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In silico identification and verification of deleterious gene mutations in B-cell acute lymphoblastic leukemia

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Abstract

The gene is a well-known regulator of the development of lymphocytes, and genetic variation in this gene has been found repeatedly to be associated with leukemogenesis and poor prognosis in B-cell ALL. Determining the neutral and functionally deleterious single nucleotide substitutions within this gene is an important step towards the use of variants in this gene as diagnostic and/or prognosis markers. This was a non-experimental, descriptive, and analytical in silico study; it did not involve wet-laboratory experiments or clinical trials. Transcript variants of the gene were retrieved from the NCBI Nucleotide database using the Homo sapiens and RefSeq filters. FASTA sequences were analyzed using BLASTx to identify amino acid substitutions. Mutations were designated based on position and residue change (e.g., R83P). Each identified mutation was analyzed with the following tools PolyPhen-2: Predicts potential impact on protein structure/function. SIFT: Predicts whether amino acid changes affect protein function. MuPRO and I-Mutant: Predict stability changes post-mutation. PhD-SNP: Assesses disease association. Study was a computational/databased study and not performed at a clinical site. The study was conducted without any human or animal subjects, and only reference gene sequences were used that are publicly available. Predicted functional effect (benign/neutral versus damaging/deleterious) and predicted stability change of each identified amino acid substitution, output by PolyPhen-2, SIFT, MuPro, I-Mutant, and PhD-SNP. Eight amino acid substitutions were detected and confirmed: V20L, E5D, I33V, P19T, R83P, D22N, Q11H and K75E. The majority of substitutions (V20L, E5D, I33V, and P19T) were predicted to be neutral, benign, and tolerated with little effect on protein function, though several predicted a decrease in protein stability. R83P, Q11H, K75E, and D22N were identified by several tools as potentially or probably damaging and have been flagged multiple times. The majority of the identified single-nucleotide polymorphisms in were predicted to be benign despite being associated with the presence of changes in the protein's stability, while the four polymorphisms (R83P, D22N, Q11H and K75E) were consistently predicted as deleterious and could affect protein function and disease progression in B-cell acute lymphoblastic leukemia. These results emphasize the relevance of alterations for leukemogenesis and resistance to therapy, and suggest that these variants are potential diagnostic and/or prognostic markers which should be further validated in experimental studies. This was an in silico bioinformatics analysis, not a clinical trial, and was not eligible for trial registration.

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Introduction

Leukemia is the most frequent childhood cancer in Pakistan. Globally, about 3 out of every 100,000 people are diagnosed with ALL each year, but it is highly treatable when managed appropriately, but access to such care remains limited. Acute lymphoblastic leukemia (ALL) is a heterogeneous condition that results from the abnormal transformation and unchecked growth of lymphoblasts within the bone marrow, lymph nodes, blood and thymus. [1,2] Even though the exact origins of ALL remain unclear, it is believed that both inherited genetic predisposition and external environmental influences play a role in its onset. [1,3] In recent years, many genome-wide association studies (GWAS) have revealed that inherited genetic variations are important markers to assess susceptibility to acute lymphoblastic leukemia (ALL), and to predict patient response and relapse risk in children.[1] Single-nucleotide polymorphisms (SNPs) are the most common type of germline difference and are generally employed to understand the genetic diversity of different populations. These variations have the potential to cause alterations in the DNA sequence, occurring within coding regions (exons), noncoding regions (introns), or between genes (intergenic regions). [1,4-6] The existence of SNPs in the vicinity of gene or its regulatory elements can potentially interfere with the gene's normal bodily processes. According to the study revealed from genome-wide association studies (GWAS), frequent genetic variations in certain genes associated with B-cell regulation (such as CEBPE, , and ARID5B) and cell cycle control (such as CDKN2A) may influence the likeliness of developing acute lymphoblastic leukemia (ALL) in children [1]. The gene (Ikaros family zinc finger 1), which plays essential roles in both T- and B-cell lineage development, has been linked to leukemogenesis. Deletions of are frequently found in ALL in childhood, and the frequency of these abnormalities differs depending on the prognostic value of the specific cytogenetic subgroups. B-cell precursor ALL (BCP-ALL) is the most common leukemia in children. It involves uncontrolled growth of early B lymphocytes in the bone marrow. [7,8] An independent cohort confirmed associations of genomic plus and exon 4-7 deletions with adverse outcomes.[9-11]
Loss-of-function mutations, including those caused by single nucleotide polymorphisms (SNPs), can interrupt this process, leading to high risk of acute lymphoblastic leukaemia (ALL) Although current treatments have reformed survival rates to nearly 90%, reoccurrence remains a serious problem, largely due to the recurrent cancer cells becoming resistive to chemotherapy. [7] Though advancements in treatment, acute leukaemia remains a second prominent cause of death due to malignancies.Germline loss-of-function variants in , IKZF2, and IKZF3 (heterozygous) cause IKAROS, HELIOS, and AIOLOS deficiencies, leading to inborn errors of immunity (IEI). [12,13] ALL is believed to occur more in males, who are also known to have a worse forecast or prognosis. [14] Several studies have identified deletions as an sovereign indicator of poor prognosis in ALL, inconsiderate of patient age or cytogenetic subtype, and have associated them to high likelihood of relapse and symbolically decreased survival rates Therefore, Early identification of gene deletions and identification and prediction of SNPs in B-ALL may enlighten the enhancement of therapy and exploration of other potential treatment strategies to rectify outcomes in this increased-risk leukemia.[15,16] Several studies have identified deletions as an sovereign indicator of poor prognosis in ALL, inconsiderate of patient age or cytogenetic subtype, and have associated them to high likelihood of relapse and symbolically decreased survival rates [17]. Therefore, Early identification of gene deletions and identification and prediction of SNPs in B-ALL may enlighten the enhancement of therapy and exploration of other potential treatment strategies to rectify outcomes in this increased-risk leukemia.[12]

Variation in the gene are casually perceived in B-cell precursor acute lymphoblastic leukemia (BCP-ALL), occurring in nearly 15% of pediatric cases. In adults, prevalence is between 30–50% and is higher in certain high-risk subtypes. Furthermore, mutations in the gene occur in up to 84% of Philadelphia chromosome-positive (Ph+) ALL cases and approximately 70% of Philadelphia-like (Ph-like) ALL.[18]

According to research conducted by a continuous cohort of 1,427 children diagnosed with BCP-ALL, they investigated the nature and prevalence of CNVs by using Multiplex Ligation-dependent Probe Amplification (MLPA). Their findings showed the diversity of CNVs, highlighting variability in deletions involving PAX5 and, along with the frequent occurrence of RB1 deletions. A higher burden of CNVs, especially involving and CDKN2A/B deletions, was found to be more common in patients classified as high-risk by National Cancer Institute (NCI) criteria. Furthermore, deletions and CRLF2 rearrangements in patients without a clearly defined karyotype may be indicative of the high-risk BCR-ABL1-like subgroup. Individuals were found to be predisposed to leukemia by rare germline missense and nonsense variants.[19,20] Ou *et al* 2016 used array comparative genomic hybridization (aCGH) analysis to identify a common genetic variation in PAX5, and CDKN2A/B in patients with B-cell acute lymphoblastic leukemia (B-Cell ALL). In addition, affected people often have variations in the gene in addition to fusions of the BCR-ABL gene.[2,21,22] Moreover, specific sequence mutations in gene have been discovered in ALL cases, which are anticipated to interrupt normal IKAROS function or generate a new dominant-negative variant, such as the G158S

isoform.[23,24] Hence, a comparative investigation of alterations is necessary in both laboratory-based and clinical environments, involving a larger patient cohort, to clarify the potential impact of different gene mutation types including SNPs[17 25]. The aim of this study was to understand and confirm changes and mutations in the gene that could be linked to the B-Cell Acute Lymphoblastic Leukemia (B-Cell ALL) disease through computational methods. This study was not of a wet lab nature or clinical.

Material and Methods

This was a non-experimental, descriptive and analytical study, undertaken completely in *silico* (i.e. without any wet laboratory experiments or clinical trials). The objective was to identify and verify alterations and mutations in the gene that may be associated with B-cell acute lymphoblastic leukemia (B-ALL) using computational tools. The acquired genetic sequence data of was retrieved from public database, and bioinformatics tools were utilized for sequence similarity analysis, mutation identification and functional analysis.

The sequences of the transcript variants of the gene were obtained from the NCBI Nucleotide database, using the filter Homo sapiens and the filter RefSeq. Five transcript variations that fulfilled the above criteria were retrieved. The translated query sequences were aligned to known protein sequences by NCBI BLASTx and the substitutions of amino acids were determined. Each substitution identified was analyzed using 5 in *silico* tools: PolyPhen-2, SIFT, MuPro, I-Mutant and PhD-SNP, to evaluate the potential impact of each substitution on protein function, structure and stability. Combined with each other, these tools enabled a comprehensive evaluation of each variant's predicted pathogenicity, structural consequences and potential contribution to leukemogenesis.

Research Population

In *silico* data derived from publicly available genetic sequences of the gene from NCBI nucleotide, particularly 5 transcript variants and analyzed using bioinformatics software to identify and verify mutations relevant to B-cell Acute Lymphoblastic Leukemia.

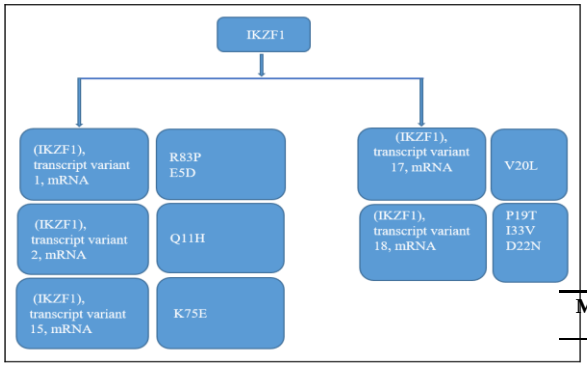


Figure 1: transcript variants with the selected single-nucleotide polymorphisms (SNPs) analyzed in this study.

Research Tools

A range of bioinformatics tools and databases were utilized to conduct this in silico analysis of the gene:

- NCBI Nucleotide Database was used to retrieve the reference nucleotide sequences of the human gene, including its various transcript variants.
- NCBI BLASTX was employed to align nucleotide sequences with protein databases to detect possible variations and mutations by comparing the translated query sequence with known protein sequences.
- PolyPhen-2, SIFT, MuPRO, I-Mutant , PhD-SNP were used to verify mutations.

These tools collectively enabled the identification, verification, and functional assessment of SNPs relevant to B-Cell Acute Lymphoblastic Leukemia (B-ALL).

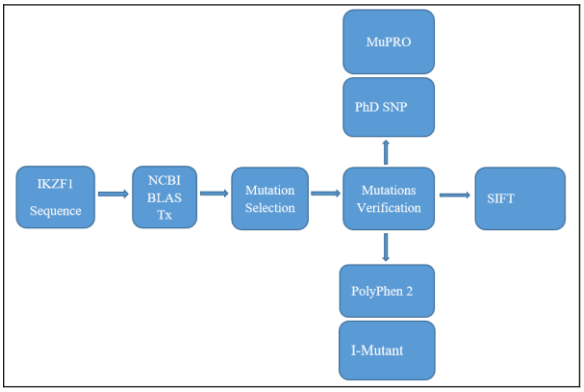


Figure 2: Overall workflow of the in silico analysis, from sequence retrieval through mutation verification.

RESULTS

Five transcript variants were identified and eight amino acid changes were confirmed with all five prediction tools. Table 1 summarizes the predictions returned by each of the five programs, which are discussed below and presented in more detail in Tables 2-6; each program is listed by the number of mutations it predicted to be damaging.Each of the five programs is presented by the number of mutations it predicted to be damaging in Table 1, and is described in the Discussion; the predictions are presented in more detail in Tables 2-6.

Table 1: Summary of predicted effects of all eight identified mutations across the five bioinformatics tools used in this study.

Mutation	PolyPhen-2	MuPro	SIFT	PhD-SNP	I-Mutant

V20L	Benign	Increased stability	Tolerated	Neutral	Decreased stability
R83P	Probably damaging	Decreased stability	Tolerated	Disease	Decreased stability
E5D	Benign	Decreased stability	Not tolerated	Neutral	Decreased stability
P19T	Benign	Decreased stability	Not tolerated	Neutral	Decreased stability
I33V	Benign	Increased stability	Tolerated	Neutral	Decreased stability
D22N	Probably damaging	Decreased stability	Not tolerated	Neutral	Decreased stability
Q11H		Possibly damaging	Decreased stability	Not tolerated	Neutral	Decreased stability
K75E		Probably damaging	Decreased stability	Not tolerated	Neutral	Decreased stability

Note: All 5 transcript variants of Homo sapiens mRNA (RefSeq) included the mutation(s); the specific transcript variant containing each mutation is listed in the Discussion.

Discussion

Query sequence was retrieved from NCBI *nucleotide* database, after applying filters of HomoSapian and RefSec ,185 results of, Sequences of Different Variants of Gene appeared. FASTA sequences of 4 variants of were copied and run on NCBI *blast X*, Allignments appeared having mutations. These mutations were named as Original AA, Position, Replacing AA e.g. V20L, which mean V at position 20 is replaced by L.
8 Mutations were selected and verified,

V20L was predicted as benign and neutral by all Tools, while I-Mutant predicted it as Decrease Stability

R83P was predicted as damaging by Polyphen, MUpro, and I-Mutant and PhD SNP, while SIFT and predicted it as neutral

E5D was predicted as neutral by PolyPhen and PhDSNP, while I-Mutant and MuPro and SIFT predicted it as Decreased protein stability

P19T was predicted as neutral by PolyPhen and PhDSNP, while MuPro, SIFT and I-Mutant predicted it as Damaging
I33V was predicted as neutral by PolyPhen, MuPro, SIFT and PhDSNP, while I-Mutant predicted it as Decreasing Stability of protein structure

D22N was predicted as damaging by all software

Q11H was predicted as damaging by PolyPhen, MUPRO, SIFT and I-Mutant, while PhDSNP predicted it as neutral

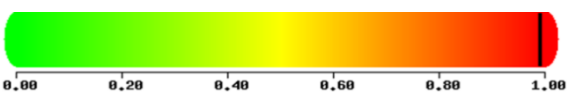
K75E was predicted as probably damaging and Not Tolerated by PolyPhen 2, SIFT, I-Mutant, and MUPRO, while PhDSNP predicted it as neutral.

Polyphen 2
Polymorphism Phenotyping version 2 (PolyPhen-2) is a widely-used tool for predicting the possible effect an Amino Acid Substitution (missense mutation) may have on the structure and function of a human protein, used in genomic studies to classify variants as benign, possibly damaging, or probably damaging. The scoring for polyPhen-2 falls between 0.0 and 1.0, with scores below about 0.15 considered to be benign, scores around 0.15 to 0.85 as possibly damaging, and scores above about 0.85 as probably damaging. Four of the eight mutations were found to be benign, two were probably damaging, and two were possibly/probably damaging (Table 2) with R83P (score 1.000), D22N (score 0.990; sensitivity 0.72, specificity 0.97), Q11H (score 0.771), and K75E (score 1.000) (Figure 3).

Table 2: shows results for all mutations by PolyPhen 2

Mutation	PolyPhen2 Score	PolyPhen 2 Sensitivity	PolyPhen 2 Specificity	Results
V20L	0.320	0.90	0.89	Benign
R83P	1.000	0.00	1.00	Probably Damaging
E5D	0.001	0.99	0.15	Benign
P19T	0.294	0.91	0.89	Benign
I33V	0.000	1.00	0.00	Benign
D22N	0.990	0.72	0.97	Probably Damaging
Q11H	0.771	0.85	0.92	Possibly Damaging
K75E	1.000	0.00	1.00	Probably Damaging

Figure 3: PolyPhen-2 output for the D22N substitution, predicted as probably damaging (score = 0.990; sensitivity = 0.72; specificity = 0.97).



MUPRO

MuPro is a computational tool used to predict the impact of single-point mutations on protein stability, reporting whether a substitution is likely to increase (positive) or decrease (negative) stability. MuPro predicted that seven of the eight mutations would destabilize the protein, whereas V20L and I33V would be stabilizing (Table 3).

Table 3: shows results for all mutations by MuPro

Mutation	Detal delta G	Method 1 Confidence Score	Method 2 Confidence score	Results
V20L	-0.48038602	-0.48835149	-0.82675714915367	Increase Stability of protein structure
R83P	-1.8916144	-1	-0.983249323674079	Decreased Stability of protein structure
E5D	-0.73908893	0.23018434	-0.555640745636039	Decrease Stability
P19T	-1.0114128	-0.56818537	-0.806122717793805	Decrease stability
I33V	-0.41160257	-0.31540826	-0.545404521570468	Increase stability of protein structure
D22N	-0.99938927	0.40155094	0.546728120828241 1	Decrease stability of protein structure
Q11H	-0.85977276	- 0.092561046	-0.092561046	Decrease stability of protein structure
K75E	-0.65266837	- 0.000625763	0.599603423989741 9	Decrease stability of protein structure

Sorting Intolerant From Tolerant

Sorting Intolerant From Tolerant (SIFT) is a computational tool predicting whether an amino acid substitution in a protein will affect its biological function. It is widely used for evaluating the functional consequences of nonsynonymous single-nucleotide polymorphisms (nsSNPs). SIFT uses sequence homology and the physical properties of amino acids to assess the impact of a mutation. It compares the mutated protein sequence against a database of similar sequences (multiple sequence alignment) to assess how conserved the affected amino acid is across species. Highly conserved positions are more likely to be functionally important, and mutations at these positions are more likely to be deleterious. SIFT Score Range Prediction Meaning < 0.05 is predicted as deleterious, and the mutation is likely to affect protein function. > 0.05 – 1.00 is predicted as tolerated and means the mutation is likely to be functionally neutral. According to SIFT, 3 SNPs were predicted to be Tolerated, while 5 were predicted to Affect Protein Function.

Table 4: shows results for all mutations by SIFT

Mutation	Score	Median Sequence Conservation	Results
V20L	0.41	4.32	Tolerated
R83P	0.25	3.27	Tolerated
E5D	0.00	4.32	Affect Protein Function
P19T	0.00	4.32	Affect Protein Function
I33V	0.83	4.32	Tolerated
D22N	0.00	4.32	Affect Protein Function
Q11H	0.00	4.32	Affect Protein Fuction
K75E	0.00	4.32	Affect Protein Function

PhD SNP

Predictor of human Deleterious Single Nucleotide Polymorphisms (PhD-SNP) is a machine learning-based software or tool used to predict whether a nonsynonymous single-nucleotide polymorphism (nsSNP) is likely to be disease-associated or neutral. A higher probability score (closer to 1) indicates greater confidence in the prediction. The output is binary (Disease or Neutral), along with a probability score to support interpretation. Mutations predicted as 'Disease' are likely to contribute to disease phenotypes, while 'Neutral' variants are likely to have no significant effect on protein function. According to the PhD SNP, 7 Mutations were predicted as Neutral, while 1 was predicted as disease-causing.

Table 5: shows results for all mutations by PhD SNP

Mutation	Prediction	Reliability Index
V20L	Neutral	7
R83P	Disease	3
E5D	Neutral	8
P19T	Neutral	8
I33V	Neutral	6
D22N	Neutral	7
Q11H	Neutral	5
K75E	Neutral	1

I-Mutant

I-Mutant is a computational tool, specifically the I-Mutant 2.0, used to predict how single-point mutations affect protein stability. It predicts the change in protein stability (free energy) when a specific amino acid is replaced with another. This tool is valuable for researchers in fields like biochemistry and biophysics who need to understand the effects of mutations on protein structure and function. It is used to predict the impact of single-point mutations on protein stability. Based on support vector machine models, the tool calculates the ΔΔG (kcal/mol) of unfolding. A negative ΔΔG indicates decreased stability, while a positive ΔΔG indicates increased stability. The prediction is accompanied by a reliability index (RI) ranging from 0 to 10. According to I-Mutant, all 8 mutations were predicted to decrease the stability of the Protein Structure

Table 6: shows results for all mutations by I-Mutant

Mutation	Stability	Reliability Index
V20L	Decrease	7.0
R83P	Decrease	6
E5D	Decrease	7.0
P19T	Decrease	8
I33V	Decrease	8
D22N	Decrease	3
Q11H	Decrease	6
K75E	Decrease	5

The findings are consistent with a growing body of evidence which indicates that IKZF1 is one of the most functionally intolerant and clinically relevant genes in B-ALL. The substitutions described here are located in areas that we expect to be critical for normal IKAROS function and changes in IKZF1 (deletions or point mutations) have been reported to affect B-cell precursor ALL (BCP-ALL) disease biology and are highly associated with poor treatment responses in BCP-ALL[13] thus, the identification and characterization of damaging IKZF1 variants as described here is clinically relevant. In children with a B-cell precursor ALL, Wang ondu *et al* demonstrated that the nature and location of the AL alteration in IKZF1 is predictive of relapse risk rather than the presence of an AL. In our study, not all AL substitutions in IKZF1 were harmful but only a subset (K75E, Q11H, D22N, R83P).

This is partly why in *silico* prediction tools were beneficial in highlighting variants of clinical interest prior to experimental studies, such as IKZF1 Δ4-7 and the R83P and D22N variants used in the present study by the group of Soltani *et al*.[12]

Copy-number level evidence also has been obtained demonstrating the relevance of IKZF1 dosage and disruption of its structure to the biology of B-ALL. Ou *et al* used array comparative genomic hybridization to identify PAX5 and IKZF1 alterations as some of the most common and clinically important alterations in B-cell precursor ALL[22], and Steeghs *et al* showed that copy number alterations of genes important for B-cell development (e.g. IKZF1) are associated with drug resistance and clinical outcome in pediatrics B-cell precursor ALL.[23] Although these studies did not include point mutations but rather deletions, the implication that loss of normal IKZF1 function, whatever its mechanism, is detrimental to B-cell development and worsens prognosis is supported by our finding that specific missense substitutions (R83P, D22N, Q11H, K75E) are predicted to be structurally and functionally damaging.

Kuehn *et al* examined the effects of germline IKZF1 mutations on immune function, highlighting the fact that mutations that affect the DNA-binding zinc-finger domains of IKAROS are especially likely to impair normal protein function and thus to be associated with IKAROS-associated disease.[24] This is relevant to our results because some of the damaging substitutions identified here happened within the N-terminal portion of IKZF1 that is associated with DNA binding, which may explain why these variants (and not V20L, E5D, P19T, or I33V) were systematically predicted to be harmful.

Finally, Kuehn *et al* examined the role of germline IKZF1 mutations on immune function, and found variants that disrupt the DNA-binding zinc-finger domains of IKAROS to be especially likely to disrupt normal protein function and to be associated with IKAROS-associated disease.[24] This may be relevant to our results as some of the damaging substitutions detected here occurred in the N-terminal part of IKZF1 linked to DNA binding, but not V20L, E5D, P19T or I33V, which led to systematic prediction as damaging.

Conclusion

The gene was screened for eight mutations using a panel of complementary bioinformatics tools. All identified SNPs were predicted to be either benign or neutral, but some were also predicted to alter protein stability. Importantly, R83P, D22N, Q11H, and K75E are consistently predicted to be deleterious and damaging, with the potential to significantly disrupt protein function, suggesting a possible association with disease progression in B-ALL and highlighting their potential role in prognosis and therapeutic response. These findings highlight the pivotal role of alterations in leukemogenesis and therapy resistance, and demonstrate how online computational tools can enable rapid, low-cost assessment of candidate genetic mutations. Therefore, these variants need to be further validated experimentally and further correlated with clinical characteristics to prove their biological significance and evaluate their diagnostic or prognostic value in B-ALL.

Conflict of Interest

The authors declare no conflict of interest

References

1. Sattarzadeh Bardsiri M, Zehtab S, Karami N, Farsinejad A, Ehsan M, Fatemi A. Association of IKZF1 and CDKN2A gene polymorphisms with childhood acute lymphoblastic leukemia: a high-resolution melting analysis. *BMC Medical Genomics*. 2022/08/05 2022;15(1):171. doi:10.1186/s12920-022-01325-6

2. Mansoor N, Javed O, Saher S, Israr T, Imran S. ALL-240: Bridging Conventional and Molecular Cytogenetics: Evaluating Concordance of Karyotyping and FISH in B-Lymphoblastic Leukemia and Its Association With Post-Induction Minimal Residual Disease. *Clinical Lymphoma Myeloma and Leukemia*. 2025;25:S342-S343.

3. Kreile M, Piekuse L, Rots D, Dobele Z, Kovalova Z, Lace B. Analysis of possible genetic risk factors contributing to development of childhood acute lymphoblastic leukaemia in the Latvian population. journal article. *Archives of Medical Science*. 2016;12(3):479-485. doi:10.5114/aoms.2016.59920

4. Vallejos-Vidal E, Reyes-Cerpa S, Rivas-Pardo JA, et al. Single-Nucleotide Polymorphisms (SNP) Mining and Their Effect on the Tridimensional Protein Structure Prediction in a Set of Immunity-Related Expressed Sequence Tags (EST) in Atlantic Salmon (Salmo salar). Original Research. *Frontiers in Genetics*. 2020-February-27 2020;Volume 10 - 2019doi:10.3389/fgene.2019.01406

5. Srinivasan S, Clements JA, Batra J. Single nucleotide polymorphisms in clinics: Fantasy or reality for cancer? *Critical reviews in clinical laboratory sciences*. 2016;53(1):29-39.

6. Wijmenga C, Zhernakova A. The importance of cohort studies in the post-GWAS era. *Nature genetics*. 2018;50(3):322-328.

7. Marke R, van Leeuwen FN, Scheijen B. The many faces of IKZF1 in B-cell precursor acute lymphoblastic leukemia. *Haematologica*. Apr 2018;103(4):565-574. doi:10.3324/haematol.2017.185603

8. Lesmana HL, Rotz S, Kuehn HS, Rosenzweig SD. Clinical Characteristics and Outcomes of Leukemia in Patients with Germline IKZF1 Mutations. *Journal of Human Immunity*. 2025;1(CIS2025)doi:10.70962/CIS2025abstract.192

9. Zhang J, Xu X-J, Liu L, et al. Clinical and Genetic Characteristics of IKZF1 Mutation in Chinese Children With B-Cell Acute Lymphoblastic Leukemia. Original Research. *Frontiers in Genetics*. 2022-March-28 2022;Volume 13 - 2022doi:10.3389/fgene.2022.822832

10. Wang'ondu RW, Ashcraft E, Chang T-C, et al. Heterogeneity of IKZF1 genomic alterations and risk of relapse in childhood B-cell precursor acute lymphoblastic leukemia: ACUTE LYMPHOBLASTIC LEUKEMIA. *Leukemia*. 2025;39(7):1595-1606.

11. Soltani I, Bahia W, Slaymi C, et al. The role of IKZF1 rs4132601 and Δ4–7 somatic deletion in acute lymphoblastic leukemia: a bioinformatics and case-control study. *Molecular Biology Reports*. 2025;52(1):487.

12. Paolino J, Tsai HK, Harris MH, Pikman Y. IKZF1 Alterations and Therapeutic Targeting in B-Cell Acute Lymphoblastic Leukemia. *Biomedicines*. Jan 1 2024;12(1)doi:10.3390/biomedicines12010089

13. Yamashita M, Morio T. IKZF-associated inborn errors of immunity. *Journal of Human Immunity*. 2025;1(3):e20250063.

14. Sahar M, Gul S, Shehzad F, Mohsin S. Demographic features of pediatric B-ALL (B Lineage Acute Lymphoblastic Leukemia) patients in Pakistani population. *The Professional Medical Journal*. 2020;27(08):1582-1589.

15. Noman Anjum Rana AM, Robert HM, Zahir S, Ali I, Riaz S. Induction chemotherapy response in childhood acute lymphoblastic leukaemia and its correlation with cytogenetic and molecular features. *Journal of the College of Physicians and Surgeons Pakistan*. 2022;32(11):1430-1434.

16. Qari MH, Alattas AA, Binkuddah SM, et al. Mutations Encountered in Acute Lymphoblastic Leukemia: A Retrospective Study in a Teaching Hospital in Jeddah, Saudi Arabia. *Cureus*. 2021;13(1)

17. Eckardt J-N, Stasik S, Röllig C, et al. Mutated IKZF1 is an independent marker of adverse risk in acute myeloid leukemia. *Leukemia*. 2023;37(12):2395-2403.

18. Feng L, Zhang H, Liu T. Multifaceted roles of IKZF1 gene, perspectives from bench to bedside. *Front Oncol*. 2024;14:1383419. doi:10.3389/fonc.2024.1383419

19. Schwab CJ, Chilton L, Morrison H, et al. Genes commonly deleted in childhood B-cell precursor acute lymphoblastic leukemia: association with cytogenetics and clinical features. *Haematologica*. Jul 2013;98(7):1081-8. doi:10.3324/haematol.2013.085175

20. Cyrus S, Panigrahi AR, Monahan AP, et al. Discovery of a germline IKZF1 deletion in a B-

lymphoblastic leukemia post-induction bone marrow specimen: a case report and review of the literature. *Journal of Hematopathology*. 2025;18(1):34.

21. Ou Z, Sherer M, Casey J, et al. The Genomic Landscape of PAX5, IKZF1, and CDKN2A/B Alterations in B-Cell Precursor Acute Lymphoblastic Leukemia. *Cytogenet Genome Res*. 2016;150(3-4):242-252. doi:10.1159/000456572

22. Popov D. Acute Lymphoblastic Leukemia: Molecular Pathogenesis and Emerging Therapeutic Paradigms, Therapeutic Innovations in Acute Lymphoblastic Leukemia: Linking Molecular Pathogenesis to Precision Medicine.

23. Steeghs EMP, Boer JM, Hoogkamer AQ, et al. Copy number alterations in B-cell development genes, drug resistance, and clinical outcome in pediatric B-cell precursor acute lymphoblastic leukemia. *Sci Rep*. Mar 15 2019;9(1):4634. doi:10.1038/s41598-019-41078-4

24. Kuehn HS, Nunes-Santos CJ, Rosenzweig SD. Germline IKZF1 mutations and their impact on immunity: IKAROS-associated diseases and pathophysiology. *Expert Rev Clin Immunol*. Apr 2021;17(4):407-416. doi:10.1080/1744666x.2021.1901582

25. Anupam K, Patel K, Andhare P, et al. The B cell receptor–mTOR signaling axis restricts the accumulation of lung tissue–resident memory B cells after influenza infection. *Science immunology*. 2026;11(117):eadw1664.