



Research Article

# Expression Profile of LncRNAs in Childhood Acute Lymphoblastic Leukemia: A Pilot Study

Zohreh Mousavi<sup>1</sup>, Saeid Ghorbian<sup>1\*</sup>, Azim Rezamand<sup>2</sup>, Leyla Roshangar<sup>3</sup>, Behboud Jafari<sup>4</sup>

<sup>1</sup>Department of Molecular Genetics, Ahar Branch, Islamic Azad University, Ahar, Iran.

<sup>2</sup>Pediatric Health Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

<sup>3</sup>Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

<sup>4</sup>Department of Microbiology, Ahar Branch, Islamic Azad University, Ahar, Iran.

## Article Info

### Article History:

Received: 22 August 2020

Accepted: 12 October 2020

ePublished: 29 October 2020

### Keywords:

- Acute lymphoblastic leukemia
- Immunophenotype
- Long non-coding RNA
- Oncogenesis

## Abstract

**Background:** Childhood acute lymphoblastic leukemia (ALL) explains 26% of pediatric malignancies and is one of the leading causes of disease-related deaths in children. A novel molecular class of non-coding genes, long non-coding RNAs (lncRNAs) having over 200 nucleotides, have been defined as regulators of different cellular processes including pluripotency, oncogenesis, and transcription. It has been demonstrated that lncRNA transcription profiles can distinguish pre B-cell subtype of ALL accurately and act as early diagnostic and prognostic biomarkers. Hence, the aim of this pilot study was the prior evaluation of expression profile of several lncRNA candidates including RP11-68I18.10, RP11-624C23.1, RP11-446E9, RP11-137H2.4, and RP11-203E8 in patients with ALL.

**Methods:** In this study, 80 blood samples were obtained from patients, definitely diagnosed by pathologists with ALL, and from healthy subjects. Total RNA was extracted from blood samples, and cDNA was synthesized. Real-time PCR was applied to determine the expression of lncRNAs. A *P*-value of 0.010 was considered statistically significant.

**Results:** Our findings revealed that the expression levels of lncRNAs RP11-624C23.1, RP11-446E9, RP11-137H2.4, RP11-68I18.10, and RP11-203E8 were significantly decreased in ALL samples compared to those of healthy samples ( $P < 0.0001$ ,  $P = 0.0616$ ,  $P = 0.0292$ ,  $P < 0.0001$ , and  $P = 0.0007$ ). Moreover, the relationship between these five lncRNA expression changes and the immunophenotype in ALL patients was not significant.

**Conclusion:** The dysregulation of lncRNAs in ALL samples could provide a novel and interesting possibility for early diagnosis and prognosis, as well as mastering the treatment of ALL.

## Introduction

Acute lymphoblastic leukemia (ALL), also called acute lymphocytic leukemia, is a tumor type characterized by the prevalence of premature lymphocytes formed by the bone marrow.<sup>1</sup>

It is a form of blood and bone marrow malignancy that damages white and red blood cells, as well as platelets.<sup>2,3</sup> Childhood ALL is highly prevalent in children and adolescents and accounts for a quartile of all childhood cancers. Leukemia is a type of hematological disorder. It is characterized by the oligoclonal expansion of hematopoietic cells that have been abnormally or poorly differentiated and hence can infiltrate the bone marrow and even invade the blood and other tissues.<sup>4,5</sup>

Generally, there are four main subtypes of leukemia, based on whether they are acute or chronic, and myeloid or lymphocytic.<sup>6,7</sup> Accordingly, chronic lymphoblastic leukemia (CLL), acute lymphoblastic leukemia (ALL),

chronic myeloid leukemia (CML), and acute myeloid leukemia (AML) have been defined.<sup>2,8,9</sup> Childhood ALL, as abovementioned, is a frequent malignancy in children and adolescents aged 1 to 14 years old and accounts for ≈25% of all childhood leukemia cases. Precursor B cell ALL (pre B-ALL) is a main type of ALL comprising 85% of ALL cases.<sup>10</sup> Several studies have reported that the expression of some molecular markers may contribute to the classification of subtypes, as well as improving the ALL prognosis.<sup>11,12</sup>

lncRNAs as a new type of molecules play controlling roles on several processes, including tumorigenesis and pluripotency. They are also identified with functional roles.<sup>2,7,13,14</sup> Some RNAs (i.e., rRNAs, non-messenger RNAs (nmRNAs), or non-protein-coding RNAs (npcRNAs)), are practical RNA molecules which do not code proteins.

Although the implication of lncRNAs has been verified in

\*Corresponding Author: Saeid Ghorbian, E-mail: [ghorbian20@gmail.com](mailto:ghorbian20@gmail.com)

©2021 The Author(s). This is an open access article and applies the Creative Commons Attribution License (<http://creativecommons.org/licenses/by-nc/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, as long as the original authors and source are cited.

ALL initiation, progression, and treatment response, their exact functional role has poorly been understood.<sup>15-17</sup>

lncRNAs, exceeding 200 nucleotides in length, are defined as regulators of different cellular processes such as pluripotency, reshaping of chromosomes, transport of intracellular molecules, oncogenesis, and transcription. In this regard, the tissue specific expression of lncRNAs has been described despite their predominantly low abundance. Therefore, the study of tissue specificity of lncRNAs could be interesting due to their role in cell migration, proliferation, reaction to cytotoxic drugs, and DNA damage especially in tumor cells. For example, Ghazavi *et al.*<sup>18</sup> reported the changes in the expression of lncRNAs in ALL cancer.

Another study also emphasized the association of lncRNAs with leukemia development. Moreover, lncRNAs BALR-2 and BALR-6 were shown to be involved in cell endurance or glucocorticoid reaction, both in human and mouse B cells.<sup>14,19,20</sup> Furthermore, it was revealed that transcription profiles of lncRNAs can distinguish pre-B ALL subtype precisely and can act as prognostic biomarkers.<sup>11,21</sup> Further investigations have explored the diagnostic potentials of lncRNAs in ALL, as well as their biological and epigenetic activities. Despite these efforts, the diversity of actions and complex molecular interactions coupled with the contrasting subtypes of lncRNAs still mask a complete comprehension of therapeutic improvement.<sup>16,17,22</sup> Intergenic lncRNA RP11-68I18.10 is located on chromosome 1. This lncRNA is overexpressed in cardiac and skeletal muscles.<sup>23</sup> Ouimet *et al.*<sup>24</sup> showed that lncRNAs *RP11-137H2.4* and *RP11-68I18.10* were downregulated in pre-B ALL. The *RP11-137H2.4* gene is located on chromosome 10 and is an antisense lncRNA type. lncRNAs *RP11-624C23* and *RP11-203E8* are respectively intronic and intergenic, and their genes are located on chromosome 8.

Previous studies have shown that overexpression of this lncRNA in pre B-ALL cells can induce malignancy. In some studies, it has been shown that three lncRNAs, *RP11-446E9*, *RP11-624C23*, and *RP11-203E8* were downregulated in pre B-ALL.<sup>11,21</sup>

In this regard, our research focused on deciphering the genetic changes and expression of lncRNAs *RP11-624c23.1*, *RP11-137H2.4*, *RP11-203E8*, *RP11-446E9*, and *RP11-68118.10* in patients with ALL in order to understand their role in disease pathogenesis and also their diagnostic potential.

## Material and Methods

### Study subjects

In this study, we obtained 160 blood samples. Eighty samples were obtained from patients, who had referred to "Tabriz Pediatric Hospital" during the years 2018-2019, and definitely diagnosed with leukemia. While, remaining 80 samples were obtained from healthy volunteers and used as control. The study subjects were children between the ages 1 and 14 who were pathologically diagnosed with lymphoblastic malignancy. The samples were taken after

a written consent form was signed by the parents or legal guardians of the children. For the inclusion criteria, patients with no history of acute viral infection, autoimmune diseases, and endocrine disorders were considered. The control group included samples obtained from healthy subjects. Approval for this study was obtained from the Ethics Committee of Tabriz University of Medical Sciences. During sampling procedures, all ethical standards were regarded to ensure the patients' safety as prescribed by the medical guidelines of Tabriz Pediatric Hospital. All of the clinical information and the initial diagnosis required from the patient's pathology results file was used as a proprietary code. The patient population underwent an initial sampling before starting chemotherapy.

### Sampling

Intravenous blood samples were obtained from each individual in both groups. Samples were put into Falcon tubes containing EDTA as an anticoagulant. Then the samples were transferred to the laboratory, where they were preserved at -80°C freezing conditions.

### RNA extraction from blood samples and cDNA synthesis

Total RNA was extracted from the blood samples using Trizol reagent kit (Macherey-Nagel Co, Germany 740304), according to the manufacturer's instructions. To test the quality of extracted RNA, it was run on 0.5% agarose gel. Completely dissolved RNA was appeared as a very low molecular weight smear. A UV spectrophotometer (NanoDrop ND-1000; Wilmington, DE) with an absorbance ratio of 260/280 was used to measure the concentration of the extracted RNA. The extracted RNA was then stored in RNase-free ultra-pure water at -70 °C. Afterward, cDNA was synthesized from the extracted RNA using the Quantitative Test Reverse Transcription Kit (Qiagen, Catalog No: 205310), with all the steps performed on ice as summarized below.

### RT-qPCR (quantitative reverse transcription-PCR)

We used the RT-qPCR technique to identify gene expression changes of lncRNAs *RP11-203E8*, *RP11-624c23.1*, *RP11-446E9*, *RP11-137H2.4*, and *RP11-68118.10* in ALL and normal samples. Specific primers for the target genes were designed to examine their expression at the RNA level in the Gene runner software (Version 3.05). To avoid binding of designed primers to other sequences similar to the target gene sequences, BLAST<sup>1</sup> was run ([www.ncbi.nlm.nih.gov/BLAST](http://www.ncbi.nlm.nih.gov/BLAST)). To detect changes in each gene expression in ALL and control samples, RT-qPCR was accomplished using the following monitored primers:

for lncRNA *RP11-624C23.1*: forward: 5'-GGGTCTTACCCGACGTGAG-3' and reverse: 5'-CGCGTGTTGAGGACTACAGA-3', for lncRNA *RP11-203E8*: forward: 5'-TGCATTATCTCCCAACACCA-3' and reverse: 5'-AGGTTCTCTCCTTCTCCA-3', for lncRNA; *RP11-446E9*: forward:

5'-CCAGAGCAGAGCATCAAGAG-3' and reverse: 5'-GAAGCACTTTGGGAAATGGA-3'; for lncRNA *RP11-137H2.4*: forward: 5'-TGCAAGTACTGGAAACCAC-3' and reverse: 5'-CGACGAAACAGTCGACTTCA-3'; for lncRNA *RP11-68I18.10*: forward: 5'-GCGAAGGCATGGTATCTTGT-3' and reverse: 5'-AAAGGGAAAGGGATTGCCTA-3'; and for GAPDH gene: forward: 5'-GCCGTCTAGAAAAACCTGCC-3' and reverse: 5'-ACCACCTGGTGCTCAGTGTA-3'.

RT-qPCR was performed to investigate the level of expression changes in ALL and healthy samples in two practical repeats. Briefly, 2 µL cDNA (2.5 ng/µL) was added to 3 µL of PCR mix (2.5 µL 2X SsoAdvanced mastermix, 0.25 µL (5µM) forward primer, and 0.25 µL (5µM) reverse primer.

Cycling factors were 95°C for 10 min, 40 cycles (95°C for 15 s, 60°C for 1 min), monitored by denaturation curvature at 60°C. GAPDH was used as the reference gene. Expression rates were also analyzed with 2-(ΔΔCT), as per Livak and Schmittgen.<sup>25</sup>

**Statistical analysis**

Statistical analysis of data was done by SPSS software (Ver 21-IBM USA). A P-value of 0.01 was considered statistically significant in ALL evaluations. Descriptive tests such as t-test and Mann-Whitney U test were used to analyze the information, with the corresponding tables, graphs, frequencies, and illustrations. For associating the relationship between the lncRNAs gene expression modifications and the clinicopathological characteristics of ALL samples and control samples, Chi-square test was used.

**Results**

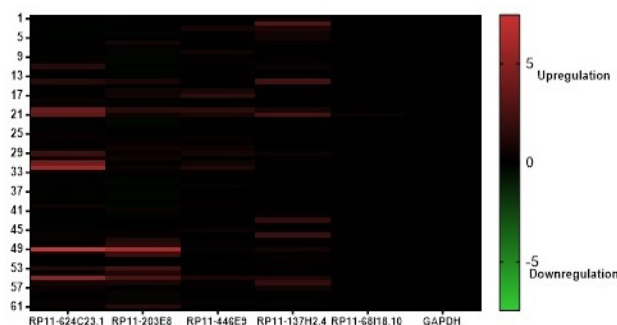
To investigate the gene expression variations of lncRNAs *RP11-624c23.1*, *RP11-203E8*, *RP11-137H2.4*, *RP11-446E9*, and *RP11-68118.10* in ALL and healthy samples, we determined the stages of lncRNAs, considering immunotype 24 (30%) T cell, 9 (11.25%) pro B-cell (early B-cell), 33 (41/25%) pre B-cell, 9 (11.25 %) B-cell, and 6(6.25 %) mixed ALL.

In this study, the association of lncRNAs *RP11-624c23.1*, *RP11-203E8*, *RP11-446E9*, *RP11-137H2.4*, and *RP11-68118.10* expression levels was assessed with clinical immune-phenotype features of ALL patients (Table 1). The median amount >1 of the lncRNAs expression levels was considered as an upregulation cluster, and the median amount ≤1 was designated as a downregulation group. The association between these two variables was not significant. The expression profile of these five lncRNAs was meaningfully downregulated in ALL patients compared to the control group (P<0.0001, P = 0.0007, P=0.0616, P=0.0292, P<0.0001) (Figure 1).

Moreover, the findings showed that the lncRNAs *RP11-624c23.1* gene expression was significantly decreased in ALL samples compared to the control samples (P <0.0001) (Figure 2).

In addition, the relationship between the lncRNAs- *RP11-624c23.1* expression changes and the immune-phenotype was studied in ALL patients. The results revealed that lncRNA *RP11-624c23.1* expression changes did not significantly associate with the immune-phenotype in ALL patients (P=0.963) (Table 1).

lncRNA *RP11-203E8* was downregulated in ALL patients compared to the healthy people (P=0.0007) (Figure 3). In addition, the association between the expression variation of lncRNA *RP11-203E8* and immune-phenotype in ALL patients was not significant (P=0.161) (Table 1).

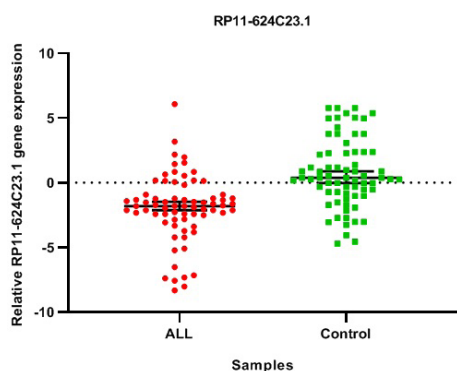


**Figure 1.** The relative expression of lncRNAs *RP11-624c23.1*, *RP11-137H2.4*, *RP11-203E8*, *RP11-446E9*, *RP11-68118.10* in patients with ALL and in normal samples

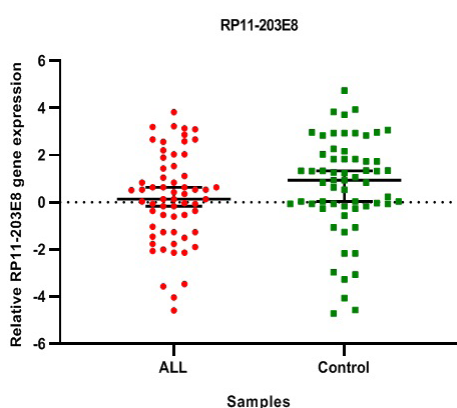
**Table 1.** Association of lncRNAs *RP11-624c23-1*, *RP11-203E8*, *RP11-446E9*, *RP11-137H2-4*, *RP11-68118-10* expression levels with clinical immunophenotypic features of ALL patients.

Regulation	RP11-203E8		RP11-624c23-1		RP11-446E9		RP11-137H2-4		RP11-68118-10	
	DR <sup>a</sup>	UR <sup>b</sup>	DR <sup>a</sup>	UR <sup>b</sup>	DR <sup>a</sup>	UR <sup>b</sup>	DR <sup>a</sup>	UR <sup>b</sup>	DR <sup>a</sup>	UR <sup>b</sup>
<b>Value</b>	≤1	<1	≤1	<1	≤1	<1	≤1	<1	≤1	<1
<b>T. Cell</b>	29.1	5.9	30.8	4.4	19.1	16.1	26.4	17.5	30	-
<b>Pro B Cell (Early B Cell)</b>	10.3	2.9	10.2	2.8	8.8	4.4	11.7	1.4	11.25	-
<b>Pre B Cell</b>	17.5	3.6	28.9	4.4	26.5	5.9	27.9	14.5	41.25	-
<b>B Cell</b>	11.8	1.5	11.6	1.4	10.3	2.9	11.8	1.5	11.2	-
<b>Mixed</b>	5.8	1.6	5.9	1.5	7.3	1.3	4.4	2.9	6.3	-
<b>P-value</b>	0.161		0.963		0.286		0.55		-	

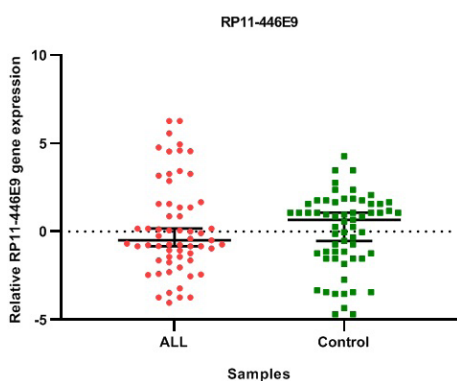
<sup>a</sup>Down regulation, <sup>b</sup>Up regulation



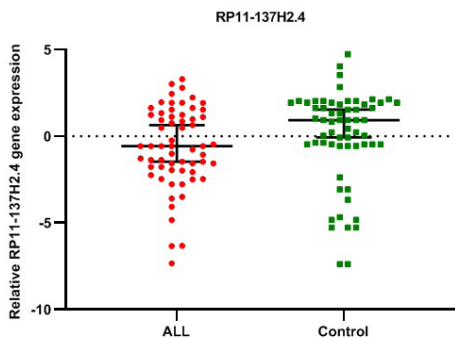
**Figure 2.** The comparison of lncRNA *RP11-624C23.1* expression level between ALL samples and normal samples.



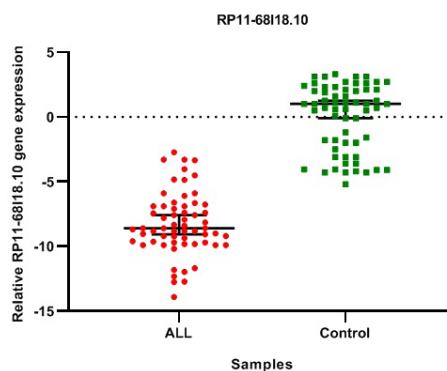
**Figure 3.** The comparison between gene expression levels of lncRNA *RP11-203E8* in ALL samples and normal samples.



**Figure 4.** Comparison of lncRNA *RP11-446E9* gene expressions in ALL samples and normal samples



**Figure 5.** Comparison of lncRNA *RP11-137H2.4* gene expression in ALL samples and normal samples



**Figure 6.** Comparison of lncRNA *RP11-68118.10* gene expressions in ALL samples and normal samples

The lncRNA *RP11-446E9* was downregulated in ALL samples compared to the control samples ( $P=0.616$ ) (Figure 4). Moreover, the association between lncRNA *RP11-446E9* expression variation and immune-phenotype in ALL patients was not significant ( $P=0.286$ ) (Table 1).

The results demonstrated the lncRNA *RP11-137H2.4* was downregulated in ALL samples compared to the control samples ( $P=0.0292$ ) (Figure 5). In addition, the association between the expression changes of this lncRNA and immune-phenotype in ALL patients was not significant ( $P=0.55$ ) (Table 1).

lncRNA *RP11-68118.10* was downregulated in ALL samples compared to the control samples ( $P<0.0001$ ) (Figure 6). Additionally, the association between the expression variation of this lncRNA and immune-phenotype in ALL patients was not significant (Table 1).

## Discussion

The current study evaluated the expression levels of lncRNAs *RP11-137H2.4*, *RP11-203E8*, *RP11-446E9*, *4RP11-624C23*, and *RP11-68118.10* in patients who were positively diagnosed with Acute Lymphoblastic Leukemia (ALL) and their possible alterations compared to the normal cases.

ALL is one of the most prevalent cancers among children with an average age of  $<10$  years<sup>26</sup> and is defined by the aggressive multiplication of white blood cells and their mal-developed stem cells.<sup>27</sup> ALL is categorized into several groups (mainly based on their CD cell surface markers) with respect to the immune-markers and clinical data, namely: T-cell, Pro B-Cell, Pre B-Cell, B-Cell, and Mixed. This categorization is done mostly based on blood lymphocyte markers (CD markers).<sup>28</sup>

Considering high mortality rate associated with this disease, early diagnosis and appropriate treatment strategies can be very helpful.

One of the most effective strategies for early diagnosis is the use of molecular biomarkers. Investigating the changes in the expression levels of the biomarkers indicates the importance of early diagnosis and treatment.<sup>29</sup> lncRNAs are regarded as one of the most reliable and effective molecular biomarkers. These non-coding molecules, which play significant roles in modifying gene expression

and epigenetic alterations, are expressed in different levels in different cancers such as breast tumor,<sup>30</sup> lung cancer,<sup>31</sup> prostate malignancy,<sup>32</sup> and ALL.<sup>33,34</sup> Therefore, evaluation of the expression levels of these molecules at different stages of cancer may present new biomarkers for cancer detection.

Changes in the expression of lncRNAs can subsequently lead to changes in the expression of target genes, in addition to stimulation of changes in the dependent signaling pathways. Thus, the study of lncRNAs can be of considerable importance while investigating the transcription level and subsequent translation. Furthermore, expression of lncRNAs can lead to changes in the induction of signal pathways such as RAS / MAP kinase, NFkB, AKT, b-catenin, and so on.<sup>35</sup>

In addition to the abovementioned, various lncRNAs have shown different expression levels in various cancers, including breast, prostate, and gastric, as well as acute lymphoid carcinoma in children, and the association with cancer phenotypes such as migration, metastasis, and apoptosis has also been observed. A study examined the changes in the expression of lncRNAs in ALL cancer and proved it.

Fernando *et al.*<sup>37</sup> displayed that CASC15 lncRNA could regulate the SOX4 gene in acute myeloid leukemia (AML). This gene confers a critical role in the development and evolution of B-cells. Furthermore, Sox4 plays an important role in the B-catenin signaling pathway, and consequently, altering the expression level of the lncRNA CASC15 can induce changes in this signaling pathway. It displays the importance of evaluating the investigated lncRNAs.<sup>37,38</sup>

In a study by Wallaert *et al.*<sup>39</sup> on important subclasses of lncRNAs for each of the T-ALL genetic subclasses, linked pattern of lncRNA expression in T-ALL subclasses with diverse stages of healthy T cell evolution in the thymus was evaluated. Similarly, Casero *et al.*<sup>40</sup> in their study, concluded that the co-expression of protein-coding genes near lncRNA genes demonstrated development for oncologies associated with the lymphoid variation.

We chose lncRNAs *RP11-68I18.10*, *RP11-137H2.4*, *RP11-446E9*, *RP11-624C23.1*, and *RP11-203E8* and investigated the expression profile of these five lncRNAs in ALL patients. Recent studies have shown that these five lncRNAs significantly affect different cellular processes in pre B-ALL. The relationship between the immune-phenotype of ALL samples and the expression level of each lncRNA was also evaluated.

In the present study, we identified and analyzed the medical and pathological indication of immune-phenotype in patients with ALL, and assessed their association with the expression levels of the abovementioned lncRNAs one by one. Analysis of samples by classifying ALL in each case and comparing the expression level of each lncRNA showed no significant relationship between immune-phenotype of ALL samples and expression level of each lncRNA.

To confirm the findings of the current study and the expression changes of lncRNAs in ALL and healthy

samples, Fang *et al.*<sup>41</sup> evaluated the expression levels of different lncRNAs in MLL-r cancer, and revealed changes in the expression levels of many of these lncRNAs. These changes in expression levels were compared between the control and unhealthy samples. The results of this study are consistent with those of our study in that they indicated a change in the expression of lncRNAs in both leukemia samples and control samples. The results of our study displayed a significant decrease in the expression of lncRNAs *RP11-68I18.10*, *RP11-446E9*, *RP11-624C23.1*, *RP11-203E8*, and *RP11-137H2.4* in ALL samples compared to the healthy control samples. These expression changes can specifically reflect expression changes in a particular type of cancer.

Consistent with the present study, a study was conducted by Gioia *et al.*<sup>11</sup> on 56 pre B-ALL cancer samples to investigate the expression changes and roles of lncRNAs *RP11-203E8*, *RP11-624C23.1*, and *RP11-446E9*. These lncRNAs, which play a role in regulating metastasis and relocation of blood leukemic cells, were down-regulated in this cancer. Fernando *et al.*<sup>37</sup>, illustrated that the gene expression levels of lncRNAs *RP11-446E9* and *RP11-624C23.1* decreased in ALL. Our findings showed that the lncRNA *RP11-624c23.1* expression was significantly decreased in ALL patients compared to the control subjects ( $P < 0.0001$ ). Additionally, we studied the association between lncRNA *RP11-624c23.1* expression variations and immune-phenotype in these ALL patients. Our findings revealed that the alteration in the expression level of this lncRNA was not significantly associated with the immune-phenotype in ALL patients ( $P = 0.963$ ).

Our results revealed that lncRNA *RP11-203E8* was downregulated in ALL patients compared to the control samples ( $P = 0.0007$ ), and the association between the expression level of this lncRNA and immune-phenotype in ALL patients was not significant ( $P = 0.161$ ). Our results also showed that the lncRNA *RP11-446E9* was downregulated in ALL patients ( $P = 0.616$ ). The results of our study about lncRNAs *RP11-203E8* and *RP11-624c23.1* corroborate the results of Fernando *et al.*<sup>37</sup> and is consistent with those of Gioia *et al.*<sup>11</sup> Furthermore, the results of these two articles are in line with those of our study in terms of lncRNA *RP11-446E9* expression in ALL cases.

Increased expression of lncRNAs *RP11-203E8* and *RP11-624C23.1* increases apoptosis and decreases phosphorylation of H2A.X, which is involved in response to DNA damage. Interestingly, the increased expression of both lncRNAs produced similar phenotypic effects, which indicate that the two lncRNAs contribute to the same molecular pathway.<sup>37,38</sup>

Moreno *et al.*<sup>38</sup> reported that the increased expression of lncRNA *RP11-446E9* reduced the rate of migration and proliferation of leukemia cells, indicating the important role of this lncRNA in signaling pathways, which are dependent on the migration and proliferation. Another study demonstrated that an increased expression of lncRNAs *RP11-624C23.1*, *RP11-446E9*, and *RP11-203E8*

results in augmented apoptosis while facing genotoxic stress, which displays the contribution of these lncRNAs in apoptosis-dependent signaling pathways. Moreover, it was shown that *RP11-203E8* and *RP11-624C23.1* play important roles in regulating DNA damage response (DDR). lncRNA *RP11-446E9* plays a role in activating cell death initiated by DNA damage, however it does not play a role in response to DNA damage. In addition, the increased expression of this lncRNA results in a decreased proliferation and cell migration.<sup>42</sup>

The studies of Ouimet *et al.* demonstrated that lncRNAs *RP11-137H2* and *RP11-68I18.10* were downregulated in pre B-ALL. Our study reflected a decrease in the expression level of lncRNA *RP11-137H2.4* in ALL samples, which agrees with previously done research.<sup>10-24</sup>

In addition, our study indicated the relationship between the lncRNA *RP11-137H2.4* expression changes and the immune-phenotype in ALL patients was not significant ( $P=0.55$ ) (Table 1). The current study further revealed that lncRNA *RP11-68I18.10* was downregulated in ALL patients compared to the control samples; this is consistent with a recent article. The association between this lncRNA expression variations and immune-phenotype in ALL patients also was not significant (Table 1). lncRNA *RP11-68I18.10* is overexpressed in cardiac and skeletal muscles and this result is not in agreement with our study results.

Previous studies have displayed that overexpression of lncRNA *RP11-137H2.4* in pre B-ALL cells can encourage malignant performances, for example, improved resistance to apoptosis, cell proliferation, and cell migration. In addition, genes of the MAPK signaling pathway are downregulated, resulting in *RP11-137H2.4* silencing. The study of Ouimet *et al.* revealed that lncRNAs *RP11-137H2* and *RP11-68I18.10* were downregulated in pre B-ALL, and describing the precise roles of *RP11-137H2.4* in cell cycle pathways and NRAS/BRAF/NF- $\kappa$ B MAPK cascade are significant enough to expand new therapeutic methods to overcome GC opposition in children treated for ALL. Furthermore, *RP11-137H2.4* knockdown significantly increases apoptosis in the cells treated with camptothecin, prednisolone, and doxorubicin. Although suppressing *RP11-68I18.10* was obligatory, it had no influence on apoptosis. Additionally, the levels of unusual effects on apoptosis are comparatively different, and they cause the deregulation of lncRNA in pre B-ALL. This effect determines specific lncRNAs despite having great effects on leukemia types. In this study, the expressions of aforementioned lncRNAs were reported to be reduced. Further studies are needed on the mentioned lncRNAs, and for defining the association of specificity and difference in lncRNA expression levels with ALL classes.

### Conclusion

Results displayed a significant decrease in the expression levels of lncRNAs *RP11-203E8*, *RP11-624c23.1*, *RP11-446E9*, *RP11-137H2.4*, and *RP11-68I18.10* in ALL patients compared to the control cases. Given the obtained results,

an important prospective prognostic assessment is required to be done on these lncRNAs. They can also be used as a new diagnostic kit, as well as therapeutic tolerance in future. Moreover, studies on the mentioned lncRNAs and other lncRNAs are needed to identify the signaling pathways and the target genes of these lncRNA and their role in the tumor progression.

### Acknowledgments

The current article was part of a Ph.D. thesis (thesis No. 22030503971013) at Ahar Branch, Islamic Azad University. We would like to thank all participants of the study, as well as their parents and guardians.

### Ethical Issues

The ethical approval was obtained from the Ethics Committee of Tabriz University of Medical Sciences (Code of Ethics: IR.TBZMED.REC.1398.732). The written informed consent form for participation in the study was also signed by the parents or legal guardians of the children.

### Author Contributions

ZM and SG: Designed the study, ZM: Performed the experiments and wrote the paper with input from all authors. AR: Contributed to sample preparation. LR and BJ: Developed the theoretical framework and analyzed the data. All authors have read and agreed to the published version of the manuscript.

### Conflict of Interest

The authors confirm that this article content has no conflicts of interest.

### References

1. Cheng H, Huang CM, Wang Y, Hu XX, Xu XQ, Song XM, *et al.* Microarray profiling and co-expression network analysis of the lncrnas and mrnas associated with acute leukemia in adults. *Mol Biosyst.* 2017;13(6):1102-8. doi:10.1039/c6mb00874g
2. Cruz-Miranda GM, Hidalgo-Miranda A, Barcenas-Lopez DA, Nunez-Enriquez JC, Ramirez-Bello J, Mejia-Arangure JM, *et al.* Long non-coding RNA and acute leukemia. *Int J Mol Sci.* 2019;20(3):735. doi:10.3390/ijms20030735
3. Wang Y, Xu G, Chen W, Pan Q, Huang K, Pan J, *et al.* Detection of long-chain non-encoding RNA differential expression in non-small cell lung cancer by microarray analysis and preliminary verification. *Mol Med Rep.* 2015;11(3):1925-32. doi:10.3892/mmr.2014.2944
4. Caron M, St-Onge P, Drouin S, Richer C, Sontag T, Busche S, *et al.* Very long intergenic non-coding RNAs transcripts and expression profiles are associated to specific childhood acute lymphoblastic leukemia subtypes. *PLoS One.* 2018;13(11):e0207250. doi:10.1371/journal.pone.0207250
5. Dahl M, Kristensen LS, Gronbaek K. Long non-coding rnas guide the fine-tuning of gene regulation

- in B-cell development and malignancy. *Int J Mol Sci.* 2018;19(9):2475. doi:10.3390/ijms19092475
6. Garitano-Trojaola A, Jose-Eneriz ES, Ezponda T, Unfried JP, Carrasco-Leon A, Razquin N, et al. Deregulation of linc-pint in acute lymphoblastic leukemia is implicated in abnormal proliferation of leukemic cells. *Oncotarget.* 2018;9(16):12842-52. doi:10.18632/oncotarget.24401
  7. Ghodousi ES, Rahgozar S. Recovery of microRNA from stored bone marrow aspirate slides. *Avicenna J Med Biotechnol.* 2019;11(1):24-7.
  8. Hu J, Han Q, Gu Y, Ma J, McGrath M, Qiao F, et al. Circular rna pvt1 expression and its roles in acute lymphoblastic leukemia. *Epigenomics.* 2018;10(6):723-32. doi:10.2217/epi-2017-0142
  9. James AR, Schroeder MP, Neumann M, Bastian L, Eckert C, Gokbuget N, et al. Long non-coding rnas defining major subtypes of b cell precursor acute lymphoblastic leukemia. *J Hematol Oncol.* 2019;12(1):8. doi:10.1186/s13045-018-0692-3
  10. Lun YZ, Sun J, Yu ZG. [Identification of differentially expressed gene core genes in early T-cell precursor acute lymphoblastic leukemia and its regulatory network analysis]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi.* 2019;27(3):673-84. Chinese. doi:10.19746/j.cnki.issn.1009-2137.2019.03.007
  11. Gioia R, Drouin S, Ouimet M, Caron M, St-Onge P, Richer C, et al. Lncrnas downregulated in childhood acute lymphoblastic leukemia modulate apoptosis, cell migration, and DNA damage response. *Oncotarget.* 2017;8(46):80645-50. doi:10.18632/oncotarget.20817
  12. Renou L, Boelle PY, Deswarte C, Spicuglia S, Benyoucef A, Calvo J, et al. Homeobox protein TLX3 activates miR-125b expression to promote T-cell acute lymphoblastic leukemia. *Blood Adv.* 2017;1(12):733-47. doi:10.1182/bloodadvances.2017005538
  13. Yang T, Jin X, Lan J, Wang W. Long non-coding RNA SNHG16 has Tumor suppressing effect in acute lymphoblastic leukemia by inverse interaction on hsa-miR-124-3p. *IUBMB Life.* 2019;71(1):134-42. doi:10.1002/iub.1947
  14. Yazdi N, Houshmand M, Atashi A, Kazemi A, Najmedini AA, Zarif MN. Long noncoding RNA pvt1: Potential oncogene in the development of acute lymphoblastic leukemia. *Turk J Biol.* 2018;42(5):405-13. doi:10.3906/biy-1801-46
  15. Kermezli Y, Saadi W, Belhocine M, Mathieu EL, Garibal MA, Asnafi V, et al. A comprehensive catalog of LncRNAs expressed in T-cell acute lymphoblastic leukemia. *Leuk Lymphoma.* 2019;60(8):2002-14. doi:10.1080/10428194.2018.1551534
  16. Lajoie M, Drouin S, Caron M, St-Onge P, Ouimet M, Gioia R, et al. Specific expression of novel long non-coding RNAs in high-hyperdiploid childhood acute lymphoblastic leukemia. *PLoS One.* 2017;12(3):e0174124. doi:10.1371/journal.pone.0174124
  17. Li S, Bian H, Cao Y, Juan C, Cao Q, Zhou G, et al. Identification of novel lncRNAs involved in the pathogenesis of childhood acute lymphoblastic leukemia. *Oncol Lett.* 2019;17(2):2081-90. doi:10.3892/ol.2018.9832
  18. Ghazavi F, De Moerloose B, Van Loocke W, Wallaert A, Helmsmoortel HH, Ferster A, et al. Unique long non-coding RNA expression signature in ETV6/RUNX1-driven B-cell precursor acute lymphoblastic leukemia. *2016;7(45):73769-80.* doi:10.18632/oncotarget.12063
  19. Tan SH, Leong WZ, Ngoc PCT, Tan TK, Bertulfo FC, Lim MC, et al. The enhancer RNA *ARIEL* activates the oncogenic transcriptional program in T-cell acute lymphoblastic leukemia. *Blood.* 2019;134(3):239-51. doi:10.1182/blood.2018874503
  20. Yu D, Wang WM, Wu FF, Ma P, Sun L, Wan DM, et al. [Expression of long-chain non-coding rna rp11-87c12.5 in acute lymphocytic leukemia and its clinical significance]. *Zhongguo Shi Yan Xue Ye Xue Za Zhi.* 2018;26(1):26-31. Chinese. doi:10.7534/j.issn.1009-2137.2018.01.005
  21. Gutierrez-Camino A, Martin-Guerrero I, Garcia de Andoin N, Sastre A, Carbone Baneres A, Astigarraga I, et al. Confirmation of involvement of new variants at CDKN2A/B in pediatric acute lymphoblastic leukemia susceptibility in the Spanish population. *PLoS One.* 2017;12(5):e0177421. doi:10.1371/journal.pone.0177421
  22. Pouyanrad S, Rahgozar S, Ghodousi ES. Dysregulation of miR-335-3p, targeted by NEAT1 and MALAT1 long non-coding RNAs, is associated with poor prognosis in childhood acute lymphoblastic leukemia. *Gene.* 2019;692:35-43. doi:10.1016/j.gene.2019.01.003
  23. Hung T, Wang Y, Lin MF, Koegel AK, Kotake Y, Grant GD, et al. Extensive and coordinated transcription of noncoding RNAs within cell-cycle promoters. *Nat Genet.* 2011;43(7):621-9. doi:10.1038/ng.848
  24. Ouimet M, Drouin S, Lajoie M, Caron M, St-Onge P, Gioia R, et al. A childhood acute lymphoblastic leukemia-specific lncRNA implicated in prednisolone resistance, cell proliferation, and migration. *Oncotarget.* 2017;8(5):7477-88. doi:10.18632/oncotarget.13936
  25. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. *Nat Protoc.* 2008;3(6):1101-8. doi:10.1038/nprot.2008.73
  26. Bhojwani D, Yang JJ, Pui C-H. Biology of childhood acute lymphoblastic leukemia. *Pediatr Clin North Am.* 2015;62(1):47-60. doi:10.1016/j.pcl.2014.09.004
  27. Tasian SK, Loh ML, Hunger SP. Childhood acute lymphoblastic leukemia: Integrating genomics into therapy. *Cancer* 2015;121(20):3577-90. doi:10.1002/cncr.29573
  28. AlGwaiz LA, Bassioni W. Immunophenotyping of acute lymphoblastic leukemia using immunohistochemistry in bone marrow biopsy specimens. *Histol Histopathol.* 2008;23(10):1223-8. doi:10.14670/HH-23.1223
  29. Deng J, Zhou Y, Cheng X, Xu M. Screening and analysis

- of lncRNA biomarkers and the relationship with the corresponding regulatory miRNA for progressive acute lymphoblastic leukemia. Proceedings of the 2019 International Conference on Modeling, Simulation and Big Data Analysis (MSBDA 2019); 23-24 June 2019; Wuhan, China. Zhengzhou: Atlantis Press; 2019. p. 332-7. doi:[10.2991/msbda-19.2019.51](https://doi.org/10.2991/msbda-19.2019.51)
30. Tian T, Wang M, Lin S, Guo Y, Dai Z, Liu K, et al. The Impact of lncRNA Dysregulation on Clinicopathology and Survival of Breast Cancer: A Systematic Review and Meta-analysis. *Mol Ther Nucleic Acids*. 2018;12:359-69. doi:[10.1016/j.omtn.2018.05.018](https://doi.org/10.1016/j.omtn.2018.05.018)
31. Esfandi F, Taheri M, Omrani MD, Shadmehr MB, Arsang-Jang S, Shams R, et al. Expression of long non-coding RNAs (lncRNAs) has been dysregulated in non-small cell lung cancer tissues. *BMC cancer* 2019;19(1):222. doi:[10.1186/s12885-019-5435-5](https://doi.org/10.1186/s12885-019-5435-5)
32. Mitobe Y, Takayama K-i, Horie-Inoue K, Inoue S. Prostate cancer-associated lncRNAs. *Cancer lett*. 2018;418:159-66. doi:[10.1016/j.canlet.2018.01.012](https://doi.org/10.1016/j.canlet.2018.01.012)
33. Gavgani MM, Nargesian M, Ghorbian S. Critical roles of non-coding RNAs in acute lymphoblastic leukemia. *Gene Cell Tissue*. 2018;5(4):e83297. doi:[10.5812/gct.83297](https://doi.org/10.5812/gct.83297)
34. Yang T, Jin X, Lan J, Wang W. Long non-coding RNA SNHG16 has Tumor suppressing effect in acute lymphoblastic leukemia by inverse interaction on hsa-miR-124-3p. *IUBMB life*. 2019;71(1):134-42. doi:[10.1002/iub.1947](https://doi.org/10.1002/iub.1947)
35. Peng W-X, Koirala P, Mo Y-Y. lncRNA-mediated regulation of cell signaling in cancer. *Oncogene*. 2017;36(41):5661-7. doi:[10.1038/onc.2017.184](https://doi.org/10.1038/onc.2017.184)
36. Fernando TR, Rodriguez-Malave NI, Waters EV, Yan W, Casero D, Basso G, et al. lncRNA expression discriminates karyotype and predicts survival in B-lymphoblastic leukemia. *Mol Cancer Res*.2015;13(5):839-51. doi:[10.1158/1541-7786.MCR-15-0006-T](https://doi.org/10.1158/1541-7786.MCR-15-0006-T)
37. Fernando TR, Contreras JR, Zampini M, Rodriguez-Malave NI, Alberti MO, Anguiano J, et al. The lncRNA CASC15 regulates SOX4 expression in RUNX1-rearranged acute leukemia. *Mol Cancer*.2017;16(1):126. doi:[10.1186/s12943-017-0692-x](https://doi.org/10.1186/s12943-017-0692-x)
38. Moreno CS, editor. Sox4: The unappreciated oncogene. *Semin Cancer Biol*. 2019;67(1):57-64. doi:[10.1016/j.semcancer.2019.08.027](https://doi.org/10.1016/j.semcancer.2019.08.027)
39. Wallaert A, Durinck K, Taghon T, Van Vlierberghe P, Speleman F. T-cell and thymocytes: A message of noncoding RNAs. *J Hematol Oncol*. 2017;10(1):66. doi:[10.1186/s13045-017-0432-0](https://doi.org/10.1186/s13045-017-0432-0)
40. Casero D, Sandoval S, Seet CS, Scholes J, Zhu Y, Ha VL, et al. Long non-coding RNA profiling of human lymphoid progenitor cells reveals transcriptional divergence of B cell and T cell lineages. *Nat Immunol*. 2015;16(12):1282-91. doi:[10.1038/ni.3299](https://doi.org/10.1038/ni.3299)
41. Fang K, Han BW, Chen ZH, Lin KY, Zeng CW, Li XJ, et al. A distinct set of long non-coding RNAs in childhood MLL-rearranged acute lymphoblastic leukemia: biology and epigenetic target. *Human molecular genetics*. 2014;23(12):3278-88. doi:[10.1093/hmg/ddu040](https://doi.org/10.1093/hmg/ddu040)
42. Jendrzewski J, He H, Radomska HS, Li W, Tomsic J, Liyanarachchi S, et al. The polymorphism rs944289 predisposes to papillary thyroid carcinoma through a large intergenic noncoding RNA gene of tumor suppressor type. *Proc Natl Acad Sci U S A*. 2012;109(22):8646-51. doi:[10.1073/pnas.1205654109](https://doi.org/10.1073/pnas.1205654109)