**RESEARCH ARTICLE**

# High-throughput Sequencing Reveals that BCR and TCR Repertoires as Potential Prognostic Biomarkers for Pediatric Patients with B-ALL

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**Abstract:** ***Background***: B-ALL is a hematologic malignancy that recurs in approximately 10-20% of children and has a poor prognosis. New prognostic biomarkers are needed to improve individualized therapy and achieve better clinical outcomes.

***Methods***: In this study, high-throughput sequencing technology was used to detect the BCR and TCR repertoires in children with B-ALL.

***Results***: We observed a gradual increase in the diversity of the BCR and TCR repertoires during the developmental stages (Pro-, Common-, Pre-B-ALL) of precursor B-ALL cells. Conversely, as minimal residual disease (MRD) levels on day 19 of induction therapy increased, the BCR/TCR repertoire diversity decreased. Furthermore, the BCR/TCR repertoire diversity was significantly greater in B-ALL patients at low risk and those harboring the ETV6/RUNX1 fusion than in patients with medium-risk disease and those harboring the ZNF384 fusion. Notably, the abundance of BCR/TCR clones varied significantly among patients with B-ALL and different clinical characteristics. Specifically, patients with Pre-B-ALL, low-risk disease, D19MRD levels <1%, and harboring the ETV6/RUNX1 fusion exhibited a predominance of BCR/TCR small clones. In our study, we noted an imbalanced occurrence of V and J gene utilization among patients with B-ALL; however, there seemed to be no discernible correlation with the clinical attributes.

***Conclusion***: BCR/TCR repertoires are expected to be potential prognostic biomarkers for patients with B-ALL.

## 1. INTRODUCTION

B-ALL is a clonal hematopoietic disorder characterized by the abnormal proliferation and accumulation of B-lymphoid progenitor cells [1, 2]. The treatment success rate for Acute Lymphoblastic Leukemia (ALL) has steadily increased since the 1960s, with a five-year event-free survival rate of nearly 80% for children with ALL [3-8]. Despite the progress in this field, approximately 10-20% of children will experience relapse, which is linked to an unfavorable prognosis [9-11]. Resistance and subsequent relapse following standard chemotherapy, targeted therapies, and immunotherapeutic approaches [12, 13] remain major factors contributing to pediatric cancer-related mortality. Better tools are needed to enhance treatment stratification and prognosis and to avoid overtreatment and adverse long-term side effects [14-16].

The mortality of ALL in Europe, the United States, and Japan has slightly decreased due to improvements in the treatment and development of new technologies [17]. The identification of new biomarkers of acute lymphoblastic leukemia, and thus a better understanding of their molecular basis, may lead to better monitoring of the course of the disease. Therefore, new prognostic biomarkers and more accurate risk stratifications are urgently needed to further improve individualized treatment approaches and achieve better clinical outcomes.

The T-cell receptor (TCR) and B-cell receptor (BCR) play crucial roles in T cells and B cells, respectively, enabling them to recognize antigens and participate in immune responses. T lymphocytes can be structurally classified into two subtypes, αβ and γδ, on the basis of heterodimers of T-cell receptors on their cell surface [18]. αβ T cells are found mainly in peripheral tissues and the circulatory system, while γδ T cells are found in the blood, lymphoid tissue, and epithelial environments [19]. The BCR is made up of two heavy chains (IGH) and two light chains (IGK, IGL). The genes that code for the BCR and TCR consist of variable

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(V), diversity (D), joining (J), and constant gene segments. The production of each BCR and TCR involves the rearrangement of multiple gene segments [20]. CDR3, or complementary determining region 3, is a short region that includes the VD and VJ junction. CDR3 is highly variable and crucial for antigen recognition [21].

Clonal rearrangements of BCRs/TCRs can be found in more than 90% of ALL patients and serve as "DNA fingerprints" for specific ALL clones. They are frequently used as sensitive targets for minimal residual disease (MRD) monitoring [22-26]. Previous studies reported that a diverse TCR repertoire in the blood is associated with a better prognosis in patients with CRC [27]. A similar association has been previously reported in tumors other than CRC, including breast [28], ovarian [29], esophageal squamous cell carcinoma [30], head and neck squamous cell carcinoma [31], and lung [32] or gastric cancer [33], among others. However, the associations between BCR/TCR clones and the clinical features of B-ALL, as well as the potential of the BCR/TCR library as a prognostic marker, remain unexplored. In this study, we characterized the TCR and BCR repertoires by detecting and analyzing CDR3 sequences in the TCR α, β, γ, and δ chains, as well as B-cell immunoglobulin (Ig) heavy (IGH) and light (IGK/IGL) chains, in B-ALL patients with different clinical characteristics. These characteristics included diverse immunophenotypes, distinct risk stratifications, varying MRD levels at day 19, and different fusion genes. Our findings provide insights into immune receptor repertoires and T-/B-cell functions in B-ALL, aiming to establish a correlation between BCR/TCR repertoires at diagnosis and prognosis in patients with B-cell lymphoblastic leukemia (B-ALL).

## 2. MATERIALS AND METHODS

### 2.1. Patients

In this study, bone marrow samples were collected from 40 newly diagnosed children with B-ALL who were admitted to the Children's Hospital of Shandong University between 2021 and 2022. Consent was acquired from the patients or their guardians before the collection of samples.

### 2.2. Sample Collection and RNA Extraction

Bone marrow samples were collected from each patient and stored in tubes containing RNAstore reagent (BioTeke, Beijing, China). Total RNA was extracted from monocytes from bone marrow samples using a blood RNA column medium extraction kit (ComWin Biotech, Beijing, China) according to the protocol recommended by the manufacturer. The quality and quantity of the extracted RNA were assessed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA), NanoDrop (Thermo Fisher, USA), and 1% agarose gel. Only samples with a total RNA amount of 2 μg and an RNA integrity number (RIN) ≥ 7 were used for subsequent RNA sample preparation.

### 2.3. BCR/TCR Library Construction

For BCR/TCR library construction, cDNA was generated using the 5' RACE cDNA amplification method [34]. Polymerase Chain Reaction (PCR) was then performed using specific primers targeting the complementary determining region 3 (CDR3) of the BCR/TCR to amplify the desired sequences. The purified PCR products were subjected to a second round of amplification to introduce adaptor sequences compatible with sequencing. The final libraries were assessed for quality using a Qubit dsDNA HS assay kit (Invitrogen, Carlsbad, CA, USA) on a Qubit 2.0 fluorometer.

### 2.4. mRNA Library Construction

The mRNA libraries were prepared using the mRNA-seq Lib prep kit for Illumina (AB clonal, Woburn, MA, USA) according to the protocols recommended by the manufacturer. Following library preparation, the quality of the libraries was assessed using the Qubit dsDNA HS assay kit (Invitrogen, Carlsbad, CA, USA) on a Qubit 2.0 fluorometer.

### 2.5. Sequencing

High-throughput sequencing was performed with a DNB-SEQ-T7 sequencer according to the manufacturer's instructions (BGI, Shenzhen, China). Sequencing was conducted by Beijing Chigene Translational Medicine Research Center Co., Ltd.

### 2.6. BCR/TCR-seq Quality Control and Clone Sequence Analysis

The raw sequencing data were preprocessed for adapter removal, and low-quality reads were filtered using ReSeq-Tools (v0.25) and FastP (v0.20.1). The original sequencing reads were aligned and assembled, and BCR/TCR sequences were obtained using MiXCR (v3.0.3). Subsequent clone sequences were analyzed, which involved annotating sequence features, comparing samples, constructing expression profiles of immune repertoires, and performing clustering analysis. To accomplish this goal, VDJtools (v1.1.4) was employed.

### 2.7. Diversity Analysis

The Chao1 index for each sample was calculated using estimate R (from the vegan package).

### 2.8. Fusion Gene Detection

The raw data were processed using fastq to remove adapters and filter out low-quality reads. The fusion genes were detected using STAR-Fusion and FusionCatcher.

### 2.9. Statistical Analysis

Statistical analysis was conducted using GraphPad Prism version 8.0 (GraphPad Software, Inc., San Diego, CA, USA). Differences among multiple groups were assessed by one-way ANOVA, followed by post hoc analyses using the Bonferroni multiple comparisons test.

## 3. RESULTS

### 3.1. Clinical Characteristics

Table **1** summarizes the specific group information for the 40 children with B-ALL who participated in the study according to different clinical characteristics. The cohort consisted of 21 male and 19 female individuals, and the treatment approach applied to most of these patients adhered to the Chinese Children's Cancer Group study ALL-2020 (CC-CG-2020) protocol (Supplementary Table **1**).

**Table 1. Specific groups according to different clinical characteristics.**

| Samples | EGIL-1995 | Samples | Risk Stratification | Samples | D19 MRD | Samples | Fusion Gene |
|---|---|---|---|---|---|---|---|
| P2702 | Pro B ALL | P2765 | Low risk | P9081 | <0.01% | P7574 | EWSR1/ZNF384 |
| P9081 | Pro B ALL | P7209 | Low risk | P2765 | <0.01% | P7994 | TCF3/ZNF384 |
| P9273 | Pro B ALL | P7574 | Low risk | P7574 | <0.01% | P0289 | TAF15/ZNF384; ZNF384/TAF15 |
| P7994 | Pro B ALL | P0131 | Low risk | P8512 | <0.01% | P9273 | TCF3/ZNF384 |
| P0289 | Pro B ALL | P4550 | Low risk | P4550 | <0.01% | P7508 | EP300/ZNF384; ZNF384/EP300 |
| P4902 | Pro B ALL | P1506 | Low risk | P1506 | <0.01% | P0754 | RUNX1/ETV6; ETV6/RUNX1 |
| P2765 | Common B ALL | P4121 | Low risk | P0514 | <0.01% | P2765 | RUNX1/ETV6; ETV6/RUNX1 |
| P2685 | Common B ALL | P5167 | Low risk | P2193 | <0.01% | P4121 | ETV6/RUNX1 |
| P4304 | Common B ALL | P0514 | Low risk | P3011 | <0.01% | P5167 | ETV6/RUNX1 |
| P7209 | Common B ALL | P3011 | Low risk | P2882 | <0.01% | P2719 | ETV6/RUNX1 |
| P7574 | Common B ALL | P2882 | Low risk | P1556 | <0.01% | P0131 | ETV6/RUNX1; RUNX1/ETV6 |
| P8512 | Common B ALL | P5335 | Low risk | P2719 | <0.01% | P0514 | ETV6/RUNX1; RUNX1/ETV6 |
| P0131 | Common B ALL | P2719 | Low risk | P0754 | <0.01% | P5335 | ETV6/RUNX1; RUNX1/ETV6 |
| P0720 | Common B ALL | P0754 | Low risk | P8104 | <0.01% | P8481 | ETV6/RUNX1; RUNX1/ETV6 |
| P4550 | Common B ALL | P8104 | Low risk | P2661 | 0.01% | P1556 | ETV6/RUNX1; RUNX1/ETV6 |
| P4770 | Common B ALL | P2661 | Low risk | P5167 | 0.04% | P2661 | ETV6/RUNX1; RUNX1/ETV6 |
| P5740 | Common B ALL | P0090 | Low risk | P5335 | 0.05% | P2882 | ETV6/RUNX1; RUNX1/ETV6; TBC1D15/RAB21 |
| P5919 | Common B ALL | P2702 | Medium risk | P2299 | 0.09% | P5740 | PAX5/JAK2 |
| P8937 | Common B ALL | P9081 | Medium risk | P4121 | 0.12% | P9499 | TCF3/PBX1 |
| P1506 | Common B ALL | P7994 | Medium risk | P9499 | 0.14% | P4902 | AFF1/KMT2A; KMT2A/AFF1 |
| P4121 | Common B ALL | P0289 | Medium risk | P0090 | 0.21% | P2702 | NA |
| P5167 | Common B ALL | P4902 | Medium risk | P8937 | 0.31% | P9081 | NA |
| P0514 | Common B ALL | P4304 | Medium risk | P8296 | 0.35% | P2685 | NA |
| P2299 | Common B ALL | P8512 | Medium risk | P7209 | 0.36% | P4304 | NA |
| P2193 | Common B ALL | P0720 | Medium risk | P0131 | 0.50% | P7209 | NA |
| P3011 | Common B ALL | P4770 | Medium risk | P4304 | 0.69% | P8512 | NA |
| P2882 | Common B ALL | P5740 | Medium risk | P4902 | 1.30% | P0720 | NA |
| P5335 | Common B ALL | P8937 | Medium risk | P7002 | 2% | P4550 | NA |
| P7002 | Common B ALL | P2299 | Medium risk | P5740 | 2.19% | P4770 | NA |
| P7508 | Common B ALL | P7002 | Medium risk | P2702 | 2.36% | P5919 | NA |
| P8481 | Common B ALL | P7508 | Medium risk | P4770 | 2.95% | P8937 | NA |
| P1556 | Common B ALL | P8481 | Medium risk | P7994 | 3.63% | P1506 | NA |
| P2719 | Pre B ALL | P1556 | Medium risk | P7508 | 3.78% | P2299 | NA |
| P9499 | Pre B ALL | P9499 | Medium risk | P8481 | 3.87% | P2193 | NA |
| P0754 | Pre B ALL | P3764 | Medium risk | P0720 | 4.93% | P3011 | NA |
| P8104 | Pre B ALL | P9273 | High risk | P3764 | 5.78% | P7002 | NA |
| P8296 | Pre B ALL | P5919 | High risk | P0289 | 26.99% | P8104 | NA |
| P2661 | Pre B ALL | P2685 | / | P9273 | 30.10% | P8296 | NA |
| P0090 | Pre B ALL | P2193 | / | P2685 | / | P0090 | NA |
| P3764 | Pre B ALL | P8296 | / | P5919 | / | P3764 | NA |

**Note:** EGIL: Leukemia immunotyping European group
NA: No gene fusion was detected

### 3.2. Correlations between BCR/TCR Repertoire Diversity and Different Clinical Features in B-ALL Patients

We conducted an analysis to examine the correlation between the richness indices (logChao1) of BCR repertoires in patients with B-ALL and various clinical features, including immune types, risk stratification, MRD levels at day 19 (D19MRD), and the presence of different fusion genes. Our findings revealed distinct patterns of BCR repertoire diversity among different immune types of B-ALL. Specifically, we observed the lowest diversity in Pro-B-ALL patients (n=6), the highest diversity in Pre-B-ALL patients (n=8), and intermediate diversity in Common-B-ALL patients (n=26) (Fig. **1A**). These results suggested that the BCR diversity in patients with B-ALL differed at different stages of B-cell differentiation, and the higher the differentiation degree of B-ALL, the greater was the BCR diversity.

We further investigated the impact of risk stratification on BCR repertoire diversity and observed that patients with low-risk B-ALL (n=17) had a significantly greater Chao 1 index compared to those with medium-risk B-ALL (n=18) (Fig. **1B**).

Additionally, we examined the association between BCR repertoire diversity and MRD levels on day 19 of induction therapy. We divided patients into three groups based on MRD on day 19 after induction therapy: those with an MRD less than 1% (n=26), those with an MRD between 1% and 5% (n=9), and those with an MRD greater than 5% (n=3). Our analysis revealed a decrease in BCR repertoire diversity as MRD levels increased (Fig. **1C**).

Moreover, we explored the potential differences in BCR repertoire diversity among patients with B-ALL and different genetic characteristics. Owing to the limited sample size, we focused on comparing patients with ETV6/RUNX1 fusion (n=12) and those with ZNF384 fusion (n=5). Our results revealed significantly greater BCR repertoire diversity in patients with ETV6/RUNX1 fusion than in those with ZNF384 fusion (Fig. **1D**).

We used the same method to analyze the correlation between the diversity of the TCR repertoire and various clinical features in patients with B-ALL. It was found that the correlation between TCR repertoire diversity and various clinical features in patients with B-ALL was similar to that observed for BCR. Specifically, with increasing B-cell differentiation, the TCR repertoire diversity gradually increased (Fig. **2A**). The Chao 1 index was significantly greater for low-risk compared with medium-risk patients (Fig. **2B**). Moreover, the TCR repertoire diversity was higher for patients with a low MRD burden after induction therapy (Fig. **2C**), and the TCR repertoire diversity of patients with ETV6/RUNX1 fusion was higher than of patients with ZNF384 fusion (Fig. **2D**).

These findings shed light on the dynamics of BCR/TCR repertoire diversity in patients with B-ALL and its association with clinical features, providing valuable insights into the immune landscape of this disease.

### 3.3. Association of Clonotype Abundance of BCR/TCR with Different Clinical Features in Patients with B-ALL

Clonal size is an important feature of lymphocytes. Normally, lymphocytes are in a positive polyclonal state without any antigenic stimulation. In the case of diseases, specific antigen stimulation leads to rearrangement. Therefore, we assessed the relative abundance of BCR/TCR clonotypes by examining their frequencies within specific ranges, spanning from rare clonotypes (<0.01%) to highly abundant clonotypes (>10%). Our analysis revealed distinct patterns of clonotype distribution across different subtypes of B-ALL. Specifically, the repertoire of Pre-B-ALL patients was predominantly composed of small BCR clonotype groups, whereas highly abundant clonotypes were primarily found in Pro-B-ALL patients and common B-ALL patients (Fig. **3A**).

Furthermore, we investigated the relationship between clonotype abundance and risk stratification. It was observed that patients with low-risk B-ALL were predominantly occupied by small BCR clonotype groups, whereas highly abundant clonotypes were more prevalent in patients with medium-risk B-ALL (Fig. **3B**).

We also explored the association between clonotype abundance and minimal residual disease (MRD) levels on day 19 of induction therapy. Our results demonstrated that patients with MRD levels exceeding 1% had a relatively high abundance of BCR clonotypes (Fig. **3C**).

Furthermore, we examined the relationship between clonotype abundance and the presence of different fusion genes. Fig. (**3D**) shows that patients carrying the ETV6/RUNX1 fusion gene presented lower BCR clonal abundance than patients carrying the ZNF384 fusion gene.

We used the same method to analyze the correlation between the clonotype abundance of the TCR repertoire and various clinical features in B-ALL patients. We found that the correlation between the TCR repertoire clonotype abundance and various clinical features in B-ALL patients was similar to that in BCR patients. Specifically, the repertoire of pre-B-ALL patients was predominantly composed of small TCR clonotype groups, especially TRA chains (Fig. **4A**). As we expected, B-ALL patients with low-risk characteristics (Fig. **4B**), with MRD levels exceeding 1% (Fig. **4C**) and with ETV6/RUNX1 fusion (Fig. **4D**), were predominantly occupied by smaller TCR clonotype groups.

These findings provide insights into clonotype abundance and its association with clinical features, including subtype classification, risk stratification, MRD levels, and fusion genes, in B-ALL patients.
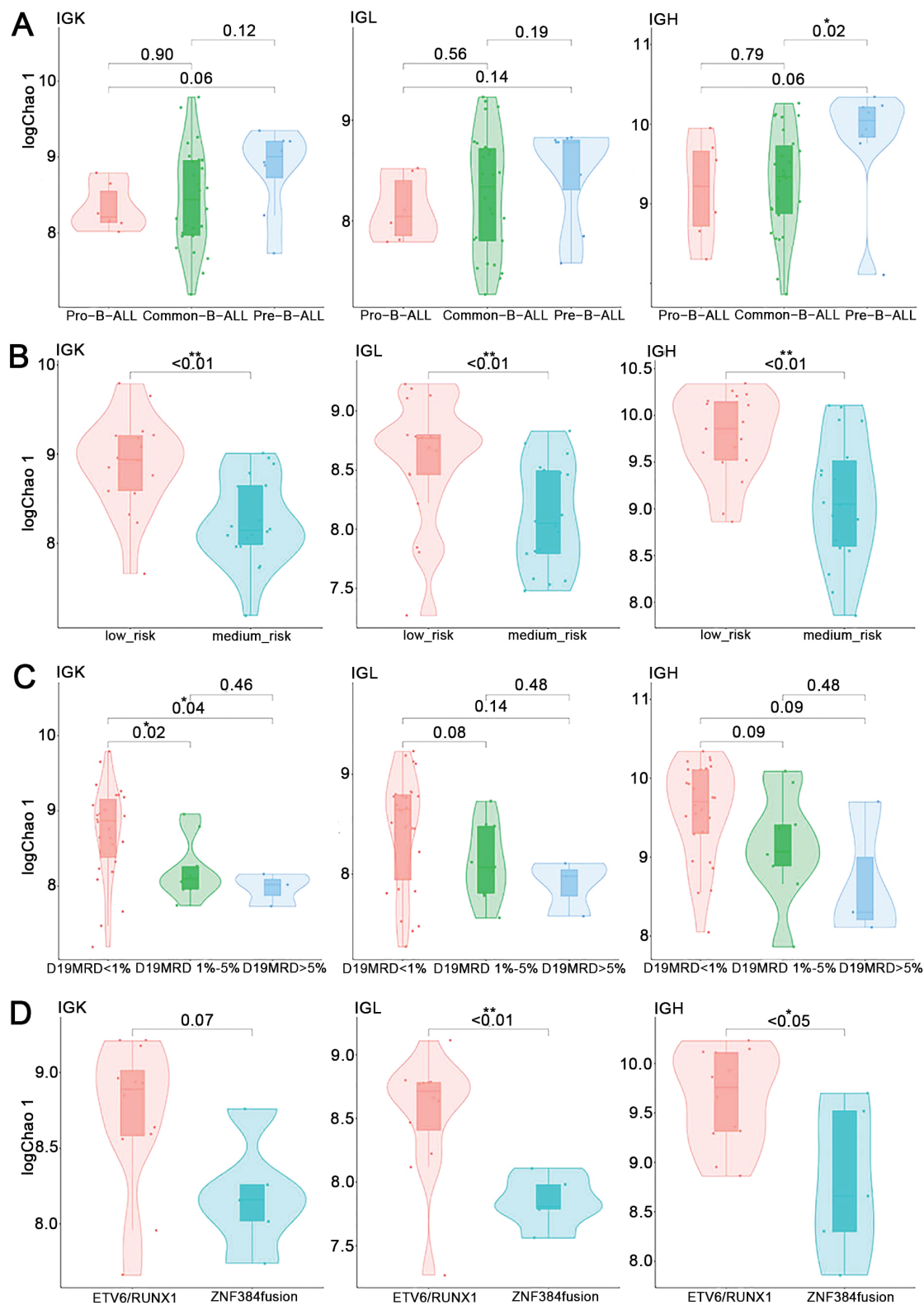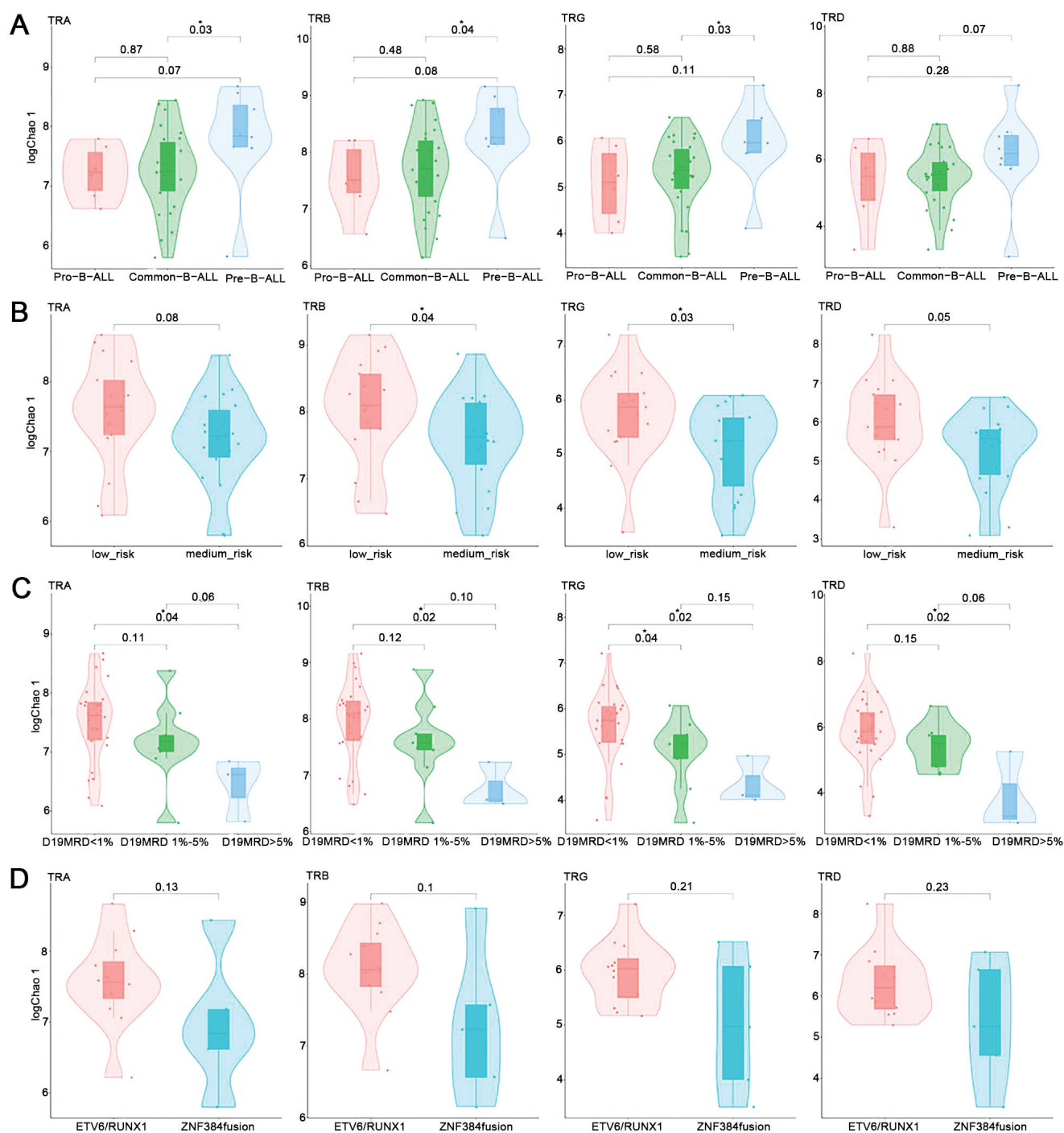
**Fig. (1).** Diversity of the BCR repertoires in patients with B-ALL with different clinical features, including (**A**) different immune types, (**B**) different risk stratification, (**C**) different MRD levels on day 19, and (**D**) carrying different fusion genes. IGK/L immunoglobulin light chain, IGH immunoglobulin heavy chain. One asterisk (*) indicates $P < 0.05$, and two asterisks (**) indicate $P < 0.01$. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

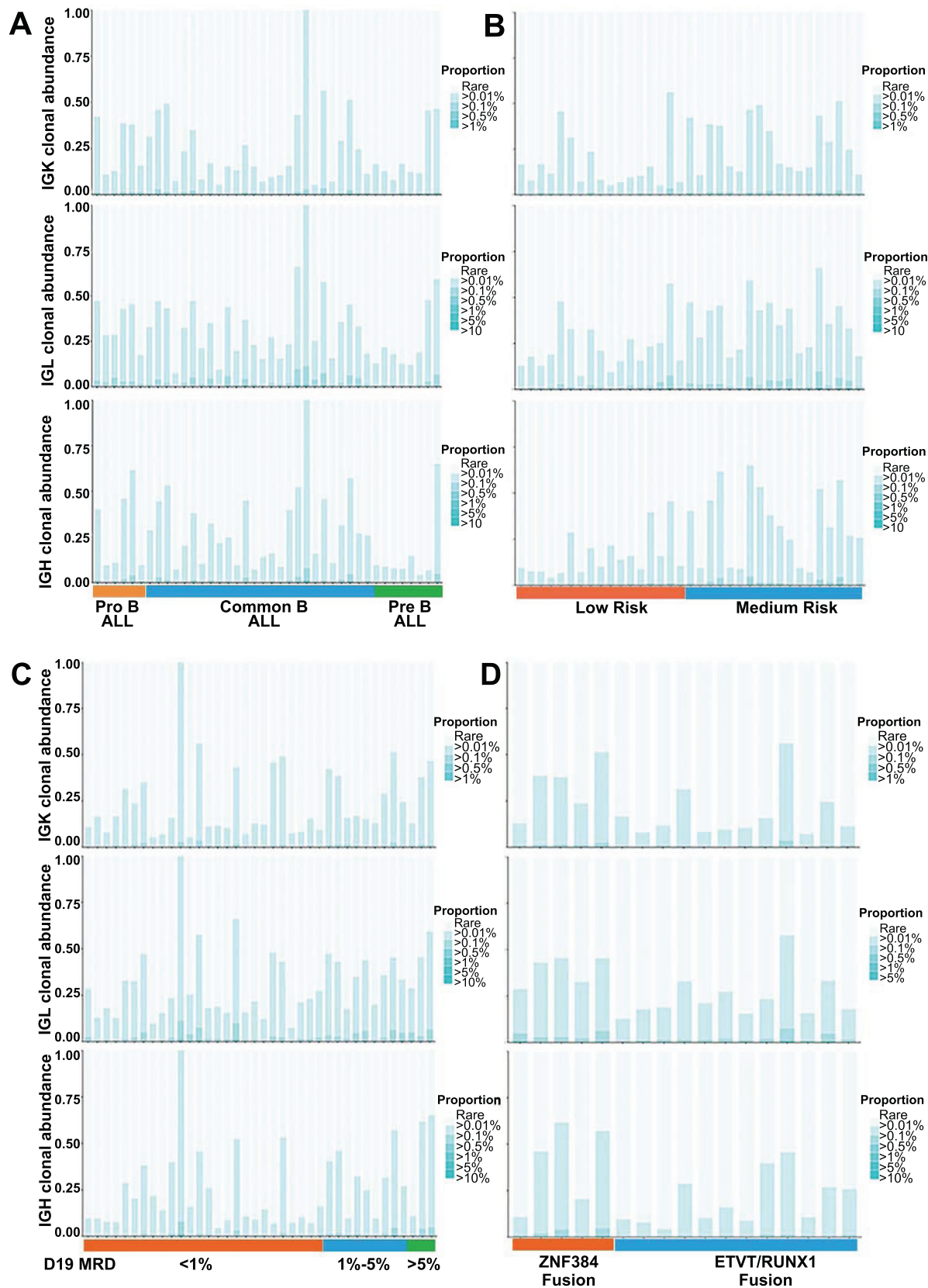**Fig. (2).** Diversity of the TCR repertoires in B-ALL patients with different clinical features, including (**A**) different immune types, (**B**) different risk stratifications, (**C**) different MRD levels on day 19, and (**D**) carrying different fusion genes. TRA T-cell receptor α, TRB T-cell receptor β, TRG T-cell receptor γ, TRD T-cell receptor δ. Asterisks (*) indicate $P < 0.05$. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

**Fig. (3).** Abundances of BCR clonotypes in B-ALL patients with different clinical characteristics, including (**A**) different immune types, (**B**) different risk stratifications, (**C**) different MRD levels on day 19 after induction therapy, and (**D**) carrying different fusion genes. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).
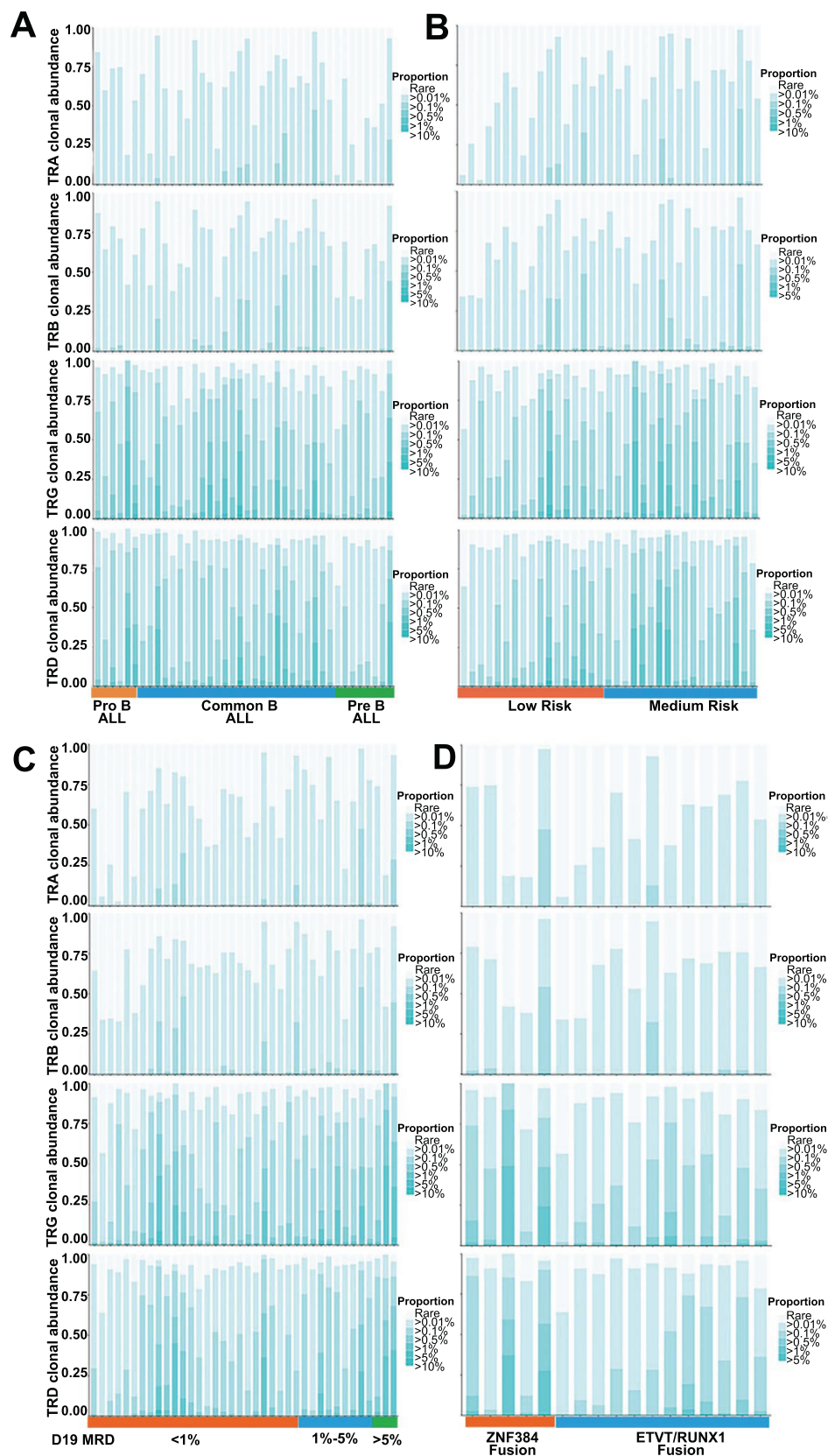
**Fig. (4).** Abundances of TCR clonotypes in B-ALL patients with different clinical characteristics, including (**A**) different immune types, (**B**) different risk stratifications, (**C**) different MRD levels on day 19 after induction therapy, and (**D**) carrying different fusion genes. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

### 3.4. The Usage Frequencies of the V and J Genes in the BCR/TCR Repertoires of B-ALL Patients with Different Clinical Characteristics

We visualized the usage frequencies of the BCR/TCR V and J genes in B-ALL patients with different clinical characteristics *via* heatmaps (Fig. **5**). It was found that several V genes, including IGKV3.20 (frequency = 0.155 ± 0.033) and IGKV3.15 (frequency = 0.151 ± 0.041), were highly prevalent in nearly all the samples (Fig. **5A**). Additionally, IGKV1.39 (frequency = 0.112 ± 0.037) (Fig. **5A**) and IGLV3.21 (frequency = 0.114 ± 0.084) (Fig. **5B**) were frequently utilized in the majority of patients. The most commonly used J genes were IGKJ2 (frequency = 0.268 ± 0.030) (Fig. **5A**), IGLJ3 (frequency = 0.491 ± 0.167) (Fig. **5B**), and IGHJ4 (frequency = 0.511 ± 0.081) (Fig. **5C**).

We also examined the usage frequencies of the TCR V and J genes in B-ALL patients with different clinical characteristics. Consistent with previous reports by other researchers [35], we observed that certain TCR V genes exhibited high-frequency usage across the samples, including TRAV13.1 (frequency = 0.100 ± 0.035), TRAV21 (frequency = 0.095 ± 0.029) (Fig. **5D**), TRBV20.1 (frequency = 0.214 ± 0.091) (Fig. **5E**), TRGV9 (frequency = 0.546 ± 0.176) (Fig. **5F**), and TRDV2 (frequency = 0.822 ± 0.143) (Fig. **5G**). Similarly, TRBJ2.7 (frequency = 0.169 ± 0.037), TRBJ2.1 (frequency = 0.166 ± 0.033) (Fig. **5E**), and TRDJ1 (frequency = 0.774 ± 0.101) (Fig. **5G**) showed high-frequency usage in all samples. These findings indicate that there are skewed patterns of TCR V and J gene usage in B-ALL patients.

Finally, we observed that the use of the V and J genes in the BCR/TCR repertoire did not significantly influence the clinical characteristics of patients with B-ALL.

### 4. DISCUSSION

Genetic and epigenetic abnormalities contribute to increased proliferation potential in leukemic cells, leading to treatment resistance and eventually resulting in relapse or treatment failure [34-36]. In the last two decades, significant genomic discoveries in ALL have emerged, including RNA sequencing (RNA-seq), gene-targeting sequencing, and whole-exome sequencing (WES) [37]. These advancements have proven crucial in risk stratification, offering therapeutic insights, and bearing prognostic implications [37-39]. However, the correlation between BCR/TCR clones and the clinical characteristics of B-ALL, along with the potential of the BCR/TCR library as a prognostic marker, remains largely unexplored.

B-ALL can be categorized into different subtypes on the basis of its developmental stage, as indicated by immunophenotyping [40]. Specifically, B-ALL is classified into pro-B-ALL (CD10-), common-B ALL (CD10+), and pre-B-ALL (cytoplasmic IgM+), each with distinct characteristics. Notably, research has shown that B-ALL patients with CD10-positive expression tend to have a favorable prognosis [41], suggesting that individuals with common B-ALL might have better outcomes than those with pro-B-ALL; as

expected, BCR/TCR diversity was greater in common B-ALL patients than in pro-B-ALL patients, suggesting a better prognosis with higher BCR/TCR diversity and lower clonal abundance. This finding was confirmed by subsequent results, which revealed that the BCR/TCR diversity was significantly greater and that the BCR/TCR clonal abundance was lower in low-risk patients than in intermediate-risk patients.

Additional studies have demonstrated that a high burden of MRD following induction therapy is generally associated with an unfavorable prognosis [42-44], and several additional prospective and nonrandomized studies have confirmed that MRD has a strong and independent effect on prognosis after induction and early consolidation of ALL in children and adults [45-50]. Additionally, ETV6/RUNX1 fusion is the most prevalent genetic abnormality in childhood ALL, accounting for approximately 25% of precursor-B phenotype cases [51]. Patients with this genetic rearrangement have been previously identified as a subgroup with a favorable prognosis [52-54]. In pediatric precursor B-ALL, the frequency of ZNF384 gene rearrangement is approximately 3% [55], and its prognostic impact is considered intermediate but can vary depending on the partner genes involved. This evidence suggests that patients with a low MRD load after induction therapy have a better prognosis and that patients with ETV6/RUNX1 fusion have a better prognosis than those with ZNF384 fusion. In our study, we observed that patients with low MRD loads (D19MRD levels less than 1%) after induction therapy and those with ETV6/RUNX1 fusions presented greater BCR/TCR clonal diversity and lower clonal abundance. On the basis of these observations, we speculate that patients with higher clonal diversity and lower clonal abundance might have a better prognosis. Nonetheless, owing to the relatively brief duration of the follow-up period, there are presently no statistically notable variances in the clinical results observed among the patients.

In a different context, chronic lymphocytic leukemia (CLL) with a stereotyped B-cell receptor (BCR) belonging to subset #1 (IGHV1-5-7/IGKV1-39) is known to be associated with a poor outcome [56]. Recently, the IGLV3.21 gene has emerged as a separate prognostic indicator associated with unfavorable consequences in CLL, regardless of its pairing with a heavy-chain counterpart [57-59]. We explored whether the BCR/TCR V gene or J gene in B ALL can serve as a prognostic indicator. The results revealed that TRAV13.1, TRAV21, TRBV20.1, TRGV9, TRDV2, TRBJ2.7, TRBJ2.1, and TRDJ1 exhibited high-frequency usage in almost all the samples. In these patients, skewed usage of the V and J genes of the BCR/TCR was frequently observed, as depicted by the high occurrence of IGKV3.20, IGKV3.15, IGKV1.39, IGLV3.21, IGKJ2, IGLJ3, and IGHJ4. This highlights a similar trend observed in the majority of patients. Nonetheless, we observed no significant differences in the frequency of V or J gene usage among patients with different clinical characteristics. As a result, further investigation is needed to determine whether these high-frequency V and J gene fragments can indeed serve as reliable indicators of B ALL prognosis.

The primary limitations of this study are the comparatively small sample size and the relatively brief duration of the follow-up period. With a larger study group and longer follow-up time, we could have conducted more sophisticated analyses specifically aimed at identifying BCR/TCR differ-ences among patients with B-ALL who exhibit diverse clinical characteristics. This could have facilitated a more in--depth exploration of novel prognostic markers, ultimately leading to the advancement of individualized therapy and the achievement of better clinical outcomes.
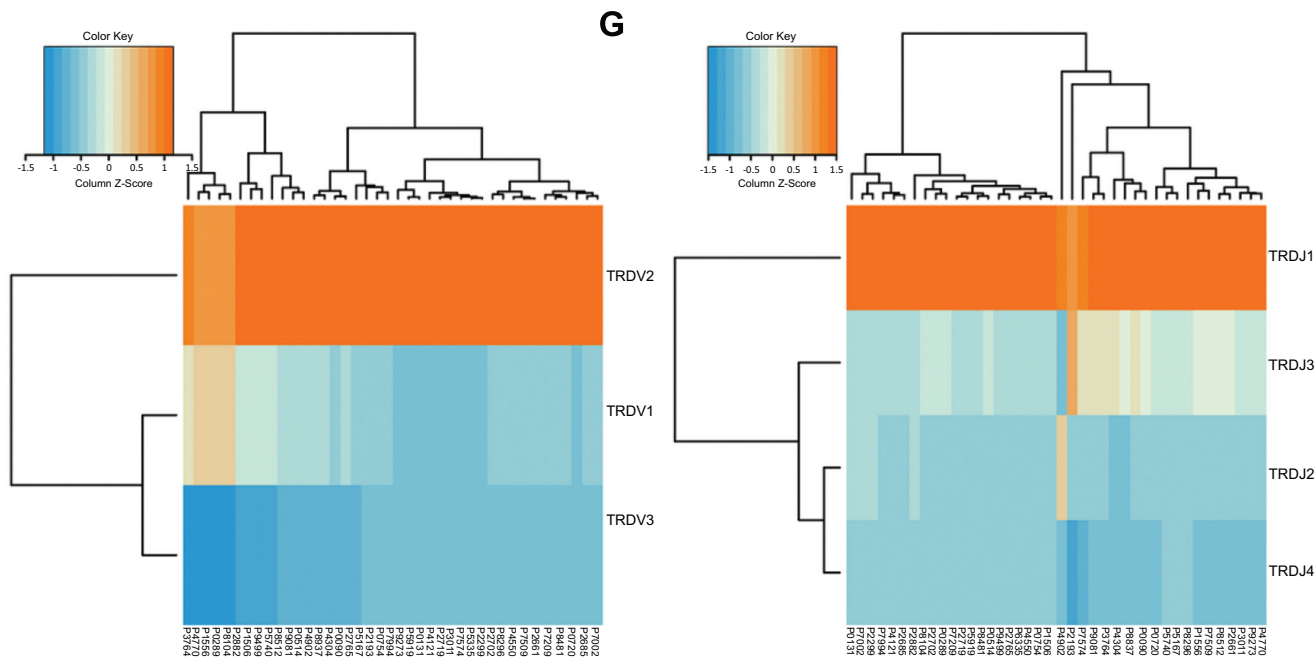
**C**



**D**



(Fig. 5) contd….

**E**



**F**



**(Fig. 5) contd….**
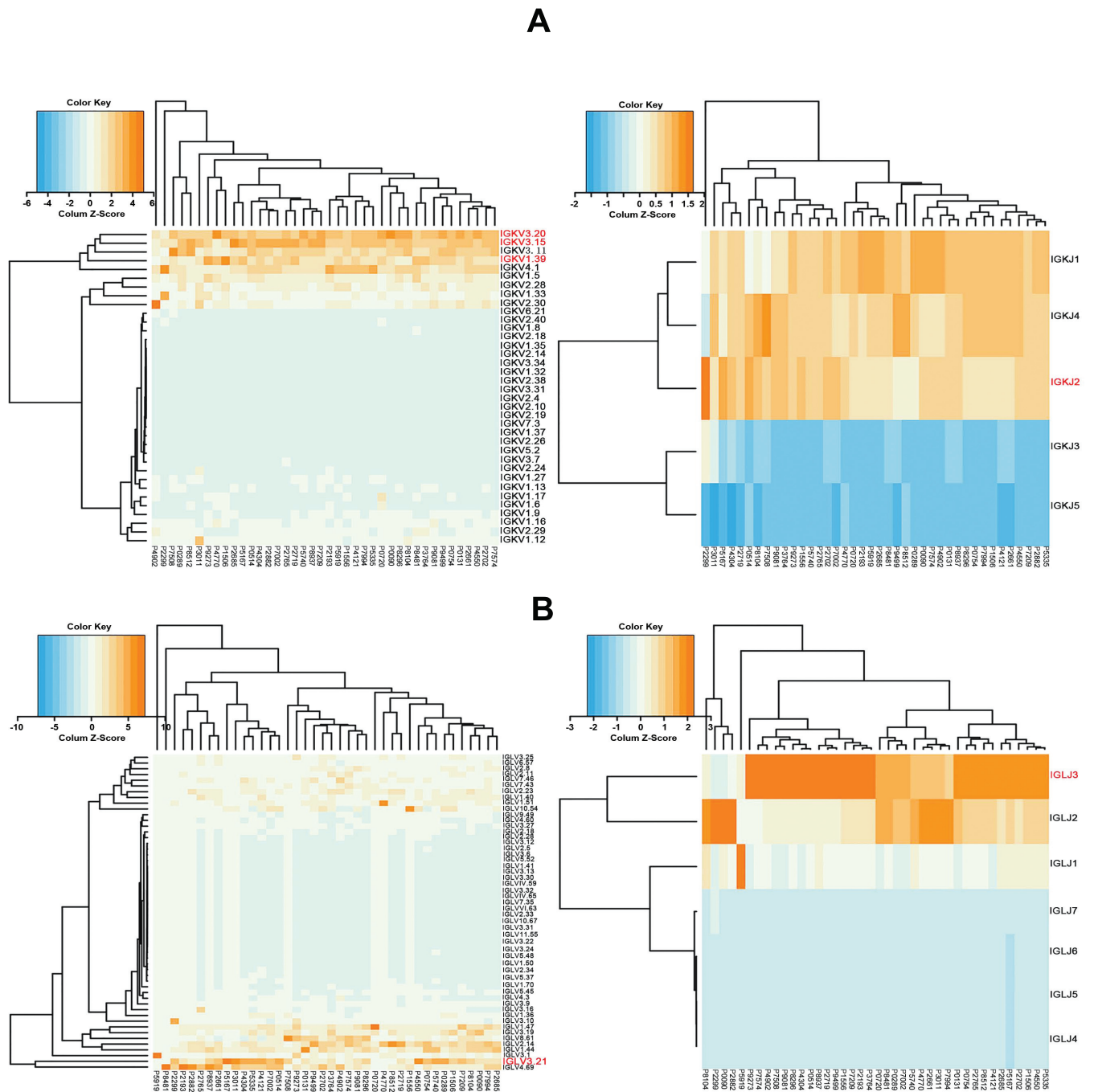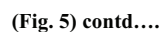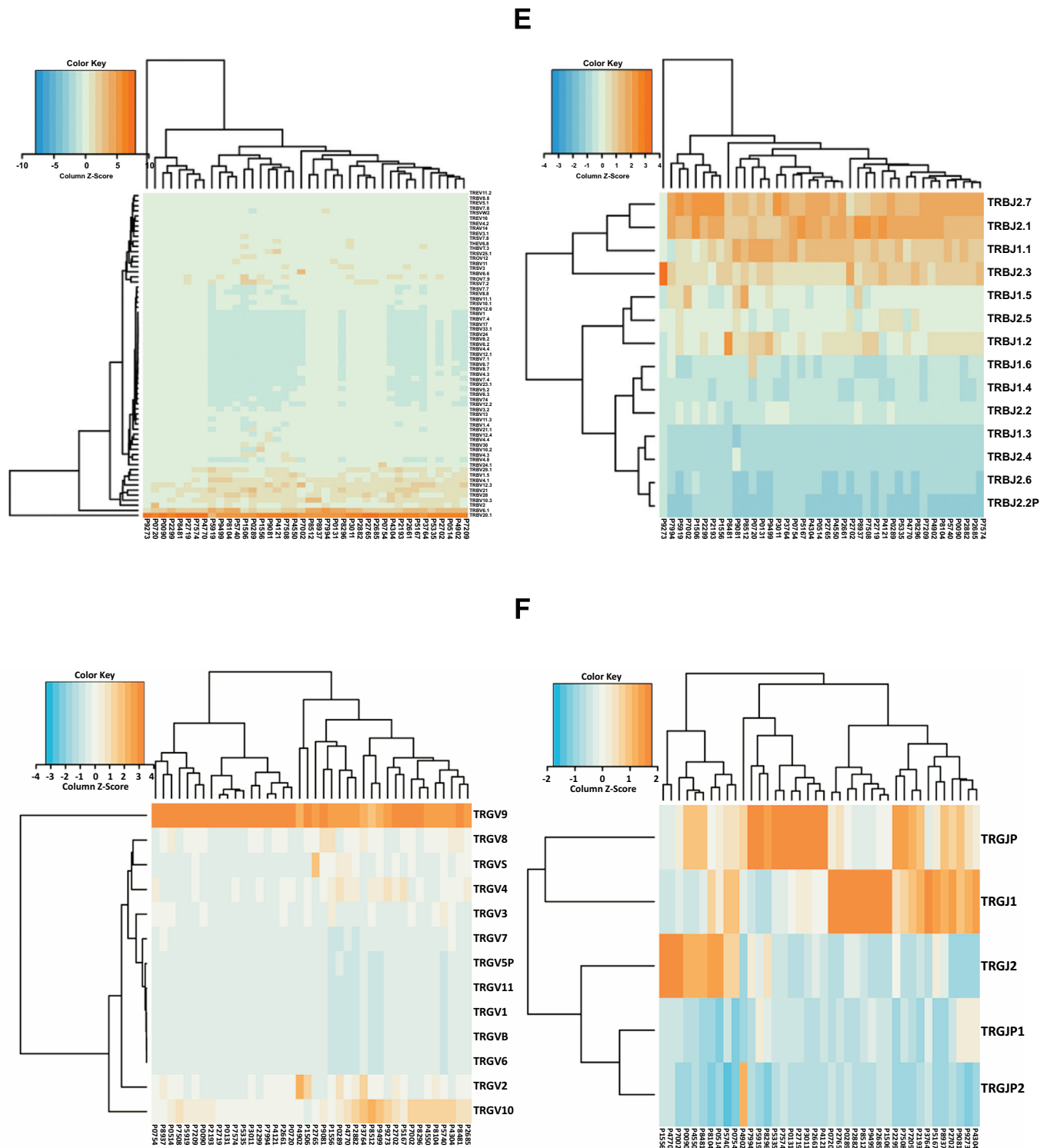
**Fig. (5).** Frequencies of V and J genes in the BCR/TCR repertoires of patients with B-ALL. (**A**) Frequency of use of V and J genes in IGK, (**B**) frequency of use of V and J genes in IGL, (**C**) frequency of use of V and J genes in IGH, (**D**) frequency of use of V and J genes in TRA, (**E**) frequency of use of V and J genes in TRB, (**F**) frequency of use of V and J genes in TRG, (**G**) frequency of use of V and J genes in TRD. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

## CONCLUSION

In conclusion, our comprehensive analysis of the TCR and BCR repertoires of B-ALL patients with different clinical characteristics highlights their potential as prognostic biomarkers. Incorporating BCR/TCR repertoire analysis at the time of diagnosis can serve multiple purposes. It can provide baseline data for monitoring MRD [60], and additionally, it offers valuable insights into potential prognostic indicators, aiding in the formulation of personalized treatment strategies to improve patient outcomes.

## HIGHLIGHTS

1. Our findings shed light on the dynamics of BCR/TCR repertoire diversity in B-ALL patients and its associations with clinical features.

2. The abundance of BCR/TCR clones varied significantly among B-ALL patients with different clinical characteristics.

3. The use of V and J genes in the BCR/TCR repertoire did not significantly influence the clinical characteristics of patients with B-ALL.

4. The BCR/TCR repertoire is expected to be a potential prognostic biomarker for patients with B-ALL.

## AUTHORS' CONTRIBUTIONS

Fu Li and Xiaomei Yang developed the concept and design of the study. Xiao Mou, Li Song, and Libo Zheng collected samples and participated in the experiments. Jiajia Mi collected and analyzed the data. Xiuxiu Wang participated in the experiments, interpreted the data, and conceptualized

and wrote the manuscript. Fu Li provided valuable input into the study and reviewed and approved the manuscript. All of the authors critically evaluated the manuscript and approved its submission.

## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| ALL | = | Acute Lymphoblastic Leukemia |
| BCR | = | B-cell Receptor |
| CDR3 | = | Complementary Determining Region 3 |
| CLL | = | Chronic Lymphocytic Leukemia |
| MRD | = | Minimal Residual Disease |
| PCR | = | Polymerase Chain Reaction |
| TCR | = | T-cell Receptor |
| WES | = | Whole-exome Sequencing |

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was reviewed and approved by the Ethics Committee of Children's Hospital, Affiliated with Shandong University and the Ethics Committee of Jinan Children's Hospital, Jinan, China with approval number SDFE-IR-B/T-2023087.

## HUMAN AND ANIMAL RIGHTS

All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

## CONSENT FOR PUBLICATION

All participants/patients (or their proxies/legal guardians) provided informed consent to participate in the study.

## AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research are available within the article.

## FUNDING

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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## SUPPLEMENTARY MATERIAL

Supplementary material is available on the publisher's website along with the published article.

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