOH (≥ 1Mbp) were screened using a chromosome analysis suite (Affymetrix). The CNV regions include several candidate genes with high frequency in ALL, such as IKZF1, CDKN1A/2B, RB1, FLT3, ETV6 and JAK2 (Table 3). The LOH regions include E2F4, JAK2, PTEN, FOXP3, NF1 and PAX5 (Table 4).

The CNV and LOH regions also included several miRNAs.

**Table 5:** The frequency of mirRNAs and its target genes in CNV and LOH regions.

| Variate | miRNA | Cytoband | B-ALL | T-ALL | Target gene |
|---|---|---|---|---|---|
| GAIN | hsa-mir-181b1 | chr1q32.1 | 28% | 14% | BCL2,CDKN2A,BCR,CEBPA,NFKB1,PTEN,TP53,IL6,STAT3,HOXA11,ERG,FLT3,WT1,ZAP70,RUNX2,MCL1 |
| | hsa-mir-516b-1 | chr19q13.41 | 36% | 43% | IL17RE,CD109,PTPRT,MLLT11,TRIB1,SPRY2,SORBS1 |
| LOSS | hsa-mir-548m | chrXq21.33 | 20% | 43% | PARP15,FAM123A,TNFRSF19,DNM3,ITPR1,CYP4F8,ELL3 |
| | hsa-mir-31 | chr9p21.3 | 20% | 29% | JAK2,TLR4,TNF,IL8,CD44,CDKN2A/2B,FASN,FOXP3,PTEN,BCL2L2,TBX1,TP53 |
| LOH | hsa-mir-877 | chr6p21.33 | 44% | 29% | PAK2,CTSC,DUS4L,TMEM139,CWF19L2,MORC3,UBL3 |
| | hsa-let-7g | chr3p21.1 | 28% | 57% | DICER1,CCND1,IL6,SOCS1,MYC,TP53,E2F1,CXCR4,FLT3,CD4,FBXW7,BCL2,INFG,NPM1 |

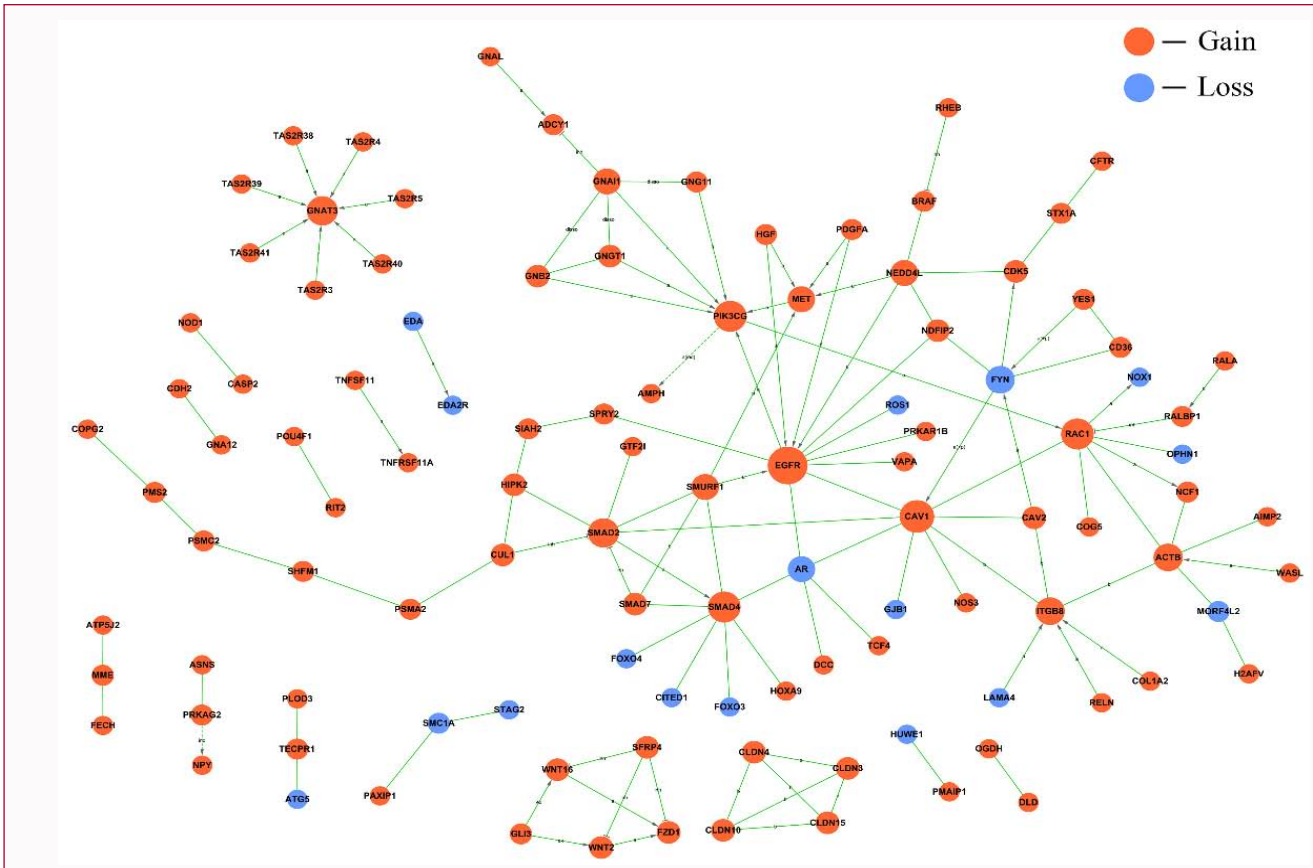


**Figure 1:** Signal-Net of CNV genes (B-ALL).

We selected the miRNAs that most frequently had variations, and assessed their likely target genes using miRanda, TargetScan, miRBase or miRWalk. The most frequently variable miRNAs, which could be associated with ALL, were mir-181b1, mir-516b1, mir-548m, mir-31, mir-877, and let-7g (Table 5).

## Signal-Net of signature genes in the initial ALL diagnosis

We constructed the Signal-Net of significant genes located in CNV regions, based on the KEGG database. In the B-ALL CNVs, EGFR and CAV1 were central genes interacting with others. CAV1 and SMAD2 were in the node position and had mediating capability (Figure 1). In the T-ALL CNVs, IKBKG interacted with most other genes. AR was in the node position with mediating capability (Figure 2).

## IKZF1 amplification confirmed by FISH

According to SNP array analysis, the IKZF1 gene on chromosome 7 is dramatically amplified (14/33). Therefore, FISH analysis of the IKZF1 gene was performed to confirm the SNP result. IKZF1 gene amplification of 3 ALL patients and the normal copy number of 1 healthy donor were identified using FISH. The amplified location identified using FISH was consistent with that of the SNP array (Figure 3).

Recently, high solution submicroscopic genomic variation analysis has been widely used in pediatric and adult ALL studies. SNP arrays and aCGH (Array Comparative Genomic Hybridization) are powerful tools to reveal the genomic-wide CNVs. Using the Affymetrix SNP6.0 array, we detected CNVs and LOH in 33 cases newly diagnosed adult ALL patients (11 patients with paired CR samples and 1 patient with a relapse sample). Compared to previous studies of genomic wide CNVs in cases of ALL, from pediatric to adult [8,9,14-16] and all ages [6,7,17], we also detected several CNV and LOH regions reported by others, such as RB1 at 13q14.2, BTLA at 3q13.2, CDKN2A, CDKN2B, JAK2, PAX5 at 9p, ETV6 at 12p13, E2F4 at 16q22.1, and MYC at 8q24.21. Moreover, we constructed a

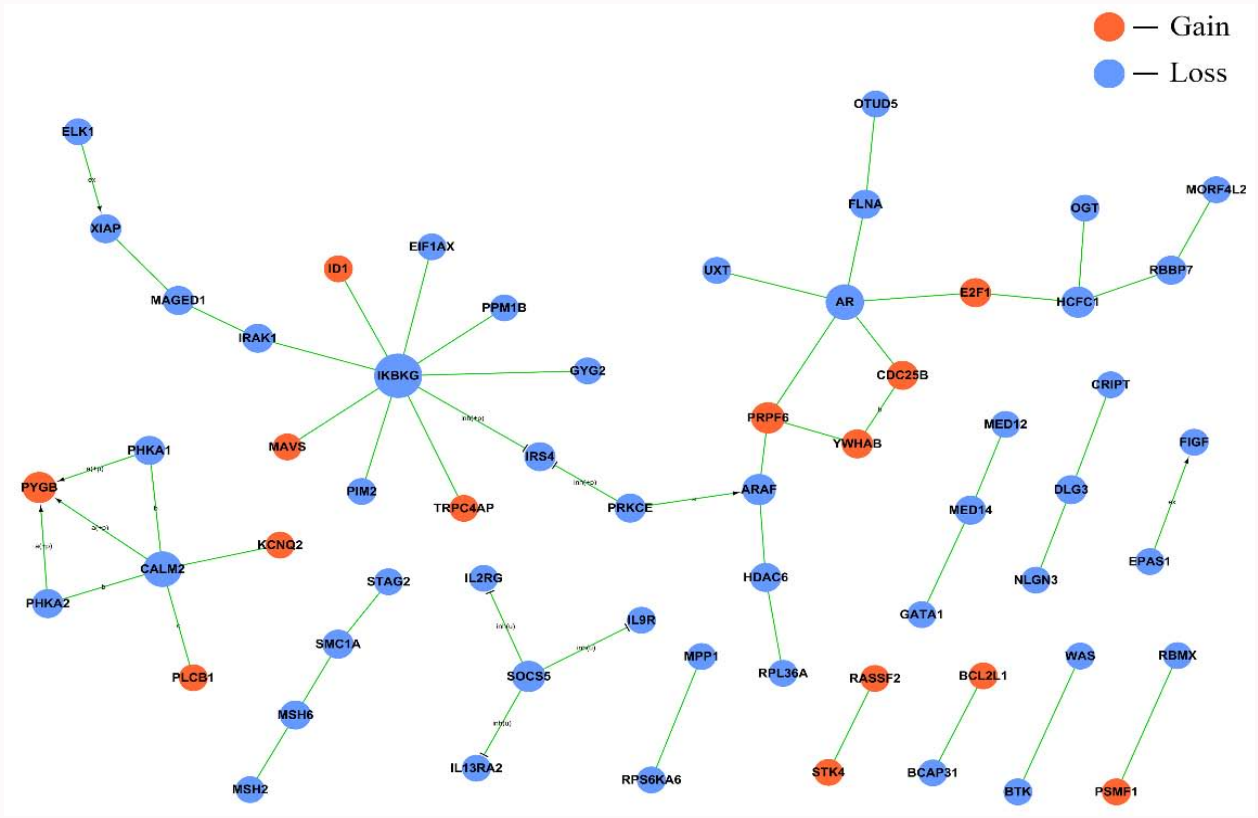


**Figure 2:** Signal-Net of CNV genes (T-ALL).

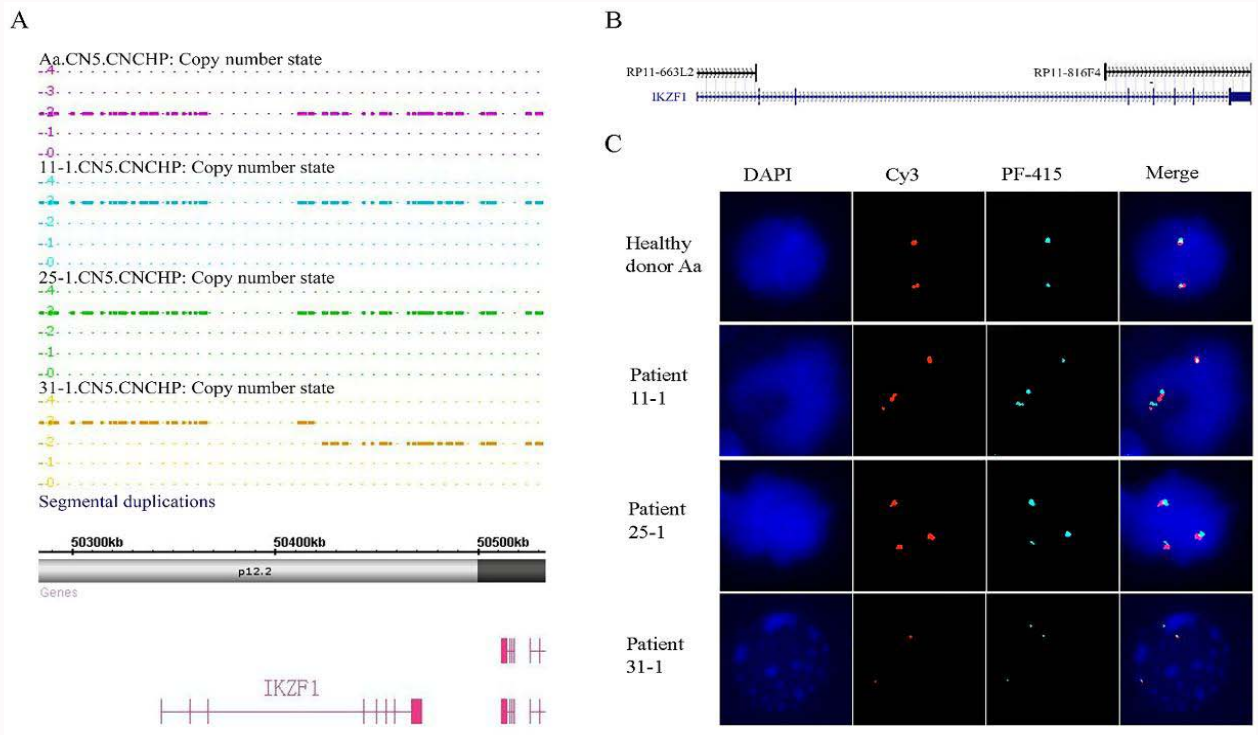


**Figure 3:** IKZF1gene DNA copy number of a healthy donor and ALL patients.
A: Representative IKZF1 amplification from Genotyping Console (Affymetrix) in 1 health donor and 3 ALL patients. Donor (a) has a normal IKZF1 copy number (DNA copy number state_2). Patient No.11 (P210+ B-ALL) and Patient No.25 (pre-B-ALL) have an IKZF1 amplification (DNA copy number state_3). Patient No.33 (T-ALL) has a partial IKZF1 amplification (DNA copy number state_3 and 2). B: Probe location covers the IKZF1 gene exon1 and exon 4-8. The probe is labeled with PF415 and Cy3. RP11-663L2-PF415 (Cascade Blue); RP11-816F4-Cy3 (Rhodamine Red). C: IKZF1gene FISH results corresponded with the SNP array. Donor (a) has a normal IKZF1 copy number (2 blue, 2 red). Patient No.11 (P210+ B-ALL) and Patient No.25 (preB-ALL) have an IKZF1 amplification (3 blue, 3 red). Patient No.33 (T-ALL) has a partial IKZF1 amplification (3 blue, 2 red).

significant gene Signal-Net from B-ALL and T-ALL patients using bioinformatics approaches, which may provide insight into relevant ALL genes and future research directions. In addition, we located the miRNAs in recurrent CNV and LOH regions, such as miR-181b1, miR-516b1, miR-548m, miR-31, miR-877, Let-7g. Some of these miRNAs were shown to be associated with leukemia [18,19], but their function needs to be further investigated. Using miRNA databases, we predicted that some of the miRNAs target genes were correlated with genes involved in ALL. For example, Let-7g displayed a high LOH frequency (57%) in T-ALL and its target genes were determined to be genes such as E2F1, FBXW7, and NPM1, which are known molecular markers of T-ALL.

Although previous comparative studies did not show critical genomic copy number differences between pediatric and adult ALL, Okamoto's research indicated that the CNVs of chromosome 7 are more common in adult ALL [16]. Similarly, we found high frequency CNVs in chromosome 7 in both adult B-ALL (21/25) and T-ALL (8/8). However, in our study, we found that more CNVs on chromosome 7 are gains/amplifications, which is different from the loss/deletions that were reported in other studies. For example, IKZF1, located in 7p12.2, is also present amplification in 57% of Ph+ B-ALL and 50% of Ph- B-ALL patients, rather than a deletion [20]. To confirm our results, we used FISH to check the IKZF1 copy number variations from 2 B-ALL patients and 1 T-ALL patient. The results were the same as the SNP arrays. The differences between Chinese and western countries may be caused by an ethnic variation. Mullighan et al. [21] and Iacobucci et al. [22] indicated that IKZF1 copy number deletions can affect the corresponding dominant negative isoform gene expression and is associated with poor outcome in Ph- B-ALL patients. Our research showed that although the DNA copy number of the IKZF1 gene was amplified, its dominant-negative isoforms were also detected using RT-PCR at the gene transcription level [20-23], which could be a result of transcription of amplified fragments by ectopic separation or formation by alternative splicing.

Adult ALL patients with complete hematological remission present with a normal chromosomal karyotype, which still contains a large number of CNV and LOH, as observed using SNP arrays. Some of the CNVs and LOHs are novel in CR patients, but they are not involved in the initial patient diagnosis, which indicates that these CNVs are not just hereditary and these CR bone marrow cells could arise from different stem cell sub-clones. We also demonstrated the sub-clone hypothesis through the disease course of a P210+ B-ALL patient's copy number evolution. The LOH of Chr16, 17p were continuously detected at newly diagnosis, complete remission and relapse states of this patient, but the gains of Chr8 at diagnosis evolved into the gains of 8q and loss of 8p at relapse. The presence of the relapse clone as a minor sub-population at diagnosis, allows escape from chemotherapy-induced cell death. These data suggest that all the clones come from stem cells but different sub-clones and genomic abnormalities contributing to ALL relapse are selected during treatment, which has also been reported by Mullighan et al. [23]. In addition, the CNVs of Ph- patients at both initial diagnosis and CR are much less than that of Ph+ patients, which is probably associated with the process of the whole disease course.

## Conclusion

Briefly, we detected CNVs and LOH in adult ALL patients, and the candidate genes and miRNAs located in these regions. The amplification of IKZF1 gene copy number on chromosome 7 was newly revealed in Chinese adult ALL patients, which was different from the gene deletions in pediatric and adult ALL patients in the studies reported from western countries. CNV and LOH identification in adult ALL patients can identify susceptible genes, which may guide diagnostic classification and help with treatment selection in the future.

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