Original Article

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Transcriptomic Profiling of Pediatric Acute Lymphoblastic Leukemia Subtypes Identifies Novel Prognostic Markers

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Abstract

Background: Pediatric acute lymphoblastic leukemia (ALL) encompasses more than 20 molecular subtypes with diverse genetic and prognostic profiles. The 11q subtype, characterized by KMT2A rearrangements, exhibits an aggressive clinical course compared with favorable subtypes such as high hyperdiploidy (HeH) and dic (9; 20). This study aimed to elucidate the distinct transcriptomic signature of the 11q-rearranged pediatric ALL subtype relative to HeH and dic (9; 20) to identify novel prognostic markers.

Materials and Methods: Comparative observational study was done by gene expression profiles of 11q ALL (n = 5) were compared with dic (9; 20) ALL (n = 6) and HeH ALL (n = 18) using the GEO2R tool on the GSE47051 microarray dataset. Differentially expressed genes (DEGs) were defined as those with $p < 0.05$ and $|\log_2 \text{fold change}| > 1.0$. Common DEGs across both comparisons were identified using Venny 2.1.0, and their protein–protein interaction networks and functional enrichment were analyzed using STRING.

Results: A total of 241 common DEGs were identified in 11q ALL, including 151 upregulated and 90 downregulated genes ($p < 0.05$). Prominent upregulated genes included members of the HOXA cluster (HOXA3, HOXA4, HOXA5, HOXA7, HOXA10) and MEIS1 ($p = 0.001$), reflecting activation of leukemogenic transcriptional programs. Conversely, genes involved in B-cell differentiation, such as BLNK and MS4A1/CD20, were significantly downregulated ($p = 0.004$). Enrichment analysis revealed that upregulated genes were significantly associated with focal adhesion, protein kinase binding, and cell migration pathways ($p < 0.01$), while downregulated genes were linked to B-cell activation, DNA repair, and chromosomal stability ($p < 0.05$).

Conclusion: The 11q-rearranged ALL subtype demonstrates a distinct transcriptional landscape characterized by HOXA–MEIS1 axis activation and suppression of normal B-cell signaling. These transcriptomic alterations likely underpin its aggressive phenotype and could inform risk stratification and targeted therapeutic development for high-risk pediatric ALL patients.

Keywords: Acute Lymphoblastic Leukemia, Chromosomal Abnormalities, Differential Expression Analysis, HOXA, Pediatric, Subtyping

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Introduction

Pediatric acute lymphoblastic leukemia (ALL), the most common childhood malignancy, is characterized by the uncontrolled proliferation of immature lymphoid cells within the bone marrow and peripheral blood. This disease exhibits substantial heterogeneity at the molecular level, with over 20 distinct subtypes of B-cell ALL (B-ALL) having been identified. Accurate subtyping is crucial due to subtypes' unique biology, treatment responses, and prognoses (1, 2).

Current diagnostics have limitations in capturing disease complexity. Therefore, identifying new molecular markers is crucial for better risk assessment, patient stratification, and treatment selection. Transcriptomic profiling has significantly advanced pediatric ALL understanding by identifying novel subtypes (Ph-like, DUX4-rearranged) and characterizing gene expression signatures of known subtypes, revealing unique biology and prognostic significance (2–4).

The 11q subtype of pediatric ALL is defined by chromosomal translocations of the KMT2A (MLL) gene on chromosome 11q23, occurring frequently in infant B-ALL (around 75%, especially under 6 months) and less commonly (around 2%) in older patients. This subtype is typically aggressive, associated with a poor prognosis marked by hyperleukocytosis, higher CNS involvement risk, early relapse, and poor survival rates. The specific outcome can be influenced by which of the over 80 identified gene partners fuses with KMT2A (5).

Gene expression studies reveal a unique transcriptomic signature for KMT2A-rearranged ALL, distinct even from MLL-germline infant ALL, with further specific signatures often associated with particular translocations like t (4; 11), indicating underlying biological variation. Novel prognostic markers identified through gene expression include high MEIS1 levels correlating with unfavorable outcomes in KMT2A-rearranged leukemia (while low levels are favorable in MLL-germline infant ALL), and high BCL11A expression being linked to poor survival in KMT2A-rearranged ALL. Specifically within t(4;11)-positive infant ALL, co-expression of the reciprocal AF4-MLL fusion and the HOXA gene cluster predicts better event-free survival, whereas the absence of HOXA expression signals a very high risk of relapse (5,6).

The dicentric chromosome dic(9;20)(p11-13;q11) is a recurrent structural abnormality found in 2-5% of pediatric B-cell precursor ALL (BCP-ALL) cases, resulting from a fusion between parts of chromosomes 9 and 20 that leads to the loss of chromosome arms 9p and 20q. This abnormality is often accompanied by deletions of key genes on 9p, such as tumor suppressors CDKN2A and CDKN2B, and the B-cell transcription factor PAX5. Recent studies have highlighted recurrent genomic rearrangements involving the DNMT3B gene with PAX5 or the adjacent gene ZCCHC7 within a subset of dic (9; 20)-positive patients; these rearrangements are strongly linked to an unfavorable prognosis and a significantly increased risk of relapse, with the precise breakpoint location within DNMT3B appearing prognostically important. Furthermore, transcriptomic analysis indicates that dic (9; 20) cases exhibit significantly higher expression levels of FGFR1 and MED12L compared to other BCP-ALL subgroups (7, 8).

High hyperdiploidy (HeH), characterized by 51 to 67 chromosomes resulting from nonrandom gains (notably X, 4, 6, 10, 14, 17, 18, 21), is a frequent subtype of pediatric ALL, occurring in 25-30% of cases and generally associated with an excellent prognosis and a distinct hypomethylation signature. Despite this favorable outlook, about 15-20% of children with HeH experience relapse, prompting research using transcriptomics and genomics to identify risk factors within this group. Whole-genome sequencing suggests chromosome gains are early drivers, and while HeH has a relatively low mutation burden, recurrent mutations in Ras pathway genes (FLT3, KRAS, NRAS, PTPN11) and chromatin modifiers like CREBBP are found and may contribute to relapse (9).

Given these differences between 11q, dic (9; 20), and HeH subtypes of ALL, a further transcriptomics analysis is warranted that compares 11q to these two other subtypes to assess the unique dysregulated transcriptomic signature of 11q.

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While the general transcriptomic features and some prognostic markers for 11q ALL are known, a specific research gap lies in comprehensively identifying the unique gene expression landscape that distinguishes the typically aggressive 11q subtype from subtypes with differing prognoses and distinct genetic origins, such as the generally favorable HeH and the structurally unique dic (9; 20), although a similar expression pattern has been reported, as mentioned earlier.

Performing a differential expression analysis comparing the 11q subtype against both the dic (9; 20) and HeH subtypes can effectively isolate the gene expression changes specifically associated with 11q ALL. Therefore, in this bioinformatics-based comparative differential expression analysis, we will pinpoint genes and pathways uniquely dysregulated in the 11q compared to the other two subtypes, thereby aiding in the identification of novel prognostic markers distinct for 11q-rearranged leukemia, which could improve risk stratification and potentially reveal new therapeutic targets for this high-risk patient group.

Material and Methods

Transcriptomics Analysis of 11q ALL Compared to Subtypes

To identify the dysregulated mRNAs in individuals with 11q ALL compared to dic (9; 20) and HeH, differential expression analysis was performed using GEO2R (www.ncbi.nlm.nih.gov/geo/geo2r). To investigate gene expression changes associated with specific chromosomal translocations in ALL, microarray data from the NCBI GEO dataset GSE47051 were analyzed. Initially, a comparative analysis was conducted between 11q ALL (n=5) and dic (9; 20) ALL (n=6) samples to identify a dysregulated gene signature characteristic of these translocation subtypes. Subsequently, to further elucidate the gene expression profile of 11q ALL, a second comparison was performed between the same 11q ALL samples (n=5) and a larger cohort of HeH ALL individuals (n=18) from the same dataset.

This dataset comprises gene expression patterns in pediatric patients with acute ALL collected utilizing Affymetrix U133 plus 2.0 GeneChips. The samples are collected at diagnosis from patients with B-cell precursor ALL, originating from either bone marrow or peripheral blood, and categorized based on chromosomal abnormalities. The analysis was conducted with threshold values of p-value < 0.05 and logFC <-1.0 and logFC > 1.0. Therefore, the dysregulated genes (DEGs) through this step were used for further analysis.

Furthermore, to identify common upregulated and downregulated DEGs between 11q vs. dic (9; 20) and 11q vs. HeH, we utilized Venny 2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/) webserver. These common DEGs were then utilized further.

Protein-Protein Network-Based Functional Enrichment Analysis

To assess how these DEGs interact with each other and to assess their functional partners. Protein-protein interaction (PPI) analysis of target genes was conducted using the STRING database (https://string-db.org/), which integrates PPI data from text-mining of scientific literature and computational predictions. The PPIs were clustered into ten clusters using the K-means clustering algorithm. Therefore, we utilized the biggest cluster for the functional enrichment analysis of these DEGs using STRING. This tool allows for the identification of Gene Ontology (GO) terms, KEGG pathways, and other functional pathways, and protein-protein interaction networks, providing insights into the biological processes, molecular functions, and cellular components. Finally, the DEGs associated with the ALL were shortlisted for further analysis.

Chromosomal Localization of Dysregulated DEGs

Given the chromosomal translocations involved in ALL, we analyzed the chromosomal locations of the shortlisted DEGs by selecting those with a logFC greater than 2 or less than -2 using the biomaRt library in the R statistical programming language.

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Results

Differential Gene Expression and Functional Insights in 11q ALL Compared to dic (9; 20) and HeH Subtypes

Differential gene expression analysis identified significantly differentially expressed genes in 11q ALL patients when compared to dic (9; 20) ALL individuals (p-value < 0.05). We observed that a total of 341 genes were upregulated and 291 downregulated. These dysregulated biomarkers are illustrated in Figure 1(A). However, for 11q ALL compared to HeH ALL individuals, a total of 441 genes were found to be upregulated while 419 genes were downregulated, illustrated in Figure 1(B). Furthermore, to assess the commonly upregulated and downregulated genes when 11q ALL individuals are compared to both dic(9;20) and HeH, we observed a total of 151 common upregulated while 90 genes were found to be commonly downregulated, shown in Figure 1(C) and Figure 1(D).

Among the commonly upregulated genes, we observed prominently featured several members of the HOXA gene cluster (HOXA3, HOXA4, HOXA5, HOXA7, HOXA10) along with MEIS1, which are well-known master regulators of embryonic development and hematopoiesis, often found dysregulated in various leukemias, driving aberrant differentiation and proliferation.

Furthermore, genes critical for cell cycle progression (CDK6, CCNA1), apoptosis regulation (BCL2L11), and extensive signaling pathways (GNA12, MAP3K5, PRKCE, TNIK, NR3C1, SOCS2-AS1). Additionally, numerous genes involved in cell adhesion, migration, and cytoskeletal organization are present, such as CD44, VCAN, SDC2, FLNA, EZR, PALLD, and MYO1G, suggesting alterations in cell-cell interactions and motility. Genes associated with immune response and inflammation (NLRP3, NOD2, FCN1, FPR1, PTPRC, TMEM173/STING, SERPINB1/B2) are also notably upregulated. Additionally, the signature includes epigenetic modifiers (JARID2, SETD6, CXXC5), metabolic enzymes (FBP1, GPT2, QPRT), cell surface markers (PROM1/CD133), ion channels (KCNE5, P2RX5), and several non-coding RNAs (MBNL1-AS1, FOXP1-IT1, LINC00092) and complex loci (e.g., PCDHG cluster, LOC identifiers), indicating a multifaceted transcriptomic shift.

In contrast, however, among the commonly downregulated genes, we observed that a significant number of these genes are involved in normal B-lymphocyte development, identity, and signaling, including critical factors like BLNK, MS4A1 (encoding CD20), BACH2, ID3, TCL1A, FCMR, LTB, SP140, and the lymphoid-specific enzyme DNTT. Their reduced expression strongly suggests a disruption of the normal B-cell differentiation program and signaling network. Additionally, genes with known potential tumor suppressor functions, such as FHIT, the signaling scaffold and cell cycle regulator AKAP12, and negative regulators like SPRY1 (inhibitor of receptor tyrosine kinase signaling) and ARHGAP21 (Rho GTPase activating protein). Downregulation of these genes could remove crucial brakes on proliferation and survival pathways. Furthermore, several downregulated genes are key players in cell cycle regulation (CDKN3, E2F2), DNA replication (GINS3, POLE2), centrosome biology (PLK4), and DNA damage repair (LIG4, XPA, PARP2), suggesting potential cell cycle dysregulation and increased genomic instability. Reduced expression is also seen for components of important signaling pathways (PIK3CG, PDK1, TIAM1, STIM2), transcription factors (TCF4, SP4, ELK3), adhesion molecules (LAMC1, COL5A1, ECM2), and apoptosis-related genes (DFNA5). The inclusion of several non-coding RNAs (LINC00865, LINC00960, ZNF667-AS1) indicates regulatory alterations at multiple levels.

Protein-Protein Interaction Clustering and Functional Enrichment Analysis in 11q ALL

Through PPI analysis, we identified a total of ten clusters for upregulated and downregulated DEGs. Among these clusters, Cluster 1, the largest with 64 genes, includes key regulators such as MYO1G, PTPRC, CD44, HOXA10, BCL2L11, and NR3C1, suggesting involvement

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in immune signaling, cell adhesion, and transcriptional regulation. Cluster 2 consists of 20 protocadherin gamma subfamily members (PCDHGA7, PCDHGA4, PCDHGB3, etc.), indicating a role in cell-cell adhesion and neural differentiation. Cluster 3, containing FNBP1, FCHSD1, MPP7, and CPNE8, points to cytoskeletal organization and membrane trafficking. Clusters 4 through 10 each consist of two genes with potential functional relationships, such as SLC27A2 and ATP8B4 in lipid metabolism, CENPV and EMB in chromosome organization, and SERPINB1 and F13A1, possibly linked to protease inhibition and coagulation, as shown in Figure 2A and Table I.

Furthermore, functional enrichment analysis of these upregulated genes in 11q ALL reveals key biological processes, molecular functions, and cellular components associated with the disease (Figure 2B. Several genes, including ADAM10, GNA12, CSPG4, EZR, FLNA, SLA, CORO1C, CD44, PTPRC, and PALLD, are linked to focal adhesion (GO:0005925), highlighting their role in cell-extracellular matrix interactions. NR3C1, ADAM10, NOD2, CSPG4, TESK1, EZR, MAP3K5, FLNA, SPRY2, RHOBTB3, BCL2L11, PTPRC, IRGM, and STING1 are involved in protein kinase binding (GO: 0019901), suggesting regulation of signal transduction pathways. Genes such as VCAN, LIPC, NOD2, ADGRE2, NLRP3, FGF9, CD44, and PTPRC participate in glycosaminoglycan binding (GO:0005539), while MYO1G, VCAN, CDK6, GNA12, LIPC, NOD2, PRKCE, ADGRE2, NLRP3, TESK1, MAP3K5, FCN1, RHOBTB3, FGF9, HPSE, CD44, TNIK, FBP1, LPAR4, PTPRC, IRGM, and STING1 are enriched in carbohydrate derivative binding (GO:0097367).

The role of HOXA5, HOXA7, MEIS1, HOXA10, HOXA4, and HOXA3 is reinforced by literature associations, such as PMID: 11840284 linking them to HOX gene expression in AML and PMID: 31681594, which identifies miR-340 as a biomarker for treatment selection in AML. MYO1G, VCAN, SDC2, CSPG4, ADGRE2, TESK1, STARD13, ITGAL, FLNA, GPR183, RHOBTB3, CORO1C, CD44, PTPRC, PALLD, and GREM1 contribute to cell migration (GO: 0016477), a key process in cancer progression. Immune-related pathways include immune response-regulating signaling pathway (GO: 0002764), involving KLRK1, MYO1G, NOD2, PRKCE, KLRC4, NLRP3, FCN1, PTPRC, IRGM, and FPR1, and activation of immune response (GO: 0002253), linked to a similar gene set. Additionally, genes such as NR3C1, ADAM10, CDK6, ITGAL, BCL2L11, CD44, and PTPRC are associated with chronic lymphocytic leukemia cells (BTO: 0001546) and lymphocytic leukemia cells (BTO: 0000744), suggesting their relevance in leukemic pathophysiology.

However, for the commonly downregulated DEGs, we observed that Cluster 1 contains 38 genes, including FCMR, MS4A1, TCL1A, and BLNK. Cluster 2 consists of four genes, GNA11, GNAI1, PIK3CG, and FHIT, associated with G protein alpha subunit signaling. Cluster 3 contains three genes, LAMC1, COL5A1, and MYLK, which are involved in MET activation of PTK2 signaling. Lastly, Cluster 4 consists of three genes, GSDME, WFS1, and JAZF1, shown in Figure 3A and Table II.

Based on cluster 1, the functional enrichment analysis highlighted key biological components, pathways, and disease associations relevant to 11q ALL (Figure 3B). Genes linked to chromosomal functions (GO:0005694 - Chromosome), including POLE2, PARP2, BACH2, SMAD2, PLK4, MIS18A, CDCA2, E2F2, CDK1, TCF4, GINS3, and LIG4, suggest roles in genomic stability and transcription regulation. B-cell activation (KW-0075 - UniProt Keywords) is associated with BLNK and MS4A1, highlighting their significance in immune function and leukemia progression. Key publications provide further insights, with PMID: 33815362 (2021) identifying TCF4, LTB, MME, MS4A1, TCL1A, and CD24 as critical in peripheral B cell subsets and developmental pathways, while PMID: 32383690 (2020) emphasizes the importance of BLNK, DNTT, MME, TCL1A, and CD24 in leukemia through a meta-learning approach. Genes involved in developmental regulation

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(GO: 0050793), such as PARP2, SMAD2, RHOF, APP, TIAM1, E2F2, ID3, CDK1, TCF4, and CD24, play key roles in leukemogenesis and differentiation. The B-lymphocyte cell line association (BTO: 0001522 - TISSUES Database) highlights genes like BLNK, FCMR, XPA, MS4A1, and TCL1A, reinforcing their relevance in B-cell malignancies. Additionally, genes such as POLE2, PARP2, XPA, and LIG4 contribute to DNA repair pathways (WP4946 - WikiPathways), suggesting a role in genomic instability, while SMAD2, APP, PDK1, and CDK1 are implicated in TGF-beta signaling (WP366 - WikiPathways), a pathway involved in cell growth and differentiation.

Chromosomal Distribution of Differentially Expressed Genes in 11q ALL.

Based on the previous analysis, the shortlisted upregulated genes are associated with various cellular processes, notably immune response and cell adhesion, featuring genes such as ADAM10, PTPRC (CD45), CD44, NOD2, and components of the HOXA cluster. Furthermore, genes involved in signal transduction (GNA12, MAP3K5, PRKCE), cytoskeletal organization (FLNA, PALLD, CORO1C, EZR), and inflammation (NLRP3, IRGM, STING1) were also upregulated. In contrast, the downregulated genes were enriched for those involved in DNA replication and repair (POLE2, PARP2, LIG4, XPA), cell cycle regulation (CDK1, CDCA2, E2F2, PLK4), and B-cell related functions (BLNK, MS4A1, TCL1A, CD24). Additionally, genes implicated in TGF-beta signaling (SMAD2, CDK1) and developmental processes (TCF4, SPRY1) were found to be downregulated, shown in Figure 4A.

Furthermore, among the upregulated genes, several are located on chromosome 13, including SPRY2, GPR183, FGF9, and STARD13, while chromosome 5 harbors VCAN, NR3C1, and STING1. Other notable upregulated genes include PRKCE on chromosome 2, FLNA on the X chromosome, and CDK6 and MYO1G on chromosome 7, which are linked to cell proliferation and cytoskeletal regulation. The HOXA cluster genes (HOXA3, HOXA4, HOXA5, HOXA7, HOXA10) are also prominently upregulated on chromosome 7, reinforcing their role in hematopoietic development. Additional upregulated genes include ITGAL on chromosome 16, CSPG4, LIPC, and ADAM10 on chromosome 15, and BCL2L11 and MEIS1 on chromosome 2, which may contribute to cell survival and leukemia pathogenesis, shown in Figure 4B.

Conversely, downregulated genes are clustered across multiple chromosomes, with chromosome 18 featuring SMAD2 and TCF4, which are involved in developmental regulation. Chromosome 21 contains MIS18A, TIAM1, and APP, while chromosome 14 harbors TCL1A, PARP2, and POLE2, all implicated in DNA repair and transcriptional regulation. Other notable downregulated genes include DNTT on chromosome 10, CD24 on chromosome 6, BACH2 on chromosome 6, and PLK4 on chromosome 4, all of which are linked to immune signaling and genomic integrity. The presence of XPA on chromosome 9 and GINS3 on chromosome 16 suggests a disruption in DNA repair mechanisms, while MS4A1 on chromosome 11 and BLNK on chromosome 10 highlight dysregulation in B-cell activation, shown in Figure 4B.

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Table I: PPI-based clustering of upregulated DEGs

Cluster	Count	Genes
1	64	MYO1G, PTPRC, PROM1, CD44, TGFA, FGF9, GREM1, LRRFIP1, SPRY2, TESK1, CSPG4, SDC2, EZR, PALLD, FLNA, CORO1C, RHOBTB3, ZC3H12C, FOSL2, PRL, HOXA10, HOXA3, HOXA4, MEIS1, LMO2, MED13L, CDK6, BCL2L11, MAP3K5, TNIK, GNA12, LPAR4, ADGRE2, PLA2G4A, FAAH, ADGRG5, CCNA1, BAALC, HOXA7, HOXA5, NR3C1, PRKCE, LYZ, FCN1, FPR1, GPR183, NLRP3, IRGM, NOD2, WIPI1, STING1, VCAN, FBP1, STARD13, LGALS1, HPSE, APBA1, ADAM10, KLRK1, KLRC4, ITGAL, LIPC, VLDLR, SLA
2	20	PCDHGA7, PCDHGA4, PCDHGB3, PCDHGB5, PCDHGB1, PCDHGA9, PCDHGA5, PCDHGA1, PCDHGC3, PCDHGA3, PCDHGA2, PCDHGB2, PCDHGA6, PCDHGA8, PCDHGA10, PCDHGB6, PCDHGB4, PCDHGB7, PCDHGA11, PCDHGA12
3	4	FNBP1, FCHSD1, MPP7, CPNE8
4	2	SLC27A2, ATP8B4
5	2	CENPV, EMB
6	2	PCDHGC4, PCDHGC5
7	2	SERPINB1, F13A1
8	2	QPRT, BOLA2
9	2	SKIDA1, JARID2
10	2	B3GALNT1, GPT2

Table II: PPI-based clustering of downregulated DEGs

Cluster	**Count**	**Genes**
1	38	FCMR, MS4A1, TCL1A, BLNK, DNTT, MME, CD24, TCF4, CDK1, RACGAP1, E2F2, ID3, BACH2, CD2AP, APP, CDKN3, CDCA2, PLK4, DEPDC1B, RHOF, AKAP12, EGLN1, PDK1, ARHGAP21, TIAM1, PARD3, POLE2, GINS3, USP28, MIS18A, SPRY1, SMAD2, NT5E, LIG4, XPA, PARP2, LTB, LST1
2	4	GNA11, GNAI1, PIK3CG, FHIT
3	3	LAMC1, COL5A1, MYLK
4	3	GSDME, WFS1, JAZF1

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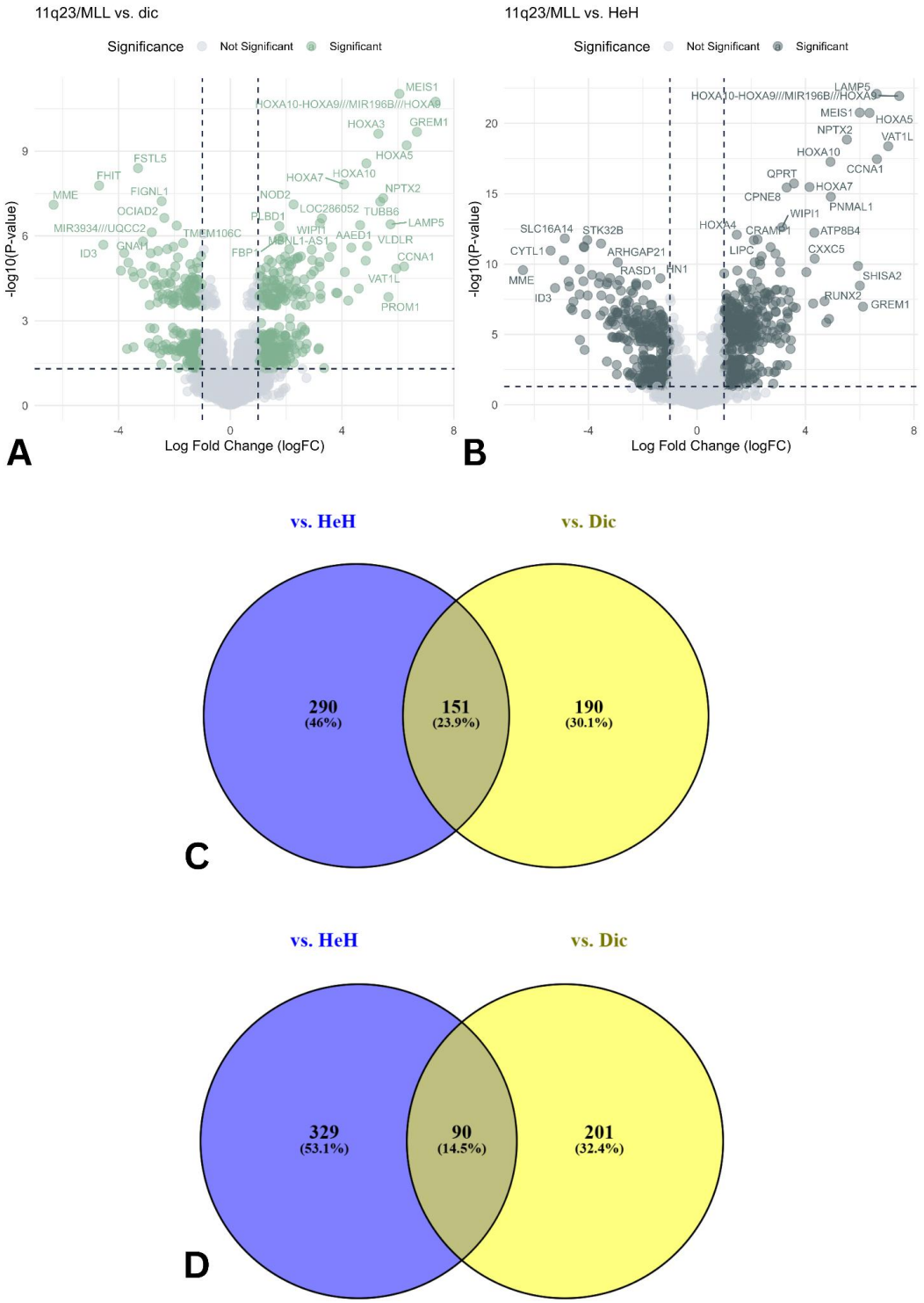

Figure 1. Illustrates the DEGs in 11q ALL compared to dic(9;20) ALL and HeH ALL individuals. Figure 1(A) shows the number of upregulated and downregulated genes in 11q ALL compared to dic(9;20) ALL. Figure 1(B) shows the number of upregulated and downregulated genes in 11q ALL compared to HeH ALL. Figure 1(C) and Figure 1(D) show the commonly upregulated and downregulated genes when 11q ALL individuals are compared to both dic(9;20) and HeH.

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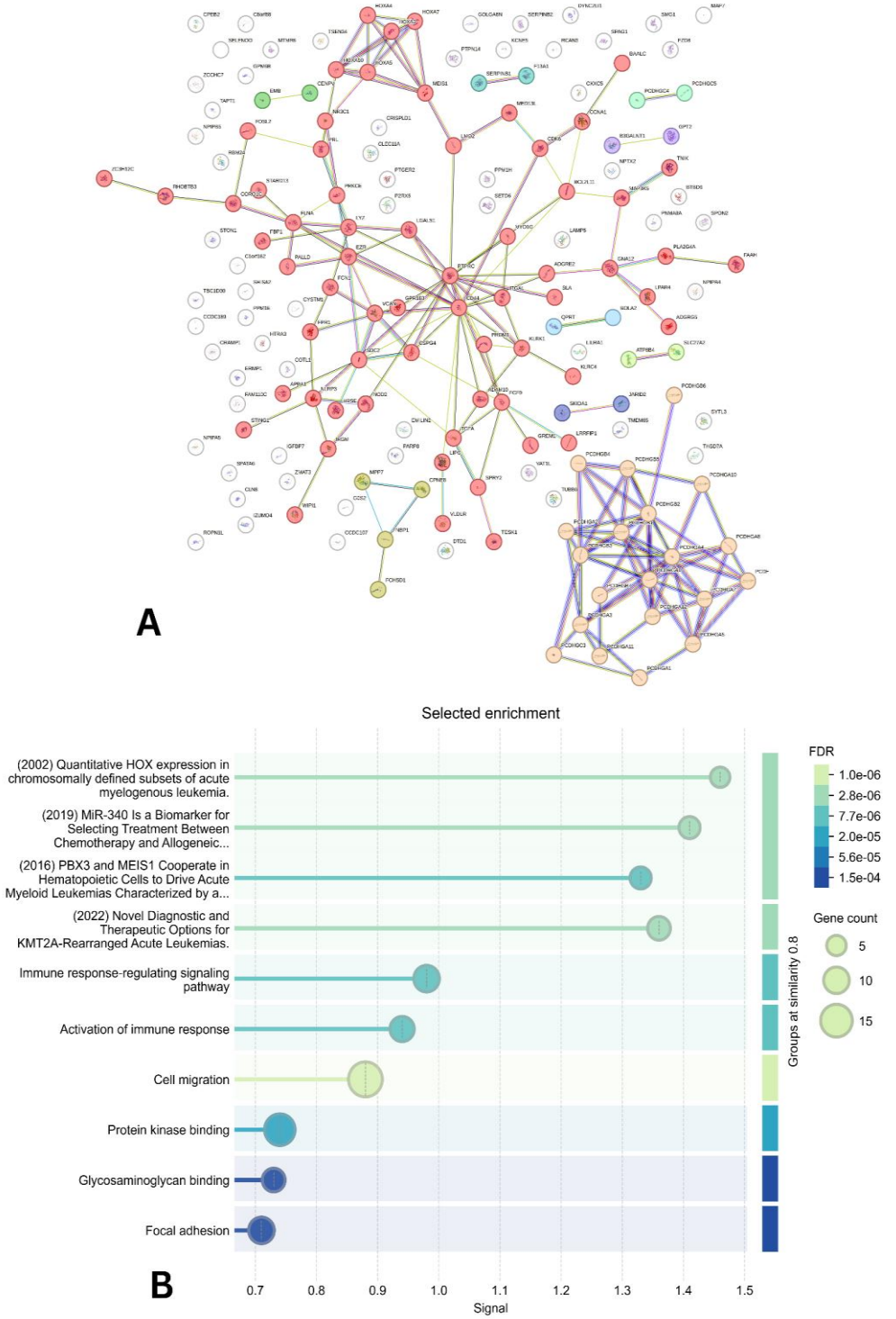

Figure 2. Shows the PPI-based clustering of upregulated DEGs (A) and the functional enrichment analysis of these upregulated genes in 11q ALL (B)

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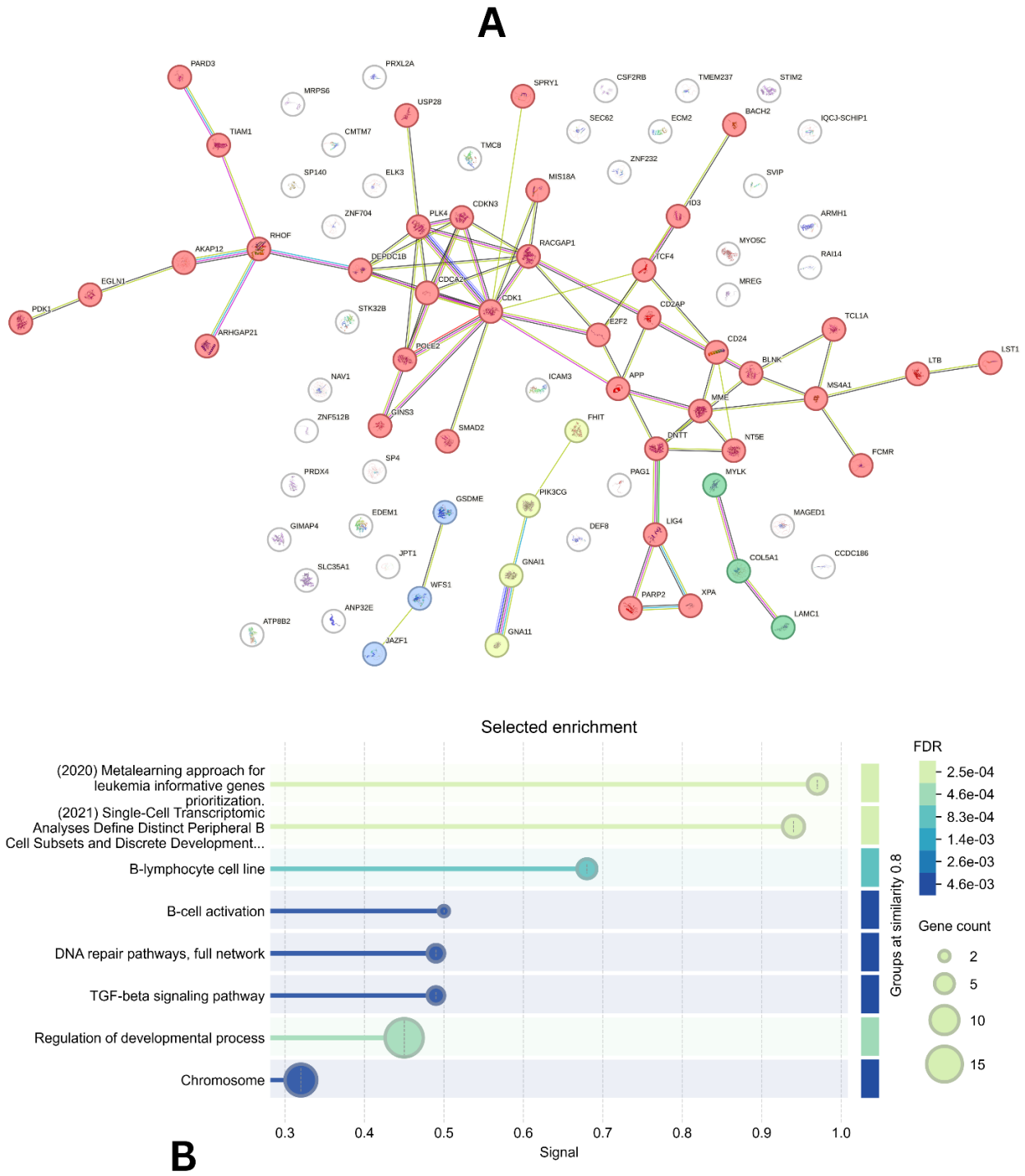

Figure 3. Depicts the PPI-based clustering of downregulated DEGs (A) and the functional enrichment analysis highlighting key biological components, pathways, and disease associations relevant to 11q ALL based on Cluster 1 (B)

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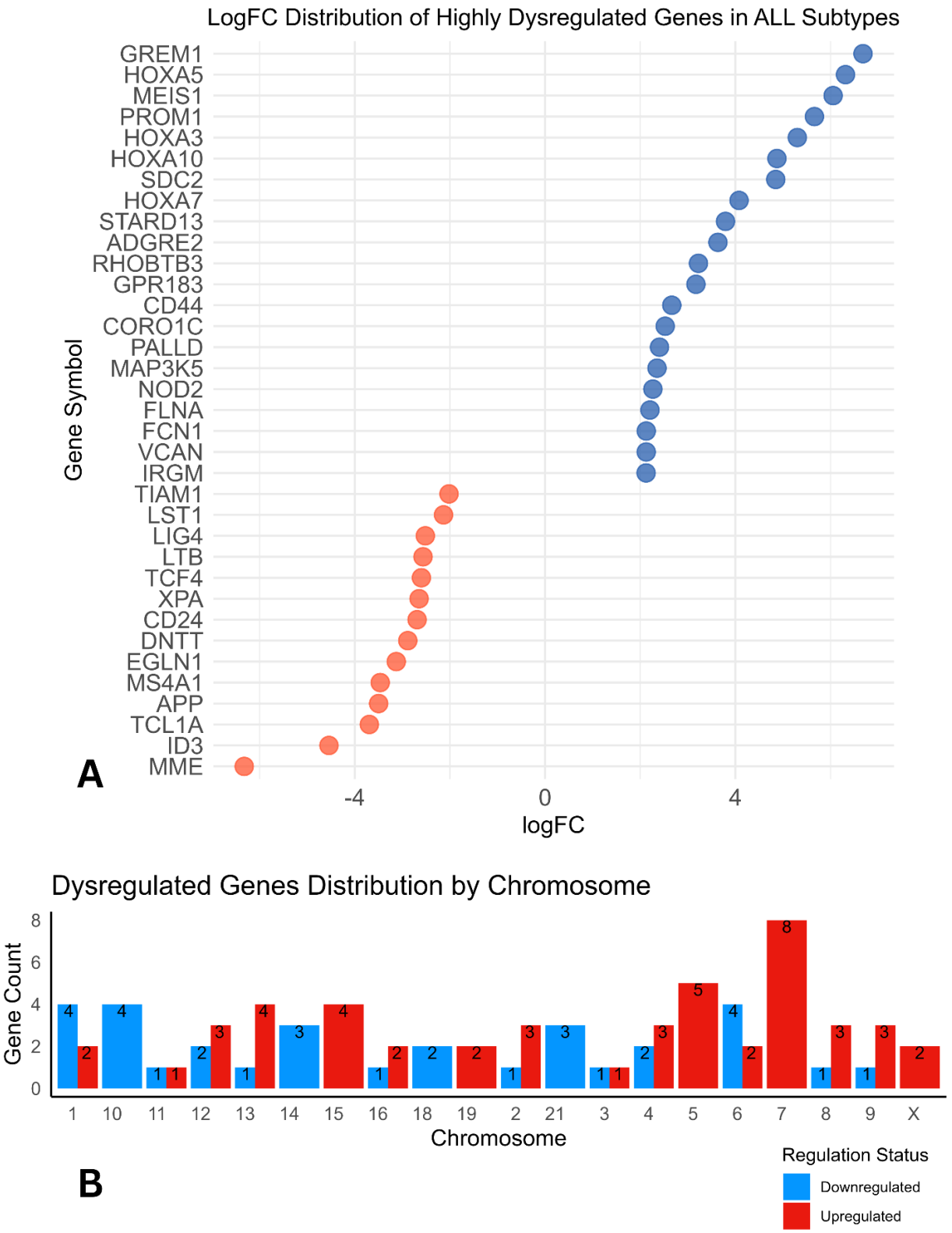

Figure 4. Shows the chromosomal distribution of differentially expressed genes in 11q ALL (A) and lists some notable upregulated and downregulated genes with their chromosomal locations (B)

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Discussion

This study aimed to elucidate the unique transcriptomic signature of 11q-rearranged ALL by comparing its gene expression profile with those of dic (9; 20) and HeH subtypes. Through differential expression analysis, we identified a distinct set of commonly dysregulated genes in 11q ALL, providing insights into the molecular mechanisms underlying its aggressive nature.

Our analysis revealed a significant number of DEGs in 11q ALL when compared to both dic(9;20) and HeH subtypes. Notably, 151 genes were commonly upregulated, and 90 genes were commonly downregulated, suggesting a robust and specific transcriptomic rewiring in 11q ALL. Previously, researchers have identified several novel B-ALL subtypes through detailed gene expression analyses, uncovering distinct molecular signatures that were previously undetectable by standard cytogenetic techniques. DUX4-rearranged ALL, characterized by DUX4 overexpression and ERG deletions, often has a favorable prognosis. BCR-ABL1-like (Ph-like) ALL mimics BCR-ABL1-positive ALL but lacks the fusion. ETV6-RUNX1-like ALL resembles ETV6-RUNX1-positive ALL with alternate ETV6 and IKZF1 alterations. ZNF384-rearranged ALL shows immature myeloid lineage markers and JAK-STAT pathway activation. MEF2D-rearranged ALL exhibits MEF2D target deregulation, with MEF2D-CSF1R cases resembling BCR-ABL1-like ALL. PAX5-altered (PAX5alt) ALL encompasses diverse PAX5 alterations, and the PAX5 P80R mutation defines a distinct subtype. The IKZF1 N159Y mutation also marks a subtype. BCL2/MYC rearranged ALL resembles "double-hit" lymphoma. Hypodiploid ALL, including near-haploid and low hypodiploid cases, shows unique gene expression and genetic alterations (3, 6, 10–15).

Among the upregulated genes, we observed a prominent enrichment of the HOXA gene cluster (HOXA3, HOXA4, HOXA5, HOXA7, HOXA10) and MEIS1. These genes are pivotal in hematopoiesis and embryonic development, and their overexpression is frequently associated with poor prognosis in leukemia. For example, studies have shown that high MEIS1 expression correlates with unfavorable outcomes in KMT2A-rearranged leukemia, supporting our findings. HOXA genes (HOXA7, HOXA9, HOXA10) and MEIS1 are consistently upregulated in KMT2A-rearranged leukemias (ALL, AML), driven by fusions like MLL-AF4 and KMT2A-MLLT3. These genes are crucial for leukemic development and maintenance, with HOXA9 and MEIS1 often acting together. Studies show overexpression induces leukemia, while knockdown impairs leukemic cell function (proliferation, engraftment, migration). Specifically, HOXA9 knockdown downregulates other HOXA genes and MEIS1, unlike knockdown of HOXA7, HOXA10, or MEIS1, indicating distinct roles. These genes are promising therapeutic targets, with efforts focused on disrupting MEIS1 interactions (16, 17).

Additionally, the upregulation of genes involved in cell cycle progression (CDK6, CCNA1), apoptosis regulation (BCL2L11), and signaling pathways (GNA12, MAP3K5, PRKCE, TNIK, NR3C1) indicates enhanced proliferation and survival mechanisms in 11q ALL. Previously, it has been noted that acute myeloid leukemia features uncontrolled immature myeloid cell proliferation and a differentiation block. This stems from disrupted cell cycle regulation (hyperactive Cdks, inactive inhibitors like p53) and apoptosis resistance (defective G2/M checkpoints, oncogenic signaling). Key deregulated pathways include Flt3, c-Kit, Jak2, and CXCR4, impacting proliferation and survival. Therapies targeting these pathways (TKIs, cell cycle inhibitors, p53 reactivation, CXCR4 disruption) show promise, improving AML treatment by exploiting its vulnerabilities (18).

Furthermore, we observed increased expression of genes related to cell adhesion, migration, and cytoskeletal organization (CD44, VCAN, SDC2, FLNA, EZR, PALLD, MYO1G). These findings suggest that 11q ALL cells may exhibit enhanced migratory and invasive capabilities, contributing to the

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observed higher risk of central nervous system (CNS) involvement and relapse. Leukemia metastasis and central nervous system (CNS) infiltration are significantly influenced by alterations in genes governing cell adhesion and migration. Specifically, chemokine signaling pathways, particularly the CXCR4/CXCL12 axis, play a critical role in promoting leukemic cell proliferation, survival, and migration, with high CXCR4 expression correlating with poor prognosis. Additionally, changes in cell adhesion molecules, such as the upregulation of CD44, VCAN, and SDC2, and the downregulation of LAMC1 and COL5A1, contribute to enhanced migratory and invasive properties of leukemia cells. KMT2A-rearranged ALL exhibits a heightened risk of CNS involvement, suggesting that specific genetic alterations predispose leukemic cells to infiltrate the central nervous system. Furthermore, DUX4-rearranged leukemia demonstrates relapse-specific gene signatures highlighting chemotaxis and cytokine environment changes, with DUX4 overexpression potentially increasing chemotaxis via CXCR4 and lineage switching affecting adhesion and migration (6).

The upregulation of immune response and inflammation-related genes (NLRP3, NOD2, FCN1, FPR1, PTPRC, TMEM173/STING, SERPINB1/B2) suggests a complex interplay between leukemic cells and the immune microenvironment. It has been shown that leukemia uses inflammation to evade immune attack. AML, ALL, and MDS show similar patterns where immunosuppressive cytokines (IL-10, TGF-β) hinder anti-tumor cells, while pro-inflammatory cytokines (IL-1β, IL-6, TNF-α) promote leukemia growth. IDO1, MDSCs, and M2 macrophages further suppress immunity. Leukemic cells also induce immune checkpoint ligands, exhausting T cells. Essentially, leukemia manipulates inflammation to create an immunosuppressive environment, enabling disease progression (19–22). Finally, the dysregulation of epigenetic modifiers (JARID2, SETD6, CXXC5) and metabolic enzymes (FBP1, GPT2, QPRT) indicates that 11q ALL may also exhibit alterations in chromatin remodeling and metabolic pathways.

In contrast, the commonly downregulated genes in 11q ALL are primarily involved in normal B-lymphocyte development and signaling (BLNK, MS4A1, BACH2, ID3, TCL1A, FCMR, LTB, SP140, DNTT). The reduced expression of these genes suggests a profound disruption of the normal B-cell differentiation program, potentially contributing to the immature phenotype and aggressive behavior of 11q ALL. B-cell development relies on precise gene regulation, with HDACs playing key roles. HDAC1/2 deletion blocks early B-cell development via p21/p57 and impairs mature B-cell proliferation. Blimp-1 recruits HDAC1/2 to repress c-myc, while Ikaros-HDAC1 repression of KDM58 is disrupted in B-ALL. HDAC3 is crucial for VDJ recombination and STAT5a regulation, while Bach2-HDAC3 represses plasma cell differentiation. In B-cell malignancies, HDAC dysfunction drives leukemia and lymphoma. HDAC9-BCL6 promotes lymphomagenesis, while HDAC4 suppresses it, countered by miR-155. HDAC7 regulates lineage commitment, and HDAC6 affects immunogenicity via PD-L1. Targeting HDAC-transcription factor interactions holds therapeutic potential, but drug resistance remains a challenge (23, 24).

Additionally, the downregulation of potential tumor suppressor genes (FHIT, AKAP12, SPRY1, ARHGAP21) and genes involved in cell cycle regulation (CDKN3, E2F2), DNA replication (GINS3, POLE2), and DNA damage repair (LIG4, XPA, PARP2) may contribute to increased genomic instability and uncontrolled proliferation. Several key genes in B-cell development also function as tumor suppressors in leukemia (22).

PPI analysis and functional enrichment revealed the interconnectedness of DEGs in 11q ALL. Upregulated genes, including MYO1G, PTPRC, CD44, HOXA10, BCL2L11, and NR3C1, play roles in immune signaling, adhesion, and transcription. Enrichment analysis linked them to focal adhesion, kinase binding, glycosaminoglycan binding, and migration.

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Downregulated genes, such as FCMR, MS4A1, TCL1A, and BLNK, indicate disrupted B-cell functions. Functional enrichment highlighted roles in chromosomal integrity, B-cell activation, development, and DNA repair.

The chromosomal localization of the DEGs revealed that several upregulated genes are located on chromosomes 13, 5, 2, 7, 15, and 16, while downregulated genes are distributed across various chromosomes, including 18, 21, 14, 10, 6, 4, and 9. This widespread chromosomal distribution reflects the complex genomic alterations associated with 11q ALL. Chromosomal abnormalities play a crucial role in both ALL and chronic lymphocytic leukemia (CLL), influencing their pathophysiology, prognosis, and treatment. In ALL, abnormalities are detected in a high percentage of cases, ranging from numerical changes like hyperdiploidy and hypodiploidy to structural alterations such as translocations, with specific translocations like t(9;22) (Philadelphia chromosome) and t(4;11) carrying significant prognostic implications. These abnormalities, along with gene mutations like IKZF1, guide risk-adapted therapy. In CLL, chromosomal aberrations, particularly deletions of 13q14, 11q23, and 17p13, and trisomy 12, are essential for risk stratification and treatment decisions. The deletion of 17p13 and TP53 mutations mark high-risk disease, necessitating alternative therapies. Clonal evolution, influenced by genetic abnormalities and treatment, further complicates CLL management (25–29). The presence of upregulated HOXA cluster genes on chromosome 7 and downregulated genes involved in DNA repair and B-cell development across multiple chromosomes further underscores the significant impact of chromosomal translocations on the transcriptomic landscape of 11q ALL.

Our findings provide a comprehensive overview of the unique transcriptomic signature of 11q ALL, highlighting potential prognostic markers and therapeutic targets. The identified DEGs, particularly those involved in cell signaling, adhesion, and DNA repair, could serve as biomarkers for risk stratification and treatment selection. Future studies should validate these findings in larger cohorts and explore the functional roles of the identified DEGs in 11q ALL pathogenesis. Additionally, investigating the potential of targeting these dysregulated genes with novel therapeutic strategies could improve outcomes for patients with this high-risk subtype. By providing a deeper understanding of the molecular underpinnings of 11q ALL compared to other subtypes of ALL, this study contributes to the ongoing efforts to improve risk assessment and develop more effective treatments for pediatric ALL.

Conclusion

The comparative transcriptomic analysis successfully identified a unique gene expression signature for the aggressive 11q-rearranged pediatric ALL subtype. Key findings include the upregulation of genes like the HOXA cluster and MEIS1, known to be associated with poor prognosis, and the downregulation of genes involved in normal B-lymphocyte development and signaling. These findings provide insights into the molecular mechanisms underlying the aggressive nature of 11q ALL and could lead to the identification of potential biomarkers and therapeutic targets for this high-risk patient group.

Availability of Data

All data are available in article body

Ethical Considerations

UOA-SCI-EC-2025/014

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ChatGPT (OpenAI, GPT-5.1 model) was used to refine language and improve readability. No AI tool was used for data analysis or scientific interpretation.

Authors' Contributions

Haneen Abdulrahman Shaban:Collected and organized the raw microarray dataset (GSE47051),Performed data preprocessing and

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initial GEO2R differential expression analyses.

Waleed Nazal Hosi: Conducted the bioinformatics pipeline including DEG filtering, Venn analysis, STRING PPI clustering, and functional enrichment.

Semaa Abdulrahman Shaban: Contributed to literature review and background writing, particularly the introduction of ALL subtypes, transcriptomic relevance, and molecular mechanisms.

Ahmed AbdulJabbar Suleiman: Conceived and designed the study framework and analytical strategy.Supervised data analysis, interpretation, and manuscript preparation.Wrote and finalized the Discussion, Conclusion, and overall manuscript structure.

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Conflict of Interest

The Authors declare that there is no conflict.

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