# The Gene Expression Levels of *ETS2*, *ADAM28*, and *GPRC5D* Genes in Acute Lymphoblastic Leukemia Patients

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#### Abstract

**Background:** Genetic biomarkers significantly influence the pathological differentiation and proliferation of lymphoid precursor cells, contributing to the development of Acute Lymphoblastic Leukemia (ALL) in children. This case-control study aimed to assess the expression levels of three potential biomarkers: *ETS2*, *ADAM28*, and *GPRC5D*, in patients with ALL.

**Materials and Methods:** In this cross-sectional study, a group of ALL patients (n=65) referred to the hematology-oncology departments of Nemazee Hospital and Amir Hospital in Shiraz, Iran, from May 2018 to May 2021 were selected as the patient group. A control group (n=65) of age- and gender-matched volunteers was also selected.

Quantitative reverse transcription polymerase chain reaction (qRT-PCR) was used to study the expression profiles of these genes in patients and compare the results to those of a control group. The study included 65 patients with ALL and 65 healthy participants. The Pfaffl method of relative quantification, which compares the threshold cycle of a constitutive gene (*TBP*) with the test gene of each sample in duplicate, was used to determine the relative levels of *ETS2*, *ADAM28*, and *GPRC5D* gene expression. SPSS software version 16 was used for statistical analysis. Nonparametric tests were used to analyze non-normally distributed data, and the Mann-Whitney test was used to compare the medians and ranges and a p-value of 0.05 was considered significant.

**Results:** The patient group showed significantly greater expression of the *ETS2* and *ADAM28* genes compared to the control group. (P <0.0001; fold changes of 2.80 and 2.14, respectively), while *GPRC5D* expression remained unchanged (P >0.05).

**Conclusion:** These genetic biomarkers in patients with ALL may play an oncogenic role in the pathogenesis of the disease and could serve as potential novel biomarkers for ALL.

Keywords: Acute Lymphoblastic Leukemia, ADAM28, Biomarker, ETS2, Pathogenesis

#### Introduction

The most common type of childhood cancer is acute lymphoblastic leukemia (ALL), which represents about a third of all pediatric malignancies. In the United States, the incidence is 3 to 4 cases per 100,000 children under 14 years and about one case per 100,000 individuals over 15. Although ALL can affectchildren and adults throughout their lives, it is most commonly diagnosed in children aged 2 to 6 years (1). Also, in Iran, it is assumed that ALL is the sixth most common type of

cancer (2). This malignancy is described by the accumulation of immature B and T lymphoblast due to a defect in these cell differentiations. Etiological studies have identified a significant association between environmental factors—such as pesticides, ionizing radiation, and certain infections and an increased risk of developing ALL. Additionally, chromosomal and genetic abnormalities, including chromosomal alterations and rearrangements, along with and epigenetic changes like genetic regulation, noncoding RNA modifications, and DNA methylation,

contribute notably to the development of ALL. Gaining insights into the roles of these genetic biomarkers in leukemia may help the patients by facilitating treatment decisions and improving risk assessment (3). The GPRC5D gene encodes a G protein-coupled receptor protein with seven transmembrane segments in cell membranes. While information regarding the role of GPRC5D in cancer is limited, two studies have reported its presence in the bone marrow of patients with multiple myeloma. The correlation of GPRC5D expression levels with factors such as bone marrow plasma cell count, plasma b2microglobulin levels, the International Staging System (ISS), cytogenetic alterations (including deletion of 13q14 and translocation t (4;14)), and overall survival has made GPRC5D a novel prognostic marker in multiple myeloma. Additionally, it has been identified as a potential new cancer antigen as it is upregulated in low-risk myeloma but downregulated in normal tissues (4, 5). GPRC5D gene association with ALL has never been investigated before. Human lymphocytes express ADAM28 in two different forms: membrane (ADAM28m) and short secreted (ADAM28s). The expression of ADAM28 mRNA serves as a marker for cell proliferation. Additionally, high expression of ADAM28 has been reported in various human including lung, breast, gastric, bladder cancers, and in ALL (6-12). Its tissue expression levels have been shown to correlate with cancer metastasis. Research indicates that ADAM28 influences the proliferation, migration, and invasion of leukemic cells in vitro. Furthermore. elevated levels of ADAM28 lead to increased degradation of IGFBP-3 and enhance IGF-I-induced cell proliferation (13). Transcriptional regulation plays a crucial role in maintaining the sequential stages of hematopoiesis as it affects

differentiation, proliferation, and lineage commitment through the regulation of lineage-specific gene expression. The Vets erythroblastosis virus E26 oncogene homolog 2 (ETS2) is a transcription factor that regulates gene expression by binding to various genes containing GGA (A/T) ETS response elements (EREs). The Ras/Raf/MAP kinase pathway and the phosphatidylinositol 3-kinase/Akt pathway both target ETS2 as a downstream effector, influencing various cellular processes such as proliferation, differentiation, migration, transformation. and apoptosis.(14). Research has demonstrated that ETS2 exhibits dual roles in cancer, functioning both an oncogene and a tumor suppressor. For instance, ETS2 acts as an oncogene in acute myeloid leukemia (AML) patients (15) while it displays tumor-suppressive properties in non-small cell lung cancer. (14). In this study, the ETS2, ADAM28, and GPRC5D gene expression levels in ALL patients were evaluated.

# Materials and Methods Patients and control group

In this cross-sectional study, a group of ALL patients referred to the hematologyoncology departments of Nemazee Hospital and Amir Hospital in Shiraz, Iran, from May 2018 to May 2021 were selected as the patient group. The inclusion criteria were newly diagnosed and untreated patients. A control group of age- and gender-matched volunteers was selected. Subjects with a history diagnosed cancer or any history chemotherapy were excluded from the study. The patients were diagnosed using WHO criteria as well as clinical, laboratory, and molecular tests. participants signed written informed consent forms from the Shiraz University of Medical Sciences Ethics Committee in

Shiraz, Iran, with the code of IR.SUMS.REC.1399.450

# Total RNA extraction and cDNA synthesis

Lymphodex (Innotrain) was used to collect peripheral blood granulocyte cells from the patient and control groups. Total RNA was extracted using RiboExTM (GeneAll) according to the manufacturer's instructions. Α NanoDrop ND1000 spectrophotometer was used to measure the concentration of RNA at 260 nm. RNA integrity was also assessed electrophoresis on a 1% agarose gel. The cDNA Kit was used to synthesize cDNA from the total RNA (500 ng) (Thermo Fisher Scientific). Then, the cDNA was used as a template for real-time PCR.

#### **Real-Time PCR**

Real-time PCR was performed by the set of primers for *ETS2*, *ADAM28*, and *GPRC5D* gene which were designed by Primer3 and AllelId software shown in Table I. Gene expression was normalized to the expression of a housekeeping gene (TATA box binding protein [TBP]). The PCR conditions were 95°C for 10 minutes, then 40 cycles of 95°C for 20 seconds, 60°C for 30 seconds, and 72°C for 20 seconds.

#### Statistical analysis

The Pfaffl method of relative quantification, which compares the threshold cycle of a constitutive gene (*TBP*) with the test gene of each sample in duplicate, was used to determine the relative levels of *ETS2*, *ADAM28*, and *GPRC5D* gene expression. SPSS software version 16 was used for statistical analysis,

and a p value of 0.05 was considered significant. Mean and standard deviation were computed for normally distributed data. Nonparametric tests were used to analyze non-normally distributed data, and the Mann-Whitney test was used to compare the medians and ranges.

#### **Results**

This study included 65 ALL patients and 65 healthy individuals matched for age and sex (27 males and 38 females with a mean age of 11±8 years old for the patient's group and 28 males and 37 females with a mean age of 13±6 years old for the control group). The group of patients had an average hemoglobin level of 10.3±2.6 gr/dl, a mean platelet count of 123±35.7 ×103/mm3, and an average WBC count of  $23.7\pm11.8 \times 103$ /mm3. The ETS2 gene and ADAM28 gene expression were significantly higher in the patients' group compared to the control group (P<0.0001 with the actual fold-change difference of 0.67 and 0.9868, respectively), whereas GPRC5D gene expression did not differ between the two groups (P > 0.05). Compared to the control group, the ETS2 and ADAM28 genes showed 2.80 and 2.14-fold greater expression, respectively. Figure 1 shows a histogram analysis of the relative expression of the ETS2 ADAM28 and GPRC5D genes in two groups. ETS2 gene expression was significantly greater in males compared to females; however, the expression level of the two other genes was not different in both genders.

Table I: Primer sequences and PCR product sizes of genes

Gene name	Primer sequence	Product length
GPRC5D-F	GCCCGAGACAGTGATGGA	
GPRC5D-R	CAACAGTCTGCGGCTGAATG	76 bp
ADAM28-F	GGTGGTTGCTATGGTAATC	
ADAM28-R	CTTCATCTGACTCATCTCTTG	154 bp
ETS2-F	CAGCGGCAGGATGAATGA	
ETS2-R	CCCATCAAAGGTGTCAAAGG	112 bp
TBP-F	CCCGAAACGCCGAATATAATC	
TBP-R	TCTGGACTGTTCTTCACTCTTG	134 bp

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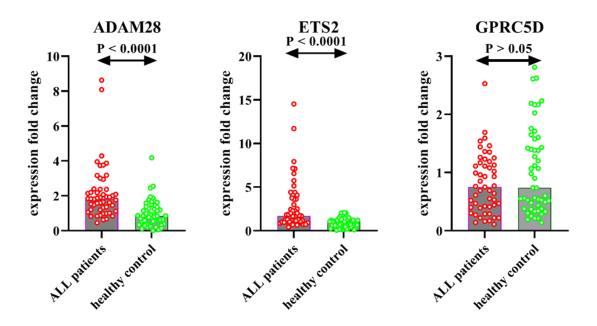


Figure 1. ETS2, ADAM28, and GPRC5D genes' expression fold change in patients and control group

#### **Discussion**

The present study examined the levels of gene expression for ETS2, ADAM28, and GPRC5D in patients with ALL and their matched control specimens using real-time PCR. Identifying biomarkers for ALL is for developing essential innovative targeted therapies and risk-based treatment plans. According to the available data, this research is among the first to investigate the expression levels of these genes in ALL patients. While the results indicated no significant difference in GPRC5D expression between the control and patient groups, both ETS2 and ADAM28 were significantly upregulated in ALL patients compared to their controls. Although the biological function of GPRC5D remains largely unclear, two previous studies have indicated that this gene is overexpressed in multiple myeloma and its expression diminishes in response to antimyeloma treatments (4, 5). Moreover, they showed

GPRC5D overexpressed that was exclusively in multiple myeloma cells, while most normal and abnormal bone marrow cells exhibited low to moderate levels of expression. These results are in the same line with the findings of their study, which indicated that this gene was expressed only at minimal levels in lymphoblastic leukemia and normal lymphocytes (5). Based on available data, there is a limited number of investigations into ADAM28 as a biomarker for ALL (16). ADAM28 is involved in several biological processes, including adhesion, proteolysis, and the growth and metastasis of solid tumors, as well as hematological malignancies (17). Research has shown that ADAM28 is significantly expressed in various human tumors, including chronic lymphocytic leukemia, B-cell ALL, as well as lung, breast, and colorectal cancers (17-20).Tissue expression levels of ADAM28 are also directly related to cancer metastasis (21). Studies have reported that ADAM28 is specifically overexpressed in advanced stages of human non-small cell lung expression is cancer (NSCLC). Its associated with several factors, including tumor size, cell growth, lymph node metastasis, and overall prognosis for patients with this type of cancer. This suggests that ADAM28 may play a critical role in the progression and aggressiveness of NSCLC, making it a potential target for therapeutic intervention or a biomarker for disease monitoring (11, 18, 22). Further have shown that studies ADAM28 facilitates lung metastasis by cleaving and inactivating the pro-apoptotic protein von Willebrand's factor (vWF) in carcinoma cells, likely enhancing the survival of these cells within blood vessels. Additionally, in human breast cancer cells, ADAM28 has been identified as being overexpressed in its active form. This overexpression promotes cell proliferation by increasing the availability of IGF-I, which is released from the IGF-I/IGFBP-3 complex that ADAM28 can specifically cleave (16). The upregulation of urinary ADAM28 in bladder cancer has been identified, suggesting that ADAM28 could play as a potential biomarker for the disease (10). In a single study examining ADAM28 in ALL, researchers discovered a direct correlation between ADAM28 expression levels and relapse rates and prognoses in novo B-ALL patients. It demonstrated that the PI3K pathway regulated ADAM28 expression in B-ALL cells, indicating that modifications to signal transduction pathways could also impact both ADAM28 transcription and traslation (12). This study demonstrated the overexpression of ADAM28, aligning with earlier findings. ADAM28 is a gene that plays a significant role in immune regulation and inflammation. is expressed lymphocytes in В overexpressed in various immune and

inflammatory conditions, as well as in adult B-ALL. Its involvement in B-ALL is likely linked to its role in B lymphocyte function immune regulation, and highlighting its potential significance in the pathology and prognosis of the disease (17). Further research is essential to fully elucidate the mechanisms underlying ADAM28's role in B-ALL and to identify potential therapeutic targets for disease. Understanding how ADAM28 contributes to the pathophysiology of B-ALL could lead to new strategies for intervention, potentially improving patient outcomes and guiding the development of targeted therapies. Investigating pathways signaling and molecular interactions associated with ADAM28 will be crucial in advancing our understanding and management of this malignancy. ETS2 is a transcription factor that plays a critical role in regulating a variety of biological processes, including cell division, development, differentiation, apoptosis, and immune modulation. Its function is highly context-dependent; ETS2 can act as both an activator and an inhibitor of transcription, depending on the specific involved and the cellular genes environment. Bvmodulating the target expression of genes, ETS2 influences essential processes in both normal physiology and disease states. Understanding its multifaceted role may important insights into mechanisms of various diseases, including cancers and immune disorders, and could inform the development of targeted therapies (23). The product of this gene serves as a downstream target for the phosphatidylinositol 3-kinase (PI3K) pathway and the Ras/Raf/MAP kinase pathways (24). Multiple researchs indicate that ETS2 plays dual roles in cancer, acting as both a tumor promoter and a tumor suppressor in different malignancies. For instance, it functions as an oncogene in megakaryocytic leukemia,

demonstrates tumor-suppressive effects in non-small cell lung cancer (14). ETS2 overexpression has been observed in hypopharyngeal cancer and is associated with the metastasis stage of malignancy. Additionally, inhibiting ETS2 may adversely affect cell viability and colony formation in hypopharyngeal cancer (26). A study found that ETS2 was highly expressed in tissues and cell lines of renal cell carcinoma, correlating to the characteristics clinicopathological patients with the disease. Downregulating ETS2 significantly decreased both in vitro invasion of renal cell carcinoma cells and in vivo metastasis. Moreover, the study revealed that renal carcinoma cells with ETS2 levels exhibited reduced significantly lower phosphorylation levels of PI3K and Akt. (27). Another study identified ETS2 as part of a novel signaling pathway. It was found that ETS2 regulated MDM2transcription through PI3K/mTOR/ETS2 pathway independently of the p53 protein. This mechanism may shed light on why MDM2 is overexpressed in over 50% of cancers when p53 is nonfunctional (28).ETS2 has reported in association with poor patients prognosis in AML Moreover, elevated ETS2 expression is associated with poor prognosis not only in patients with acute myeloid leukemia (AML) but also in those undergoing hematopoietic allogeneic stem transplantation. Consequently, it has the potential to serve as a novel biomarker for AML (15). The findings of this study indicate that ETS2 gene overexpression is present in patients with ALL as compared healthy control group. observation is consistent with previous reports identifying ETS2 as an oncogenic factor various in cancer types. Additionally, Bhatia et al. reported comparable results for ETS2 in a smaller cohort of patients (29).

#### **Conclusion**

The findings of this study suggest that the ETS2 and ADAM28 genes may contribute to the oncogenic processes involved in the pathogenesis of ALL. Given that the PI3K pathway has been recognized as a crucial signaling pathway in **ALL** and significant target for antileukemic therapies, these genes warrant further investigation. Consequently, these genes may serve as potential biomarkers for ALL. Further research involving larger study populations is recommended to thoroughly assess the roles of these genes in the disease development and prognosis for a more comprehensive evaluation.

#### **Ethical Considerations**

Informed patient consent was obtained for publication of this report. IR.SUMS.REC.1399.450

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### **Authors' Contributions**

Study design: Nader Cohan and Mani Ramzi; Methodology: Farnoush Farokhian, Mohamad Moghadam and Farzaneh Fakhraei; Data collection and data analysis: Nader Cohan, Elham Abedi and Shirin Parand; Writing—original draft preparation: Nader Cohan and Elham Abedi; Writing — review and editing: Nader Cohan, Mani Ramzi and Elham Abedi. All authors have read and agreed to the published version of the manuscript.

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None.

#### **Conflict of Interest**

None.

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