Evaluation of Expression Levels of Relapse-Associated Survivin, S100A8, GPM6B, NUDT15, AURKA and CASP1 Genes at Time of Diagnosis: A Case-Control Research Study in Childhood Pre-B- Acute Lymphoblastic Leukemia

Oznur TOKTA[1], Sibel BERKER KARAUZUM[1], Ayse Esra MANGUOGLU[1],
Osman Alphan KUPESIZ[2], Funda KUPESIZ[2], Elif GULER[2], Cigdem SIVRICE[2],
Ayse Nur AKINEL[2], Kemal Hakan GULKESEN[3]

[1] Akdeniz University Faculty of Medicine, Department of Medical Biology and Genetics
[2] Akdeniz University Faculty of Medicine, Department of Pediatric Hematology and Oncology
[3] Akdeniz University Faculty of Medicine, Department of Biostatistics and Medical Informatics

ABSTRACT

Childhood pre-B acute lymphoblastic leukemia (pre-B-ALL) can be identified through routine genetic diagnostic methods in approximately 70-75% of cases. However, in 20-25% of cases, genetic abnormalities remain undetected at diagnosis, complicating the prediction of relapse risk, treatment response, and therapy-related cytotoxicity. Identifying reliable biomarkers is crucial for early diagnosis and optimized treatment strategies. Bone marrow samples were collected from 15 pediatric pre-B-ALL patients and 5 healthy child donors. Following cDNA synthesis, mRNA expression levels of AURKA, CASP1, GPM6B, NUDT15, S100A8, and Survivin genes were analyzed using qRT-PCR. AURKA, CASP1, and Survivin genes showed significantly increased expression in childhood pre-B-ALL cases ($p< 0.05$). However, no significant difference was observed for GPM6B, NUDT15, and S100A8. Kaplan-Meier analysis revealed no correlation between gene expression levels and relapse time. Spearman's rho test showed a strong positive correlation between AURKA and Survivin expression ($p< 0.0001$). AURKA and Survivin overexpression are significantly correlated and may serve as potential biomarkers for predicting relapse in pediatric pre-B-ALL. CASP1 overexpression may indicate glucocorticoid resistance, potentially affecting treatment response; GPM6B, NUDT15, and S100A8 did not show significant relationships with relapse. Further validation in larger cohorts is required. Protein-level studies are needed to confirm the functional significance of these gene expression changes.

Keywords: Pediatric B-ALL, qRT-PCR, Survivin, CASP1, AURKA

INTRODUCTION

Approximately 85% of childhood ALL cases originate from B-cell precursors. The most important factor determining the biological behavior of these leukemic cells and their sensitivity to treatment is their genetic characteristics.[1] Recurrent chromosomal abnormalities, identifiable through conventional cytogenetic methods, are observed in approximately 70-75% of childhood B-ALL cases. Most of these abnormalities have prognostic significance and are used to determine risk when applying certain treatment protocols. Additionally, 25-30% of ALL cases remain uncharacterized by routine genetic methods at the time of diagnosis, making the relapse risk uncertain. In addition, in recent studies secondary somatic mutations that occur during treatment also gain great importance in treatment resistance and relapse.[2]

In childhood ALL, risk group classification is based on factors such as age, leukocyte count, cytogenetic findings, response to initial induction chemotherapy, and central nervous system or testicular involvement.[3]

However, even when these criteria are considered together, accurately identifying high-risk patients with potential treatment resistance and relapse remains challenging.[4]

Table 1. Real-Time PCR Primers

Genes	Forward 5'-3'	Reverse 5'-3'
ß-Actin	5′-CCTGGCACCCAGCACAAT-3′	5′-GCCGATCCACACGGAGTACT-3′,
AURKA	5'-TCTTCCCAGCGCATTCCTTT-3'	5'-GCTGCTTGCTCTTTTGGGTG-3'
CASP1	5'-GCCTGTTCCTGTGATGTGGAG-3'	5'TGCCCACAGACATTCATACAGTTTC-3'
GPM6B	5'-GGTGCCCGTGTTTATGTTCTA-3'	5'-CACACAGATCTGCTCCACAC 3'
NUDT15	5'-AAGCAAATGCAAAGCATCAC-3'	5'-GGCTGAAAGAGTGGGGGATA-3'
S100A8	5′- TGTCTCTTGTCAGCTGTCTTTCA-3′	5′- CCTGTAGACGGCATGGAAAT-3′
Survivin	5′-AGGACCACCGCATCTCTACAT-3′	5′-AAGTCTGGCTCGTTCTCAGTG-3′

Further studies on the molecular mechanisms underlying relapsed ALL are needed to develop more effective treatment strategies and improve patient survival rates.

More than 100 genes associated with relapse have been identified in ALL studies. Specifically AURKA, CASP1, GPM6B, NUDT15, S100A8 and Survivin genes have been reported to be linked to relapse in the literature, with their expression significantly altered in treatment-resistant patients.[5]

However, no data exist regarding the relationship between AURKA, CASP1, GPM6B, NUDT15, and S100A8 genes and pre-B ALL relapse in Turkiye.

This study aims to identify biomarkers that can determine the risk of relapse at the time of diagnosis by analyzing the expression levels of relapse-related genes (Survivin, S100A8, GPM6B, NUDT15, AURKA, and CASP1) in 15 Turkish children newly diagnosed with pre-B ALL and 5 healthy control individuals using the quantitative real-time PCR (qRT-PCR) method. The differences in gene expression levels between the control and patient groups were assessed.

PATIENTS AND METHODS

RNA samples were obtained from bone marrow material collected at the time of diagnosis during routine clinical evaluation of 15 pre-B ALL patients who applied to the Akdeniz University Pediatric Hematology and Oncology Department between 2009 and 2019. These samples were stored at -20°C and included in the study

As a control group, RNA samples were isolated from bone marrow materials obtained from five healthy children who served as bone marrow donors were isolated by using Qiamp RNA Blood Mini Kit (Qiagen).

cDNA Synthesis

RNA samples of 15 patients and total RNAs obtained from 5 healthy control groups were translated into cDNA using the Blue-Ray PCR device in accordance with the protocol with the Applied BiosystemsTM High-Capacity cDNA Reverse Transcription Kit (4368814). Quantity and purity measurements of isolated cDNA samples were determined by spectrophotometer (Quawell/Q9000B).

qRT PCR

Applied BiosystemsTM StepOnePlusTM Real-Time PCR device and PowerUpTM SYBRTM Green Master Mix (A25742) kit for determination of expression levels of relapse-associated target genes (AURKA, CASP1, GPM6B, NUDT15, S100A8 and Survivin genes) and β-Actin gene as housekeeping gene were used in accordance with the protocol specified by the manufacturer. Primer sequences are given in Table 1. The 96-well plate was designed to include patient and control groups in triplicate, with each plate containing a negative control without cDNA. qRT-PCR conditions were as follows: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation at 95°C for 15 seconds, annealing at 60°C for 1 minute, and final extension at 95°C for 15 seconds. The melt curve analysis was performed with an initial step at 95°C for 15 seconds, followed by cooling to 60°C for 1 minute, and a final heating step at 95°C for 15 seconds.

Table 2. Clinical data, cytogenetic analyses, and FISH results on bone marrow from patients with pre B-ALL

UPN	Age at Diagnosis (Years)	Gender	Diagnosis-Relapse Range (month)	Out-Come	Cyto-Genetics	FISH
1	3	M	116	Alive	46, XY, -12, der(13) t(12;13)(q11;p11), +i(21)(q10) [2]/ 46, XY [3]	nuc ish 12p13(ETV6x2), 21q22 (RUNX1x4)(ETV6 con RUNX1x2)[82]
2	3	M	30	Alive	46,XY [14]	nuc ish 12p13(ETV6x2), 21q22(RUNX1x4)(ETV6 con RUNX1x2)[8/400]
3	6	M	11	Alive	45, XY, der(9) t(9;12)(p13;p13),-12 [18]/ 45, XY, del(3)(q21), der(9) t(9;12)(p13;p13),-12 [5]	nuc ish 12p13(ETV6x1), 21q22(RUNX1x2)[356/400]
4	8	M	37	Alive	46, XY, add(6)(p25) [1]/ 46, XY [24]	nuc ish 12p13(ETV6x2), 21q22(RUNX1x4)(ETV6 con RUNX1x1)[268/400]
5	5	M	35	Alive	56,XY,+X,+Y,+4,+5,+6,+10,+14,+18,+21, +21[3]/56, XY, +X, +Y, inv(1)(p13q32), +4, +5, +6,+10, +14, +18, +21, +21[8]/46, XY[10]	nuc ish 12p13(ETV6x2), 21q22(RUNX1x4)[340/400]
6	6	M	29	Alive	46,XY[15]	nuc ish 9p21(p16x0), 9q12(D9Z3x2)[228/400]
7	4	M	40	Alive	46, XY, +X, -7, t(7;12;14)(q10;p12;q32)[5]/ 46, XY [1]	nuc ish 12p13(ETV6x3), 21q22(RUNX1x2)[244/400]
8	4	M	59	Alive	57,XY,+3,+4,+5,+8,+9,+11,+14,+15,+17, +21,+21[8]	nuc ish 12p13(ETV6x2), 21q22(RUNX1x4)[380/400]
9	4	M	8	DOD	46,XY [9]	nuc ish 9p21(p16x3), 9q12(D9Z3x3)[50/100]
10	9	M	15	Alive	45, X, -Y, add(9)(p24), add(19)(q13), del(20)(q12) [1]/46, XY [24]	nuc ish 9p21(p16x0), 9q12(D9Z3x2)[220/400]
11*	10	M	40	Alive		
12*	13	M	24	Alive		
13	10	M	42	Alive	46, XY [20]	nuc ish 12p13(ETV6x3), 21q22(RUNX1x2)[252/400]
14	7	M	108	Alive	46, XY, del(7)(q11) [1]/ 46, del(X)(q11), Y, del(3)(p12), del(7)(q22) [3]/ 46, XY, fra(10)(p13) [2]/ 46, XY, fra(3)(p22/p24/q25) [4]/ 53, XY, +X, +4, +6, +14, +i(17)(q10), +21, +21 [1]/ 46, XY [7]	nuc ish 12p13(ETV6x2), 21q22(RUNX1x4)[320/400]
15*	7	M	25	Alive		

*UPN= unique patient number; M= male; DOD= dead of disease; *: Chromosome analysis and FISH could not be performed due to insufficient material*

Ethical Approval: The approval of Akdeniz University Faculty of Medicine Clinical Research Ethics Committee (Decision No:781/28.08.2019) and informed consent form were obtained from the families.

Statistical Analysis

The difference in gene expression between the patient and control groups was examined by the $^{\Delta\Delta}$CT method. Kolmogorov Smirnov Z, Kaplan-Meier and Spearman's rho tests PASW Statistics version 18 (IBM, NY, USA) software program was used for analysis. A significance level of $p< 0.05$ was considered statistically significant.

RESULTS

In this study, all 15 patients diagnosed with pre-B ALL in the Pediatric Hematology and Oncology unit, where gene expression analysis was performed, were male, and the gender distribution of the control group was 4 girls (80%) and 1 boy (20%). The patients age distribution ranged from 3 to 13 years, with a mean age of 6.6 years.The age distribution of the control group was between 3-16,

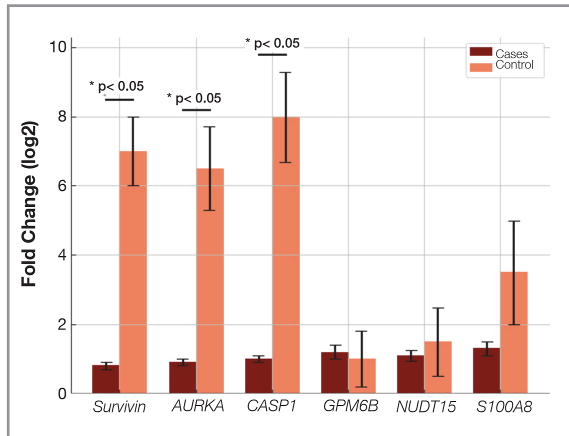

Figure 1. The relative mRNA expression levels. Except for the GPM6B gene, an increase in expression levels was observed in the Survivin, AURKA, CASP1, NUDT15, and S100A8 genes compared to the control group. However, this increase was found to be statistically significant only for the Survivin, AURKA, and CASP1 genes.

with a mean age of 8 years. Age, gender, genetic findings at the time of diagnosis, diagnosis-relapse range and survival status of the cases are given in Table 2.

As a result of the analysis with the $2^{-\Delta\Delta}$CT method; when the gene expression levels were compared between the control and pre B-ALL case groups, there was a statistically significant difference (p= 0.035) in the Survivin, AURKA, CASP1 genes, while no statistically significant difference was found in the GPM6B, NUDT15, S100A8 genes (Figure 1).

Survival analysis using the Kaplan-Meier test determined the relationship between gene expression and relapse duration. As a result of the statistical analysis, no correlation was found in terms of the duration of relapse in the samples taken at the time of diagnosis.

Statistical analysis with Spearman's rho test revealed a positive correlation between AURKA and Survivin expressions (r= 0.934; p< 0.0000).

DISCUSSION

The overall survival of childhood pre-B-ALL patients with relapse is still poor, and many children suffer from treatment side effects.[6] Therefore, it is necessary to investigate candidate biomarkers and appropriate new treatments in order to improve outcome, reduce toxic side effects, and ultimately predict the risk of relapse in childhood B-ALL patients. For this purpose, the first of the genes that we performed gene expression analysis in cases diagnosed with pre-B-ALL is the AURKA gene. It has been reported that this gene is overexpressed in many solid tumours.[7] Studies with hematological cancers are limited, and it has been reported that it is overexpressed especially in AML and is associated with poor prognosis.[8-10] In a meta-analysis study in childhood B-ALL, AURKA was found to be overexpressed during relapse compared to diagnosis.[5] In another study, it was found to be overexpressed in newly diagnosed childhood B-ALL patients compared to the control group, but functionally less important for childhood ALL compared to AML.[11]

Consistent with these studies, in this study, it was found that the AURKA gene was overexpressed in our childhood pre-B ALL cases at the time of diagnosis, with a 6-fold difference compared to the control group. This statistically significant difference (p= 0.035) shows that the AURKA gene can be an important marker in determining the risk of relapse at the time of diagnosis. When the samples taken for the AURKA gene at the time of diagnosis were examined in terms of expression levels and time to relapse, no statistically significant results were found in terms of relapse times.

Another gene that we included in this study is the Caspase-1 (CASP1) gene. CASP1 has been associated with many cancers, including melanoma, prostate, CLL, AML, colon and breast cancer.[12,13] Paugh et al. showed that CASP1 gene expression was higher in leukemia cells resistant to glucocorticoid (GC), which is one of the main components of ALL treatment, in their study on primary leukemia cells of 444 newly diagnosed childhood ALL patients. They also determined that CASP1 modulates the biological and pharmacological effects of GC by cleaving and inactivating GC receptors. They also showed that when they inhibited CASP1 activity in leukemia cells overexpressing CASP1, the cellular G-receptor number and sensitivity to GCs increased significantly.[14] In addition, Laskowska et al. obtained results supporting the study of Paugh et al. in mononuclear cells taken from 155 AML and ALL cases. Also, they report-

ed that expression changes of CASP1 may affect the immune response in leukemia.[15] In the present study, we observed that the CASP1 gene was overexpressed more than 7 times in pediatric pre-B ALL cases compared to the control group, and this difference was statistically significant ($p= 0.035$). Based on this result, which supports previous studies, we suggest that CASP1 expression could serve as a biomarker at the time of initial diagnosis to assess the risk of relapse. Although there was a difference in the expression levels of CASP1 associated with GC resistance compared to the control group, there was no statistically significant result when the expression levels and relapse times were examined ($p> 0.05$). Further investigation is needed to determine the relapse caused by drug resistance of expression differences in the CASP1 gene.

Another gene related to pre-B-ALL relapse, Glycoprotein M6B (GPM6B), has been observed to be overexpressed in solid tumors and has been proposed as a biomarker for early diagnosis and prognosis in liver cancer.[16] Additionally, it has been suggested as a biomarker involved in tumor vascularization in breast[17] and ovarian cancers.[18] In a study by Charfi et al. in 2011, GPM6B was first proposed as a potential marker for B-ALL.[19] Further research in 2012 showed that GPM6B expression was specifically increased in pre-B-ALL cases at the time of diagnosis,[20] while another study by Charfi et al. in 2014 indicated that GPM6B may contribute to both adult and childhood B-ALL, potentially functioning as a proto-oncogene.[21]

Contrary to the literature, our study showed a decrease in GPM6B expression levels in pre-B-ALL cases diagnosed with relapse compared to the control group. However, no statistically significant difference was observed ($p> 0.05$). Furthermore, when examining the relationship between GPM6B mRNA expression levels and relapse duration in bone marrow samples taken at the time of diagnosis, no statistically significant correlation was found. This may be due to the limited sample size, and future studies with larger patient cohorts are needed to confirm these findings. More extensive research incorporating functional assays and proteomic analysis could provide deeper insights into the role of GPM6B in leukemia progression and relapse mechanisms.

Based on our findings and previous studies, a possible hypothesis for the lower incidence of GPM6B expression in our cohort could be linked to its regulatory role in pre-B cell differentiation and leukemogenesis. It is plausible that epigenetic modifications, such as DNA methylation or histone modifications, could suppress GPM6B transcription in relapsed cases, leading to decreased gene expression. Additionally, microRNA-mediated post-transcriptional regulation may also contribute to the observed downregulation of GPM6B in our study. Given that GPM6B has been implicated in neural and hematopoietic cell differentiation, its altered expression in leukemic cells could be context-dependent and influenced by disease stage or treatment-related factors. Future research should investigate these regulatory mechanisms to clarify the role of GPM6B in pre-B-ALL relapse.

Nudix hydrolase 15 (NUDT15) abolishes the cytotoxic effects of 6-mercaptopurine(6-MP).[22,23] Since the NUDT15 variant (c.415C>T, rs116855232) associated with thiopurine toxicity causes amino acid substitution (p.R139C) in both NUDT15*2 and NUDT15*3 haplotypes, in patients with this variant require a reduction in the standard administered dose.[22-32] Although studies have generally focused on the dose adjustment and cytotoxicity of the SNP, there are no studies on the risk of relapse in early diagnosis. In our study, as a result of qRT-PCR analysis performed to determine early diagnosis and relapse risk by using a primer specific for the c.415C> T variant, an increase in expression level was observed in childhood pre-B ALL cases compared to the control group, while very low levels of expression were found in 6 cases (25%). However, no significant difference was found as a result of statistical analysis ($p> 0.05$). Since the most important factor, in these results, is the small sample size and the frequency of this SNP, more research is needed to define the correlation between the relapse rate in ALL and the SNP of NUDT15 gene.

There is increasing data on the pathological role played by S100A8 in the development of hematological malignancies, particularly acute leukemias. Although the S100A8 gene is expressed by normal myeloid cells, studies have generally focused on AML because it is not expressed in lymphocyte cells.[33-39] However, in a meta-analysis study,

S100A8 was shown to be the most expressed gene in relapsed B-ALL compared to diagnosis. In addition, in this study, S100A8 was determined as a biomarker and therapeutic target for the early diagnosis of relapsed B-ALL.[5] Saint Fleur-Lominy et al. obtained similar results in a study in childhood B-ALL cases.[40] In the other two studies, a significant increase in the expression of the S100A8 gene was found in the most aggressive forms of B-ALL, as well as in prednisolone-resistant ALL with MLL gene rearrangement.[41,42] Two separate studies have suggested that S100A8 may play a role in the development of drug resistance in leukaemia cells through autophagy in childhood ALL and that S100A8 may be promising as a new marker.[43,44]

On the other hand, in another study in ALL, it was shown that S100A8 gene expression decreased in 26 childhood pre-B ALL cases with glucocorticoid resistance.[45] It was found that S100A8 mRNA expression levels in 14 patients with childhood ALL did not differ significantly between the control group.[36] No statistically significant difference was found in S100A8 gene expression as a result of gene expression analysis performed in samples taken at 3 different periods (diagnosis, 4 weeks after starting treatment, 8 weeks after starting treatment) from 73 patients with B-ALL.[46] Similar to the last two studies, statistically significant difference in the expression level of the S100A8 gene was not found ($p > 0.05$) in our 15 patients with childhood pre-B ALL at the time of diagnosis compared to the control group. The clinical significance of S100A8 in childhood pre-B-ALL cases is still not fully known and further studies are needed at both mRNA and protein levels to obtain results that are more realistic.

Survivin is the smallest member of the inhibitor of apoptosis protein (IAP) family and has been proposed as a promising target and prognostic marker for cancer therapy due to its overexpression in cancer cells compared to normal tissues.[47] Studies in childhood B-ALL cases have shown that Survivin expression levels increase at relapse compared to diagnosis and Survivin has been proposed as a potential biomarker for determining the prognosis of B-ALL in the future.[5,48] In a study by Troeger et al. in 66 cases, overexpression of Survivin in samples taken at the time of diagnosis was defined as a strong risk factor for relapse in childhood pre-B-ALL.[49] In this study, Survivin expression level was found to be statistically significantly higher at the diagnosis time of our 15 childhood B-ALL patients when compared to the control group ($p = 0.35$). The fact that this result is also compatible with the study of Troeger et al., in which they showed an increase in the expression of Survivin at the time of diagnosis, shows that the Survivin gene may be the most important marker in determining the risk of relapse at the time of diagnosis. There is strong evidence that AURKA and Survivin are simultaneously overexpressed in various malignancies such as gastric[50] breast[51] and chronic lymphocytic leukemia.[52] In a study in gastric cancer, it was reported that overexpression of the AURKA gene played a role in the stability of Survivin by upregulating Survivin expression.[45] Parallel to these studies, a very strong positive correlation was found between AURKA and Survivin expressions in childhood pre-B ALL patients in our study ($r = 0.934$; $p < 0.0001$).

In conclusion, AURKA, CASP1, and Survivin genes are suggested as candidate biomarkers for relapse prediction at the time of diagnosis based on the expression levels of six relapse-related genes in pediatric pre-B ALL. However, these findings should be confirmed in larger patient cohorts. In addition, it is thought that these results should be strengthened with studies at the protein level showing the conversion of these genes into products.

Funding: *This study was funded by Akdeniz University Research Foundation (TYL-2020-5172).*

REFERENCES

1. Tasian SK, Loh ML, Hunger P. Childhood acute lymphoblastic leukemia: Integrating genomics into therapy. Cancer 121: 3577-3590, 2015.
2. Tasian SK, Hunger SP. Genomic characterization of paediatric acute lymphoblastic leukaemia: an opportunity for precision medicine therapeutics. Br J Haematol 176: 867-882, 2017.
3. Heikamp EB, Pui CH. Next-Generation Evaluation and Treatment of Pediatric Acute Lymphoblastic Leukemia. J Pediatr 203:14-24.e2, 2018.

4. Schultz KR, Pullen DJ, Sather HN, et al. Risk- and response-based classification of childhood B-precursor acute lymphoblastic leukemia: a combined analysis of prognostic markers from the Pediatric Oncology Group (POG) and Children's Cancer Group (CCG). Blood 109: 926-935, 2007.

5. Chow YP, Alias H, Jamal R. Meta-analysis of gene expression in relapsed childhood B-acute lymphoblastic leukemia. BMC Cancer 17: 120, 2017.

6. Sun W, Malvar J, Sposto R, et al. Outcome of children with multiply relapsed B-cell acute lymphoblastic leukemia: a therapeutic advance in childhood leukemia & lymphoma study. Leukemia 32: 2316-2325, 2018.

7. Durlacher CT, Li ZL, Chen XW, et al. An update on the pharmacokinetics and pharmacodynamics of alisertib, a selective Aurora kinase A inhibitor. Clin Exp Pharmacol Physiol 43: 585-601, 2016.

8. Lucena-Araujo AR, de Oliveira FM, Leite-Cueva SD, et al. High expression of AURKA and AURKB is associated with unfavorable cytogenetic abnormalities and high white blood cell count in patients with acute myeloid leukemia. Leuk Res 35: 260-264, 2011.

9. Long M, Hao M, Dong K, et al. AEG-1 overexpression is essential for maintenance of malignant state in human AML cells via up-regulation of Akt1 mediated by AURKA activation. Cell Signal 25: 1438-1446, 2013.

10. Heo SK, Noh EK, Jeong YK, et al. Radotinib inhibits mitosis entry in acute myeloid leukemia cells via suppression of Aurora kinase A expression. Tumour Biol 41: 1010428319848612, 2019.

11. Hartsink-Segers SA, Zwaan CM, Exalto C, et al. Aurora kinases in childhood acute leukemia: the promise of aurora B as therapeutic target. Leukemia 27: 560-568, 2013.

12. Fusco R, Siracusa R, Genovese T, et al. Focus on the Role of NLRP3 Inflammasome in Diseases. Int J Mol Sci 21: 1-15, 2020.

13. Sharma BR, Kanneganti TD. NLRP3 inflammasome in cancer and metabolic diseases. Nat Immunol 22: 550-559, 2021.

14. Paugh SW, Bonten EJ, Savic D, et al. NALP3 inflammasome upregulation and CASP1 cleavage of the glucocorticoid receptor cause glucocorticoid resistance in leukemia cells. Nat Genet 47: 607-614, 2015.

15. Laskowska J, Lewandowska-Bieniek J, Szczepanek J, et al. Genomic and transcriptomic profiles and in vitro resistance to mitoxantrone and idarubicin in pediatric acute leukemias. J Gene Med 18: 165-179, 2016.

16. Stefanska B, Bouzelmat A, Huang J, et al. Discovery and validation of DNA hypomethylation biomarkers for liver cancer using HRM-specific probes. PLoS One 8(8): e68439, 2013.

17. Bilecova-Rabajdova M, Urban P, Gregova K, Varga J, et al. Breast carcinoma progression and tumour vascular markers related to apoptotic mechanisms. Dis Markers 156034, 2014.

18. Buckanovich RJ, Sasaroli D, O'Brien-Jenkins A, et al. Tumor vascular proteins as biomarkers in ovarian cancer. J Clin Oncol 25: 852-861, 2007.

19. Charfi C, Voisin V, Levros LC, et al. Gene profiling of Graffi murine leukemia virus-induced lymphoid leukemias: Identification of leukemia markers and Fmn2 as a potential oncogene. Blood 117: 1899-1910, 2011.

20. Tesfai Y, Ford J, Carter KW, et al. Interactions between acute lymphoblastic leukemia and bone marrow stromal cells influence response to therapy. Leuk Res 36: 299-306, 2012.

21. Charfi C, Edouard E, Rassart E. Identification of GPM6A and GPM6B as potential new human lymphoid leukemia-associated oncogenes. Cell Oncol (Dordr) 37: 179-191, 2014.

22. Singh M, Bhatia P, Khera S, Trehan A. Emerging role of NUDT15 polymorphisms in 6-mercaptopurine metabolism and dose related toxicity in acute lymphoblastic leukaemia. Leuk Res 62: 17-22, 2017.

23. Cheng J, Zhang H, Ma HZ, Li J. Homozygous mutation in NUDT15 in childhood acute lymphoblastic leukemia with increased susceptibility to mercaptopurine toxicity: A case report. Exp Ther Med 17: 4285-4288, 2019.

24. Tanaka Y, Kato M, Hasegawa D, et al. Susceptibility to 6-MP toxicity conferred by a NUDT15 variant in Japanese children with acute lymphoblastic leukaemia. Br J Haematol 171: 109-115, 2015.

25. Kodidela S, Dorababu P, Thakkar DN, et al. Association of NUDT15*3 and FPGS 2572C>T variants with the risk of early hematologic toxicity during 6-MP and low-dose methotrexate-based maintenance therapy in Indian patients with acute lymphoblastic leukemia. Genes (Basel) 11: 594, 2020.

26. Yang JJ, Landier W, Yang W, et al. Inherited NUDT15 variant is a genetic determinant of mercaptopurine intolerance in children with acute lymphoblastic leukemia. J Clin Oncol 33: 1235-1242, 2015.

27. Cargnin S, Genazzani AA, Canonico PL, Terrazzino S. Diagnostic accuracy of NUDT15 gene variants for thiopurine-induced leukopenia: a systematic review and meta-analysis. Pharmacol Res 135: 102-111, 2018.

28. Moriyama T, Nishii R, Lin TN, et al. The effects of inherited NUDT15 polymorphisms on thiopurine active metabolites in Japanese children with acute lymphoblastic leukemia. Pharmacogenet Genomics 27: 236-239, 2017.

29. Puangpetch A, Tiyasirichokchai R, Pakakasama S, et al. C. NUDT15 genetic variants are related to thiopurine-induced neutropenia in Thai children with acute lymphoblastic leukemia. Pharmacogenomics 21: 403-410, 2020.

30. Chiengthong K, Ittiwut C, Muensri S, et al. NUDT15 c.415C>T increases risk of 6-mercaptopurine induced myelosuppression during maintenance therapy in children with acute lymphoblastic leukemia. Haematologica 101: e24-26, 2016.

31. Relling MV, Schwab M, Whirl-Carrillo M, et al. Clinical Pharmacogenetics Implementation Consortium Guideline for Thiopurine Dosing Based on TPMT and NUDT15 Genotypes: 2018 Update. Clin Pharmacol Ther 105: 1095-1105, 2019.

32. Texis T, Guzman-Cruz C, Rodriguez-Dorantes M, et al. Genotyping NUDT15*3 rs1166855232 reveals higher frequency of potential adverse effects of thiopurines in Natives and Mestizos from Mexico. Pharmacol Rep 74(1): 257-262, 2022.
33. Damm F, Thol F, Hollink I, et al. Prevalence and prognostic value of IDH1 and IDH2 mutations in childhood AML: a study of the AML-BFM and DCOG study groups. Leukemia 25: 1704-1710, 2011.
34. Nicolas E, Ramus C, Berthier S, et al. Expression of S100A8 in leukemic cells predicts poor survival in de novo AML patients. Leukemia 25: 57-65, 2011.
35. Yan-Fang T, Dong W, Li P, et al. Analyzing the gene expression profile of pediatric acute myeloid leukemia with real-time PCR arrays. Cancer Cell Int 12: 40, 2012.
36. Yang L, Yang M, Zhang H, et al. S100A8-targeting siRNA enhances arsenic trioxide-induced myeloid leukemia cell death by down-regulating autophagy. Int J Mol Med 29: 65-72, 2012.
37. Yang XY, Zhang MY, Zhou Q, et al. High expression of S100A8 gene is associated with drug resistance to etoposide and poor prognosis in acute myeloid leukemia through influencing the apoptosis pathway. Onco Targets Ther 9: 4887-4899, 2016.
38. Karjalainen R, Liu M, Kumar A, et al. Elevated expression of S100A8 and S100A9 correlates with resistance to the BCL-2 inhibitor venetoclax in AML. Leukemia 33: 2548-2553, 2019.
39. Mondet J, Laurin D, Lo Presti C, et al. Increased S100A8 expression in bone marrow plasma by monocytic cells from acute myeloid leukemia patients. Hematol Oncol 38: 114-118, 2020.
40. Saint Fleur-Lominy S, Evensen NA, Bhatla T, et al. Evolution of the epigenetic landscape in childhood B acute lymphoblastic leukemia and its role in drug resistance. Cancer Res 80: 5189-5202, 2020.
41. Mousavian Z, Nowzari-Dalini A, Stam RW, et al. Network-based expression analysis reveals key genes related to glucocorticoid resistance in infant acute lymphoblastic leukemia. Cell Oncol (Dordr) 40: 33-45, 2017.
42. Spijkers-Hagelstein JA, Schneider P, Hulleman E, et al. W. Elevated S100A8/S100A9 expression causes glucocorticoid resistance in MLL-rearranged infant acute lymphoblastic leukemia. Leukemia 26: 1255-1265, 2012.
43. Ouyang MF, Wang D, Liu YT, et al. [Value of S100A8 in evaluating the prognosis of children with acute lymphoblastic leukemia]. Zhongguo Dang Dai Er Ke Za Zhi 21: 359-364, 2019.
44. Yu R, Zhang J, Zang Y, et al. iTRAQ-based quantitative protein expression profiling of biomarkers in childhood B-cell and T-cell acute lymphoblastic leukemia. Cancer Manag Res 11: 7047-7063, 2019.
45. Sarang Z, Gyurina K, Scholtz B, et al. Altered expression of autophagy-related genes might contribute to glucocorticoid resistance in precursor B-cell-type acute lymphoblastic leukemia. Eur J Haematol 97: 453-460, 2016.
46. Garza-Veloz I, Martinez-Fierro ML, Jaime-Perez JC, et al. Identification of differentially expressed genes associated with prognosis of B acute lymphoblastic leukemia. Dis Markers 828145, 2015.
47. Chen X, Duan N, Zhang C, Zhang W. Survivin and tumorigenesis: Molecular mechanisms and therapeutic strategies. J Cancer 7: 314-323, 2016.
48. Mohammadi M, Amirmahani F, Goharrizi KJ, et al. Evaluating the expression level of Survivin gene in different groups of B-cell acute lymphoblastic leukemia patients of Iran. Mol Biol Rep 46: 2679-2684, 2019.
49. Troeger A, Siepermann M, Escherich G, et al. Survivin and its prognostic significance in pediatric acute B-cell precursor lymphoblastic leukemia. Haematologica 92: 1043-1050, 2007.
50. Kamran M, Long ZJ, Xu D, et al. Aurora kinase A regulates Survivin stability through targeting FBXL7 in gastric cancer drug resistance and prognosis. Oncogenesis 6: e298, 2017.
51. Roylance R, Endesfelder D, Jamal-Hanjani M, et al. Expression of regulators of mitotic fidelity are associated with intercellular heterogeneity and chromosomal instability in primary breast cancer. Breast Cancer Res Treat 148: 221-229, 2014.
52. de Paula Careta F, Gobessi S, Panepucci RA, et al. The Aurora A and B kinases are up-regulated in bone marrow-derived chronic lymphocytic leukemia cells and represent potential therapeutic targets. Haematologica 97: 1246-1254, 2012.

Correspondence:

Dr. Sibel BERKER KARAUZUM

Akdeniz University, Faculty of Medicine,

Department of Medical Biology and Genetics,

07058 Konyaalti

ANTALYA / TURKIYE

Tel: (+90-532) 727 89 41

e-mail: sibelberkerkarauzum@gmail.com

ORCIDs:

Oznur Tokta	0000-0003-2414-1601
Sibel Berker Karauzum	0000-0001-6415-3215
Ayse Esra Manguoglu	0000-0002-6214-622x
Osman Alphan Kupesiz	0000-0001-8827-5567
Funda Tayfun Kupesiz	0000-0003-2513-7188
Elif Guler	0000-0001-8072-2561
Ayse Cigdem Sivrice	0000-0002-3332-8120
Ayse Nur Akinel	0000-0001-6869-8802
Kemal Hakan Gülkesen	0000-0002-2477-2481