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Original Article

Novel *RUNX1* Variation in B-cell Acute Lymphoblastic Leukemia

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Abstract. Acute lymphoblastic leukemia (ALL) is a malignant disease of hematopoietic stem cells. B cell ALL (B-ALL) is characterized by highly proliferative and poorly differentiated progenitor B cells in the bone marrow. Chromosomal rearrangements, aberrant cell signaling, and mutations lead to dysregulated cell cycle and clonal proliferation of abnormal B cell progenitors. In this study, we aimed to examine hot spot genetic variations in the *RUNX1, IDH2,* and *IL2RA* genes in a group of (n=52) pediatric B-ALL. Sanger sequencing results revealed a rare *RUNX1* variant p.Leu148Gln in one B-ALL patient with disease recurrence. Additionally, common intronic variations rs12358961 and rs11256369 of IL2RA were determined in two patients. None of the patients had the IDH2 variant.

***RUNX1, IDH2,* and *IL2RA* variations were rare events in ALL. This study detected a novel pathogenic RUNX1 variation in a patient with a poor prognosis. Examining prognostically important genetic anomalies of childhood lymphoblastic leukemia patients and the signaling pathway components will pilot more accurate prognosis estimations.**

Keywords: B-ALL; *RUNX1; IL2RA; IDH2*.

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Introduction. Acute lymphoblastic leukemia (ALL) is the most common pediatric cancer caused by the malign transformation of hematopoietic progenitor B- or T-cells.[1] ALL is commonly associated with acquired chromosomal translocations and other genetic or epigenetic abnormalities, which lead to aberrant expression of transcription factors.[2] Eighty percent of ALL cases are B cell ALL, and five years survival rates exceed 90% in pediatrics in high-income countries.

One of the most frequent chromosome translocations

is *ETV6::RUNX1* in B-ALL patients.[3] *RUNX1* is a transcription factor that regulates the maturation of blood cells.[4] The recurring genetic alteration of *RUNX1* characterizes a rare poor prognostic subgroup of B-ALL. Additionally, germline *RUNX1* mutations were associated with a predisposition to familial leukemia.[5]

IDH2 is an isocitrate dehydrogenase that catalyzes the oxidative decarboxylation of alpha-ketoglutarate. *IDH2* gene variations are rare events in pediatric ALL (approx. 0.5%) and AML (3%) (acute myeloid *IDH1/2* mutations in AML and T-ALL were associated with a high cumulative incidence of relapse and poor response to therapy. Importantly targeted inhibitors of *IDH1/2* are available, and preliminary clinical trials results are promising for refractory or relapsed AML patients.[6,7] IL2RA controls many different cellular functions, including proliferation, differentiation, and cell survival/apoptosis but are also involved in several pathological processes. IL2RA (CD25) expression has been associated with Ph-positive B-ALL in pediatrics and adults.[8]

The aim of this study was to determine the variants of *RUNX1*, *IDH2*, and *IL2RA* genes associated with leukemia development and to evaluate their association with the prognosis of B-ALL.

Materials and Methods

Patient Group. B-ALL cases (n=52) diagnosed in the pediatric hematology clinic of Istanbul University Medical Faculty were included in the study. Patients were treated with B.F.M. (Berlin-Frankfurt-Munich protocol for pediatric ALL) protocol. The median age of the patients was five years (min: 0,9 years and max: 15 years), and the gender distribution of male-female was 30:22 (1.3:1) (**Table 1**).

Clinical characteristics such as Bone marrow (B.M.) blast percentage, white blood cell count (WBC) at diagnosis, hemoglobin levels (Hb), platelet count (Plt), translocation, and organ involvement of the cohort were given in **Table 1**. *BCR::ABL1* (n=3), *MLL::AF4B* (n=1), and *TEL::AML1* (n=3) fusions were detected in B-ALL patients. This study is approved by the Local Ethics Committee (*Istinye University Clinical Research Ethics Committee (2017-KAEK-120)/2/2019.G-009*).

DNA isolation and Sanger Sequencing. According to the manufacturer's instructions, Genomic DNA was isolated from diagnostic bone marrow and peripheral blood samples using a Gentra Puregene Blood Kit, Qiagen. Exxon's of the *RUNX1, IDH2, and IL2RA* genes were amplified with specific primers (**Table 2**). The PCR reactions comprised 50 ng of template DNA, 10 pmol of each primer, and 2×*PCR* master mix (Hibrigen, Turkey).

In a final volume of 50µl, PCR was performed using a T100 Thermal Cycler (Bio-Rad, U.S.A.) using the following conditions: 94°C denaturation for 5 minutes, 94°C denaturation for 30 seconds, 60°C of annealing for 30 seconds, 94°C of elongation for 30 seconds, 37 cycle in total and 5 minutes of 72°C for the last extension.

Table 1. Clinical features of B-ALL patients.

	B-ALL patients (n=52)
Median age (min-max)	5 (0.9-15)
Gender (Male: Female)	30:22 (1.3:1)
B.M. blast (%) average	93
WBC (x109/L) median (min-max)	22000 (1700-629000)
Hb (dL) median (min-max)	7.8 (5-19)
PLT (x109/L) median (min-max)	40000 (8000-284000)
Extramedullary features (n)	
C.N.S. involvement	
Yes	1
No	29
NA	22
Lymphadenopathy	
Yes	12
NO	40

Sequential alterations were determined using bidirectional sequencing. PCR products have been treated with ExoSAP-IT (GML A.G., Wallerau, Switzerland) enzyme. Bigdye Terminator v3.1 cycle sequencing kit and A.B.I. 3500xL genetic analyzer device have been used for sequencing. Amplicon sequences were evaluated using a CLC workbench 3.6.1 (Denmark) (NM_001001890.3, NM_000417.3, NM_001289910).

Open source programs such as Sorting Tolerant From Intolerant release 63 (SIFT, http://sift.jcvi.org), Polyphen (http://genetics.bwh.harvard.edu/pph2), Combined Annotation Dependent Depletion (CADD, http://cadd.gs.washington.edu), and Mutation Taster (http://www.mutationtaster.org) were used to predict the functional impact of the gene variants. Also, the Database of Single Nucleotide Polymorphism (dbSNP, https://www.ncbi.nlm.nih.gov/SNP), 1000 Genomes Project samples (http://www.1000genomes.org), The Human Gene Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php), and the Exome Aggregation Consortium (ExAC, http://exac.broadinstitute.org) were used for frequency data.

Results. The hotspot regions of *RUNX1, IDH2, and IL2RA* genes were examined in 52 diagnostic samples of B-ALL cases. A pathogenic *RUNX1* variation c.443T>A, p.Leu148Gln was detected in one B-ALL (1.9%) patient (**Figure 1A**). This novel variant, p.Leu148Gln was located in the Runt homology domain of the *RUNX1* gene, and **Figure 1B** shows the *RUNX1* gene variations distribution in the St. Jude's Children's Research

Table 2. Primer sequences.

Chr 21.	GTAAAACGACGGCCAGTCTTCCTGTTTGCTTTCCAGC	CAGGAAACAGCTATGACCCACGCGCTACCACACCTAC
Chr 21.	GTAAAACGACGGCCAGTACCACGTCGCTCTGGTTC	CAGGAAACAGCTATGACCATCCTCGTCCTCTTGGGAGT
Chr 21.	GTAAAACGACGGCCAGTAAGAAAATCAGTGCATGGGC	CAGGAAACAGCTATGACCACCCTGGTACATAGGCCACA
Chr 21.	GTAAAACGACGGCCAGTTGTTACGACGGTTTGCAGAG	CAGGAAACAGCTATGACCGGAAGGGAAGGGAAATCTTG
Chr 21.	GTAAAACGACGGCCAGTAGTTGGTCTGGGAAGGTGTG	CAGGAAACAGCTATGACCGGAAAGACAAGAAAAGCCCC
Chr 21.	GTAAAACGACGGCCAGTGCAACTTTTTGGCTTTACGG	CAGGAAACAGCTATGACCGGTAACTTGTGCTGAAGGGC
Chr 21.	GTAAAACGACGGCCAGTCCGAGTTTCTAGGGATTCCA	CAGGAAACAGCTATGACCCATTGCTATTCCTCTGCAACC
Chr 21.	GTAAAACGACGGCCAGTAGAAAGCTGAGACGAGTGCC	CAGGAAACAGCTATGACCGCAGAACCAGAACGTTTTCC
Chr 21.	GTAAAACGACGGCCAGTGGAATCAGCAGAAACAGCCT	CAGGAAACAGCTATGACCAACCACGTGCATAAGGAACA
Chr 21.	GTAAAACGACGGCCAGTGGTGAAACAAGCTGCCATTT	CAGGAAACAGCTATGACCTTTGGGCCTCATAAACAACCT
Chr 10.	CAGAGAAAGACCTCCGC	TCCCTTCTTGGAACCATCTAC
Chr 10.	AAGAAATATGTGATTAAGTCATTATAGGAT	AAGAAATATGTGATTAAGTCATTATAGGAT
Chr 10.	GTGCTTCTCAAGTGAATGAATAC	GTCCGCTAGCAGGAGTTA
Chr 10.	CAACCTGGACTCACTCG	AGCCTGATGGAGCAAAG
Chr 10.	GCCCTGACTCCTGTGTTTA	TCTGTGGTCCAGCGTTT
Chr 10.	CTCGTGCTGTCCTAAAGTC	CTCAGCCTGGTGTACAT
Chr 10.	5-CCCTTTGCTGAACTCCC-3	GGTACAGGACTTTGATCTGAC
Chr 15.	CTGGGCGACCATTTAGCA	CACTGGGTTTAGAGTGAGGAC
Chr 15.	CAGGAAACAGCTATGACCAAC ATG-CAAAATCACATTATTGCC	TGTAAAACGACG-GCCAGTGGGTTCAAATTCTGGTTGAA

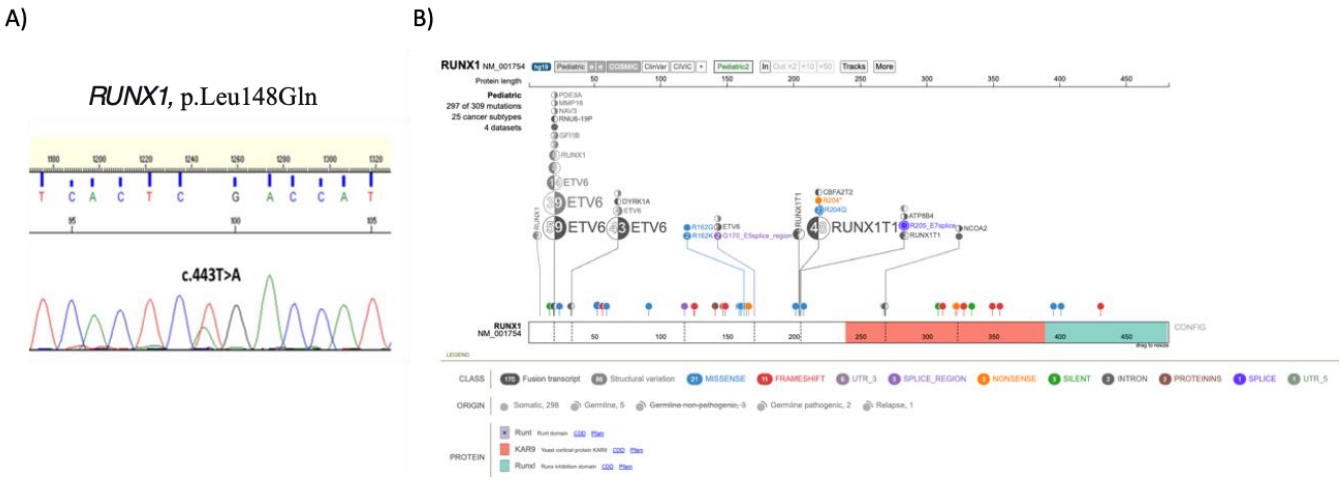

Figure 1. Sanger sequencing results for *RUNX1* mutant patient. **A**) Electropherogram image of a patient with *RUNX1* c.443T>A, p.Leu148Gln variant. **B**) Hotspot *RUNX1* gene variations in PeCan (St.Judes Children's Research Hospital Data Portal).

Hospital Pediatric Cancer Data Portal (PeCan). The Mutation Taster prediction was Disease Causing with a score of 1. Varsome (The Human Genomics Community) prediction was likely pathogenic; SIFT prediction was pathogenic with a score of 0, and the MutPred prediction was Pathogenic with a 0.87 score, PROEVAN (Protein Variation Effect Analyzer) prediction was Pathogenic with -5.31 score. Pathogenicity meta scores based on the combined evidence from multiple other in-silico predictors is 6 (BayesDel addAF, BayesDel noAF, MetaLR, MetaRNN, REVEL).

The patient with *RUNX1* variation was 9 years old boy with high WBC counts (132400/mm^3). He had lymphadenopathy at diagnosis. He had a 33-day treatment response to therapy *without any BCR/ABL1*, *MLL-F4B*, or *TEL-AML1* translocations. He relapsed in 16 months and died due to the recurrence 30 months after diagnosis.

Two common intronic variants of *IL2RA* c.367+12A>T, rs12358961, and c.367+7G>C, rs11256369, were detected in two patients (**Figure 2**). According to the prediction tools, these variants were benign, and patients had a standard risk for B-ALL treatment response.

Additionally, we screened the hotspot region of *IDH2*, and none of the patients showed *IDH2* variation.

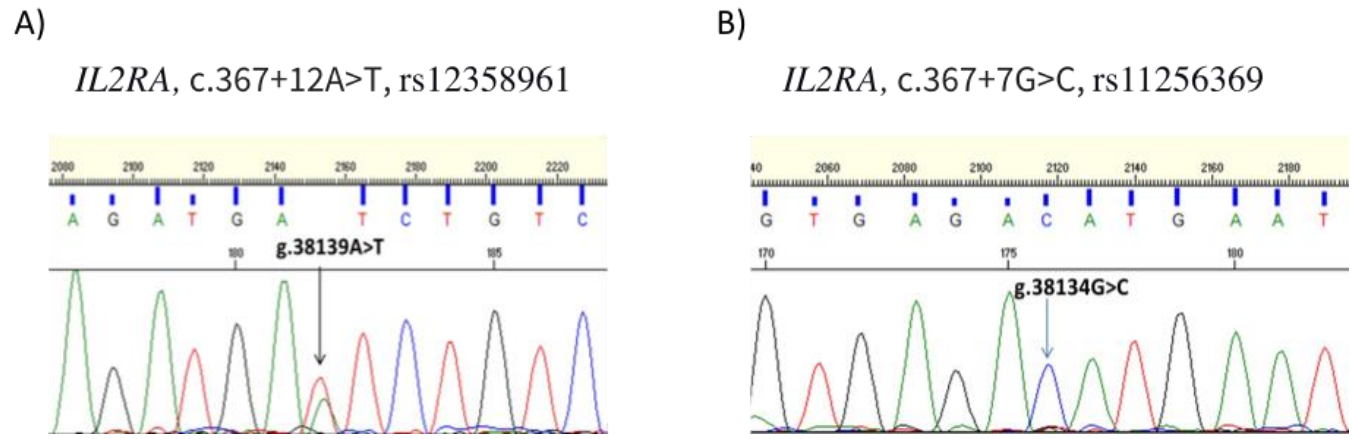

Figure 2. Sanger sequencing results of patients with *IL2RA* variants. **A**) *IL2RA* c.367+12A>T, rs12358961, **B**) *IL2RA* c.367+7G>C, rs11256369 variants.

Discussion. In this study, we aimed to analyze *RUNX1*, *IDH2*, and *IL2RA* hotspot gene variations described as rare and poor prognostic events in ALL. Out of 52, one B-ALL patient was found to carry a pathogenic *RUNX1* variation (1.9%). *IL2RA* and *IDH2* genes did not found mutated in the pediatric B-ALL cohort. *RUNX1* p.Leu148Gln variation is located on the Runt domain of the *RUNX1* gene that mediates binding to the core-binding factor (CBFbeta). Heterodimerization of RUNX1 and CBFbeta increases the DNA binding affinity. Previous studies showed that mutations on the Runt domain stabilize the DNA binding ability of the *RUNX1* gene. *RUNX1* mutated B-ALL patient had a poor prognosis and dead after early relapse. It is known that *RUNX1* mutations have been associated with poor prognosis in myeloid malignancies.[9] *RUNX1* gene mutations were found in 18.3% of patients with T-ALL, 5-15% in cytogenetically normal acute myeloid leukemia (CN-AML), and 3.8% in patients with B-ALL.[10] Garza-Veloz et al. reported the association between the high *IL2RA*, *SORT1*, *FLT3*, and *DEFA1* gene expression levels and relapse risk and poor survival rates in B-ALL patients.[11] We also found two common variations, rs12358961 and rs11256369 in the *IL2RA* gene in two B-ALL cases. Although both variations are classified as benign, both were placed in the splicing machinery sites, and the functional impacts are unknown. *IDH2* mutation is rather more often seen in AML (8%) than in ALL patients (<1%).[12] None of our patients was found to carry pathogenic mutations in *IL2RA* and *IDH2* genes, which is in concordance with the previous data.

Our study confirmed that the pathologic variants of *RUNX1* act as a poor prognostic factor in pediatric B-ALL, which is uncommon. A limited number of B-ALL patients were reported for the hotspot regions of three genes (*RUNX1*, *IL2RA, and IDH2*), which took part in the early developmental stages of lymphocytes, particularly B-cells. According to the previously published manuscripts, mutation ratios are not significantly different compared to whole gene screening studies. Larger independent patient cohorts are required to confirm the findings of this study.

Gene expression profiling and genome-wide sequencing analyses have made great advancements in understanding B-ALL genetics over the past few years. High-throughput analysis of big ALL cohorts has been very helpful in subclassifying B-ALL patients with different risks, identifying novel therapeutic targets, and improving overall clinical outcomes. Biomarkers with prognostic and predictive value and targeted therapeutic agents have emerged as promising approaches in the clinical care of B- in the era of personalized medicine.

References:

1. Bellavia D, Palermo R, Pia Felli M, Screpanti I, Checquolo S. Notch signaling as a therapeutic tar- get for Acute Lymphoblastic Leukemia. Expert Opin Ther Targets Sci 2018; 12 :331-342 https://doi.org/10.1080/14728222.2018.1451840 PMid:29527929
2. Kruse A, Abdel-Azim N , Kim HN, Ruan Y, Phan V, Ogana H, Wang W, Lee R, Gang E, Khazal S, Kim YM. Minimal Residual Disease Detection in Acute Lymphoblastic Leukemia. Int Jou Mol Sci 2020; 6: 1054 https://doi.org/10.3390/ijms21031054 PMid:32033444 PMCid:PMC7037356
3. Cooper S L, Brown P A. Treatment of Pediatric Acute Lymphoblastic Leukemia. Pediatr Clin North Am 2015:19: 61-73. https://doi.org/10.1016/j.pcl.2014.09.006 PMid:25435112 PMCid:PMC4366417
4. Sun C, Chang L, Zhu X. Pathogenesis of ETV6/RUNX1-positive childhood acute lymphoblastic leukemia and mechanisms underlying its relapse. Oncotarget 2017:18: 35445-35459. https://doi.org/10.18632/oncotarget.16367 PMid:28418909 PMCid:PMC5471068
5. Lam K, Zhang D E. RUNX1 and RUNX1-ETO: roles in hematopoiesis and leukemogenesis.Front Biosci. 2012:1: 1120-1139. https://doi.org/10.2741/3977 PMid:22201794 PMCid:PMC3433167
6. Triplett T, Curti B D, Bonafede P R, Miller W L, Walker E B, Weinberg A D. Defining a functionally distinct subset of human memory CD4+ T cells that are CD25POS and FOXP3. Eur. J. Immunol 2012:5: 1893-1905. https://doi.org/10.1002/eji.201242444 PMid:22585674

7. Simonin M, Schmidt A, Bontoux C, Dourthe M E, Lengline E, Andrieu G P, Lhermitte L, Graux C, Grardel N, Cayuela J M, Huguet F, Arnoux I, Ducassou S, Macintyre E, Gandemer V, Dombret H, Petit A, Ifrah N, Baruchel A, Boissel N, Asnafi V. Oncogenetic landscape and clinical impact of IDH1 and IDH2 mutations in T-ALL. J Hematol Oncology 2021:5: 1-7.
https://doi.org/10.1186/s13045-021-01068-4
PMid:33941203 PMCid:PMC8091755
8. Leonard W J, Donlon T A, Lebo R V, Greene W C. Localization of the Gene Encoding the Human interleukin-2 Receptor on Chromosome 10. Science.1985.28: 1547-1549.
https://doi.org/10.1126/science.3925551
PMid:3925551
9. Smith BM, Arthur D , Camitta B, Carroll AJ, Crist W, Gaynon P, Gelber R, Heerema N, Korn EL, Link M, Murphy S, Pui CH, Pullen J, Reaman G, Sallan SE, Sather H, Shuster J, Simon R, Trigg M, Tubergen D, Uckun F, Ungerleider R. Uniform Approach to Risk Classification and Treatment Assignment for Children With Acute Lymphoblastic Leukemia. J Clin Oncol. 1996.14: 18-
https://doi.org/10.1200/JCO.1996.14.1.18
PMid:8558195
10. Grossmann V, Kern W, Harbich S, Alpermann T, Jeromin S, Schnittger S, Haferlach C, Haferlach T, Kohlmann A. Prognostic relevance of RUNX1 mutations in T-cell acute lymphoblastic leukemia. Haematologica. 2011:12:1874-1877.
https://doi.org/10.3324/haematol.2011.043919
PMid:21828118 PMCid:PMC3232273
11. Veloz IG, Martinez-Fierro ML, Jaime-Perez JC,MCarrillo-Sanchez K, Ramos-Del Hoyo MG, Lugo-Trampe A, Rojas-Martinez A, Gutierrez-Aguirre CH, Gonzalez-LlanoO, Salazar-Riojas R, Hidal- go-Miranda A, Gomez- Almaguer A, Ortiz-Lopez R. Identification of Differentially Expressed Genes Associated with Prognosis of B Acute Lymphoblastic Leukemia.Disease Markers.2015:24:1-11.
https://doi.org/10.1155/2015/828145
PMid:25802479 PMCid:PMC4354728
12. Andersson AK, Miller DW, Lynch JA, Lemoff AS, Cai Z, Pounds SB, Radtke I, Yan B, Schuetz JD, Rubnitz JE, Ribeiro RC, Raimondi SC, Zhang J, Mullighan CG, Shurtleff SA, Schulman BA, Downing JR. IDH1 and IDH2 mutations in pediatric acute leukemia. Leukemia.2011:7:1-15.
https://doi.org/10.1038/leu.2011.133
PMid:21647154 PMCid:PMC3883450