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Altered Arylamine N-acetyltransferase 1 and miR-1290 Levels in Childhood Acute Lymphoblastic Leukemia: A Pilot Study

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Abstract. *Background/Aim: Arylamine N-acetyltransferase 1 and 2 (NAT1 and NAT2) are drug-metabolizing enzymes that play a key role in the development of acute lymphoblastic leukemia (ALL). Materials and Methods: This study evaluated NAT1 and NAT2 mRNA and protein expression and their enzymatic activity in peripheral blood mononuclear cells (PBMC) from patients with ALL (n=20) and healthy children (n=19) and explored the mechanisms that regulate these enzymes in ALL such as microRNAs (miR-1290, miR-26b) and SNPs. Results: PBMC from patients with ALL showed a decrease in NAT1 mRNA and protein expression. In addition, NAT1 enzymatic activity was decreased in patients with ALL. There was no influence of SNP 559 C>T or 560 G>A on low NAT1 activity. The lower expression of NAT1 might be related to the loss of acetylated histone H3K14 in the NAT1 gene promoter in patients with ALL and the higher relative expression of miR-1290 in the plasma of patients with relapsed ALL compared with healthy controls. There were significantly fewer CD3+/NAT1+ double-positive cells in patients who relapsed compared with control subjects. Based on a t-distributed stochastic neighbor embedding algorithm, CD19+ cells that reappeared in patients with relapse showed low NAT1 expression. In contrast, for NAT2, there were no significant results. Conclusion: The expression and function of NAT1 and miR-1290 levels could be involved in modulating immune cells altered in ALL.*

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Acute lymphoblastic leukemia (ALL) is the most frequent form of cancer diagnosed in pediatric patients and represents 25%-30% of all types of childhood cancer (1-3). In Mexico, this malignancy is the main cause of mortality in children from 5 to 14 years of age (4, 5), and it is estimated that 650-780 cases of ALL are detected per year. However, the biological mechanisms and etiology of this disease are not entirely clear. Several studies have shown that genetic (caused by a mixture of Indigenous and European heritage miscegenation) and lifestyle-related factors contribute to this disease significantly (1, 6). However, additional epigenetic modifications such as microRNAs (miRNAs), DNA methylation, or histone acetylation might participate (7).

Children are more susceptible than adults to developing ALL due to their physiological immaturity and exposure to chemical agents, annealing of meat foods, smoke pollutants, and parental smoking (8, 9). The compounds derived from this exposure are metabolically activated and generate carcinogenic metabolites. Phase 2 enzymes detoxify these metabolites or transform them into less potent compounds (3, 10). Therefore, alterations in these metabolic pathways, particularly in the enzymes associated with the metabolism of carcinogens, could lead to the accumulation of active carcinogenic metabolites that can increase the formation of DNA adducts and, consequently,

Table I. *NAT1 and NAT2 primers that were used for the experiments of this study.*

mRNA expression	NAT1 Fw	5'-GAATTCAAGCCAGGAAGAAGCA-3'
	NAT1 Rv	5'-TCCAAGTCCAATTTGTTCCTAGA CT-3'
	NAT2 Fw	5'-GATCACTTCCCTTGCAGACTTT-3'
	NAT2 Rv	5'-AGGCTGAATGCAATCCTCTTG-3'
mir-1290 expression	Mir-1290 Fw	5'-CATGCAGTATGGATTTTTGGA-3'
	Mir-1290 probe	5'-CTGTGCTAGACGACACTCCCTGA-3'
ChIP	NAT1 Fw	5'-CACCAGCATAAACAAAGCCATA-3'
	NAT1 Rv	5'-AAGCAGAACTGGTAACCTAGAG-3'
	NAT2 Fw	5'-AGAGGACAGAAATCTGGC AG-3'
	NAT2 Rv	5'-TGATTGCCTCCTACTCCTGG-3'
SNP's NAT1	NAT1 Fw	Fw 5'-CCATTGATGGCAGGAACTACA-3'
	NAT1 Rv	5'-GATAACTGGTGAGCTGGATGAC-3'

Fw: Forward primer; Rv: reverse primer.

elevate the risk of developing some types of cancer, including ALL (11, 12).

The phase 2 xenobiotic-metabolizing enzymes arylamine *N*-acetyltransferase 1 (NAT1), and NAT2 are encoded by the genes *NAT1* and *NAT2*, respectively, located on chromosome 8 (13, 14). Modifications in the expression or activity of enzymes like NAT1 could be associated with cancer risk, including ALL. NAT1 is involved in the cell cycle and apoptosis by regulating p53, a tumor suppressor protein, and generating reactive oxygen species (15).

Several single nucleotide polymorphisms (SNPs) have been described in both these genes, and their presence is related to the probability of developing ALL (3, 10, 16, 17). Our group has demonstrated significant associations between ALL development and the presence of the *NAT1*3* [odds ratio (OR) 2.1], *NAT1*4* (OR 1.9), *NAT2*6B* (OR 3.3), *NAT2*6J* (OR 3.2), and *NAT2*7A* (OR 2.4) haplotypes, and the NAT1 rapid (OR 6.7) and NAT2 slow (OR 2.9) phenotypes in peripheral blood mononuclear cells (PBMC) from the Mexican population (17). On the other hand, epigenetic regulation plays an essential role in the development and progression of this disease, and this regulation includes miRNAs that serve as important modulators of gene expression. Aberrant expression of miRNAs could affect the expression of their target genes, like *NAT1*, contributing to ALL. *NAT1* is a target of miRNAs such as miR-1290 (18) and miR-26b (19).

Although NAT1 and NAT2 are molecules that could be key in the development and progression of ALL, their expression and function have not been explored in this childhood cancer. Moreover, there is a need to determine the activity and function of the xenobiotic-metabolizing enzymes in this pediatric neoplasm and to establish whether they have any implication in the development of ALL. Therefore, the objective of this study was to evaluate NAT1 and NAT2 mRNA and protein expression and to determine their enzymatic activity in PBMC and $CD3^+$ or $CD19^+$ lymphocytes obtained from patients with ALL compared with control subjects. We also explored the possible molecular mechanisms—SNPs and miRNAs—that could modulate the expression and function of these metabolizing enzymes.

Materials and Methods

Subjects. Twenty pediatric patients aged 3-15 years, with a confirmed diagnosis of ALL and 19 clinically healthy children (the control group) were recruited from the Hospital Central "Dr. Ignacio Morones Prieto" of San Luis Potosí, Mexico. The ALL diagnosis was confirmed by flow cytometry analysis, using monoclonal antibodies against CD10, CD19, CD20, CD22, CD34, CD79a, TdT, IgMs, and IgMc. All patients were classified as subtype B and received the oncological treatment described in the Mexican clinical guidelines: mercaptopurine, methotrexate, and L-asparaginase (20). The parents gave written informed consent; however, informed consent was obtained directly from the patient for patients aged 12 to 17 years. The study was approved by the research committee and the research ethics committee of Hospital Central "Dr. Ignacio Morones Prieto" (Number 25-17) and performed following the ethical standards in the 1964 Declaration of Helsinki and its later amendments.

Isolation of PBMC. Blood samples were collected from both groups in 4-ml EDTA Vacutainer tubes (BD) for the NATs expression and enzymatic activity analyses. PBMC were isolated from the whole blood of both groups by density gradient using Ficoll-Histopaque (Sigma, St. Louis, MO, USA).

NAT1 and NAT2 mRNA expression assay. We used reverse transcription–quantitative polymerase chain reaction (RT-qPCR) to determine *NAT1* and *NAT2* mRNA expression. Total RNA isolated from PBMC from each participant was purified using the TRIzol® reagent. Complementary DNA (cDNA) was generated using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA). NAT1 and NAT2 mRNA expression was normalized against the level of the endogenous control β-actin using the specific primers listed in Table I and by the $2^{-\Delta\Delta Ct}$ method (21, 22).

Expression of NAT1 and NAT2 proteins in $CD3^+$ or $CD19^+$ lymphocytes by flow cytometry. The percentage of double-positive cells was determined using flow cytometry, a FACS Canto II Cytometer, and FlowJo V10.6.1 software (BD). We used the monoclonal antibodies, anti-CD3-PE (eBiosciences®, San Diego,

CA, USA) or anti-CD19-FITC (eBiosciences®) as cell surface markers, primary antibodies to NAT1 [rabbit anti-NAT1 (Abcam, Cambridge, UK)] and NAT2 [mouse anti-NAT2 (Abcam)], and secondary antibodies anti-rabbit APC or anti-mouse APC secondary antibodies (eBiosciences®), respectively. Ten thousand lymphocyte cell gate events were counted for the analysis. The hematic biometry data were used from all the participants to represent NAT1 and NAT2 expression in absolute values.

In situ NAT assay. PBMC (2×10^5 cells) from patients with ALL and control subjects were cultured in RPMI 1640 medium supplemented with 50 U/ml penicillin and 50 μg/ml streptomycin (Sigma-Aldrich) and maintained at 37˚C in a humidified atmosphere of 5% CO_2. The medium contained a specific substrate for each enzyme—100 μM acid para-aminobenzoic (PABA) (Sigma-Aldrich) for NAT1 and 100 μM isoniazid (INH) (Sigma-Aldrich) for NAT2 (23, 24). The cells were incubated for 24 h; after this time, the supernatant was removed and frozen at –80˚C until high-performance liquid chromatography (HPLC) analysis.

In situ NAT enzymatic activity determination. NAT1 and NAT2 activities were determined based on HPLC using supernatants from PBMC cell cultures of patients and control subjects to quantify the concentrations of the substrates and metabolites for each enzyme: PABA and acetyl-PABA (AcPABA) for NAT1, and INH and acetyl-INH (AcINH) for NAT2. We optimized the methods previously carried out by our research group (23) and validated them analytically according to the International Conference on Harmonization (25). The concentration of each analyte in the sample was calculated using the respective calibration curve. We determined PABA or INH *N*-acetylation by measuring nanomoles of AcPABA or AcINH per milliliter over 24 h.

Barnes-Hut t-distributed stochastic neighbor embedding (t-SNE) analysis. To display subpopulations of interest ($CD3^+$, $CD19^+$) and marker expression, we generated heat maps ($NAT1^+$, $NAT2^+$) of PBMC from the participants. Barnes-Hut t-SNE was performed using FlowJo V10.6.1 with perplexity=50, θ=0.5, interactions=1000, and Euclidean distance (26, 27). For this analysis, patients were classified as those who had relapsed and those who had received their initial diagnosis. We concatenated and down-sampled events from six samples of each group. This approach provided a typical t-SNE map distribution, allowing sample comparison.

Expression of miR-1290 in plasma. We obtained plasma from the whole blood samples and then purified total RNA from 500 μl of plasma using the TRIzol® reagent. We analyzed miR-1290 and miR-26b by using the probes and primers listed in Table I. Relative expression was calculated with the $2^{-\Delta\Delta Ct}$ method and normalized with the endogenous control.

PCR amplification of NAT1 and sequencing study. Total DNA was isolated using a Wizard® Genomic DNA Purification Kit (Promega Corporation). The region of interest was amplified using the Phusion® High Fidelity DNA polymerase (New England BioLabs®) and the primers listed in Table I. Sequencing of the purified amplicons was performed in an AB 3130 instrument.

Chromatin immunoprecipitation (ChIP) assay. Crosslinked protein–DNA complexes were immunoprecipitated from PBMC at 4˚C overnight using magnetic beads (Dynabeads Protein G, Thermo Fisher) and the following antibodies: anti-histone H3 (Abcam® ab1791), anti-histone H3 trimethyl K27 (Abcam® ab6002) and anti-acetyl histone H3 (K14) (Merck Millipore®). Immunoprecipitated DNA was amplified using the primers listed in Table I and the kit Phusion® High Fidelity DNA polymerase (New England BioLabs).

Statistical analysis. The normality of the data was analyzed with the Shapiro-Wilk test. For normally distributed data, means were compared using Student's *t*-test. For non-normally distributed data, medians were compared with the Mann-Whitney *U*-test. We performed principal component analysis (PCA) with the study variables (graph matrix). The level of statistical significance was set at $p<0.05$. All the statistical tests were performed using GraphPad Prism V 7.00 (GraphPad Software Inc., La Jolla, CA, USA).

Results

Study groups. We included 20 patients with ALL and 19 control subjects, with a mean age of 9.4±3.4 years and 9±3.8 years, respectively. The similar mean weight, body mass index (BMI), and height of patients with ALL and control subjects indicated homogeneity in the demographic and anthropometric characteristics in both groups (Table II). Ninety percent of patients with ALL were in the maintenance phase. We assigned patients to a risk group based on previously established predictive factors (20, 28, 29): 19 out of 20 were at high risk, and only one was low risk. After a 2-year follow-up, 5 patients had died, and 7 out of 20 patients with ALL had a relapse, 4 at the bone marrow and 3 at the central nervous system. Three relapses were due to treatment discontinuation, and the other four to an unknown cause. Among the concomitant diseases in ALL patients, one presented with Down syndrome and another with hyperthyroidism. The patients with ALL and those in the control group were not related to each other.

Decreased NAT1 protein and mRNA in PBMC and $CD3^+$ T cells from patients with ALL. Because this is the first study of NATs expression in immune cells from ALL, we examined their mRNA and protein levels using RT-qPCR and flow cytometry. We found low *NAT1* mRNA expression in patients with ALL compared with control subjects (Figure 1A, $p=0.001$). In contrast, *NAT2* mRNA expression was similar between the groups (Figure 1B, $p=0.51$). NAT1 and NAT2 protein, measured using flow cytometry, indicated decreased NAT1 expression in patients with ALL (Figure 1C, $p=0.0003$) and in $CD3^+/NAT1^+$ double-positive cells (Figure 1E, $p=0.0049$) compared with control subjects. The absolute values of NAT2 (Figure 1D) and $CD3^+/NAT2^+$ cells (Figure 1F) were not significantly different between the groups. Due to the high variability in the data regarding the percentage of positive cells, we used the hematic biometry data of each participant from the clinical record and calculated the absolute

values of NAT1 and NAT2 as well as the double-positive cells ($CD3^+/NAT1^+$, $CD3^+/NAT2^+$). There was a low number of B lymphocytes ($CD19^+$) in patients with ALL compared with that in control subjects (p=0.0001, data not shown). However, it is important to note that patients were undergoing chemotherapy when the samples were taken. Thus, the cytotoxic activity of the drugs used influenced the results.

Identification of NAT1 and NAT2 in lymphocyte subpopulations. We next compared the expression of NATs in lymphocytes from patients who had received their initial ALL diagnosis and lymphocytes of patients with relapse.

We analyzed the distribution of NAT1 and NAT2 in $CD3^+$ and $CD19^+$ cell subpopulations using a t-SNE algorithm. Figure 2 and Figure 3 show the resulting maps. The control group showed two separate islands for $CD3^+$ cells and a single small island of $CD19^+$ cells (Figure 2A). In contrast, in patients who had received their initial ALL diagnosis, $CD3^+$ lymphocytes were concentrated in only one island; however, there were three clusters of different types of cells in this island (Figure 2C). It was expected that the patients who had received their initial ALL diagnosis had no $CD19^+$ cells, but this subpopulation reappeared in patients with relapse in a smaller and more uniform distribution compared to the control group (Figure 2E).

NAT1 distribution analysis in $CD3^+$ and $CD19^+$ cells showed the presence of this enzyme in all lymphocyte subpopulations (Figure 2B, D, and F). However, there was high heterogeneity in its expression within patients with ALL and the control group. For $CD3^+$ lymphocytes, NAT1 expression was more significant in patients compared with control subjects. There was a small island in the control group that belonged to $CD3^+$ cells that clearly comprises two clusters of cells, those with low NAT1 expression (blue color, left) and those with variable expression (different colors, right) (Figure 2B). This island disappeared in patients who had received their initial diagnosis and returned in patients with relapse, but interestingly only as a cluster of cells with low NAT1 expression (Figure 2F, arrow). Consistently, $CD19^+$ cells in control subjects showed an interesting pattern with low, medium, and high NAT1 expression (Figure 2B), in contrast to $CD19^+$ cells that reappear in patients with relapse (low NAT1 expression). It is important to note that other PBMC populations express NAT1 at various degrees and intensities (Figure 2B, D, and F).

The distribution pattern of NAT2 expression in $CD3^+$ cells was uniform within all studied groups (Figure 3), a pattern consistent with the analysis of $CD3^+/NAT2^+$ cells (Figure 1F). When analyzing the $CD19^+$ cells, we found that only the $CD19^+$ lymphocytes from the control subjects express higher levels of NAT2 than $CD3^+$ lymphocytes (Figure 3B). As described above, we did not detect $CD19^+$ cells in patients who had received their initial diagnosis; however, in patients with relapse, these cells returned, although with a different NAT2 expression pattern. Only those cells with high levels of NAT2 reappeared, possibly as the first signal of relapse (Figure 3F). As in $CD3^+$ cells, we detected high NAT2 expression in the $CD3^-/CD19^-$ cells, both in patients with their first diagnosis and with relapse (Figure 3D and F).

High plasma miR-1290 levels and decreased NAT1 expression in patients with relapse. To explore possible mechanisms involved in the low expression of NAT1 in patients with ALL, we evaluated the plasma levels of its

Table II. *Demographic and anthropometric data of the study groups*.

	Control group	Patients
n	19	20
Sex		
Male	10 (52%)	12 (60%)
Female	9 (48%)	8 (40%)
Age group (years)		
3-9	11 (58%)	11 (55%)
≥10	8 (42%)	9 (45%)
Risk classification		
Low risk	NA	19 (95%)
High risk		1 (5%)
Relapse		
No	NA	13 (65%)
Yes		7 (35%)
Relapse site		
Bone marrow	NA	4
Central nervous system		2
Weight (Kg)	37 (15-82)	35 (10.8-66)
Height (m)	1.4 (1.1-1.74)	1.3 (0.88-1.63)
Body index mass	21 (13.3-29)	20.17 (8.8-29.3)
BMI (Kg/m^2)	3 (16%) underweight 10 (53%) normoweight 1 (5%) overweight 5 (26%) obese	2 (10%) underweight 9 (45%) normoweight 3 (15%) overweight 6 (30%) obese
Concomitant diseases	1 Gastroenteritis 1 Kidney stones	1 Down's Syndrome 1 Hyperthyroidism
Treatment stage	NA	17 (85%) Maintenance treatments, 1 (5%) Consolidation therapy, 2 (10%) Reinduction chemotherapy
EGIL		
ALL pro-B (B1)		2 (10%)
ALL common (B2)		9 (45%)
ALL pre-B (B3)	NA	9 (45%)
Death		
No	19	15
Yes	0	5

BMI: Body index mass; EGIL: European Group for the Immunological Characterization of Leukemias; ALL: acute lymphoblastic leukemia; NA: does not apply. Values presented with median (range from minimum to maximum).

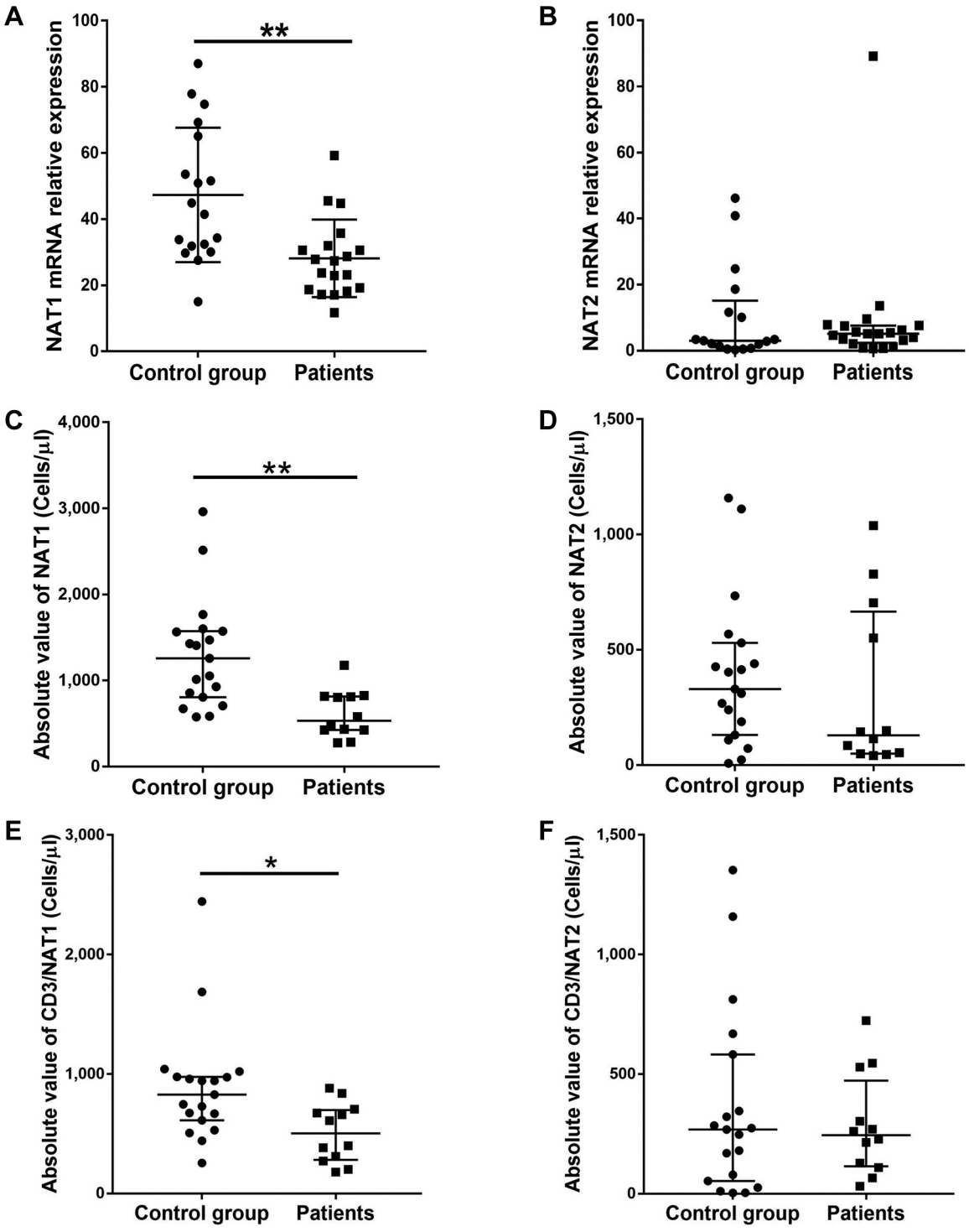

Figure 1. *NAT1 and NAT2 expression at the mRNA and protein levels. (A) Relative expression of NAT1 mRNA in patients with ALL (n=19) (mean±standard deviation, 28.1±11.7) and control subjects (n=18) (47.3±20.3). Student's t-test was employed. (B) Relative expression of NAT2 mRNA in patients with acute lymphoblastic leukemia (ALL) (n=20) (median 5.11; interquartile range=2.37-7.57) and control subjects (n=17) (2.98; 1.05-15.12). The Mann-Whitney U-test was employed. (C) Absolute values of NAT1 in patients with ALL (n=12) (mean±standard deviation, 611±274) and control subjects (n=19) (1,302±635). Student's t-test was employed. (D) Absolute values of NAT2 from patients with ALL (n=12) (median 129; interquartile range=49.5-665) and control subjects (n=19) (329; 130-530). The Mann-Whitney U-test was employed. (E) Absolute values of $CD3^+/NAT1^+$ PBMC from patients with ALL (n=12) (509±247) and control subjects (n=19) (894±484). (F) Absolute values of $CD3^+/NAT2^+$ from patients with ALL (n=12) (245; 114-473) and control subjects (n=19) (268; 53-582).*

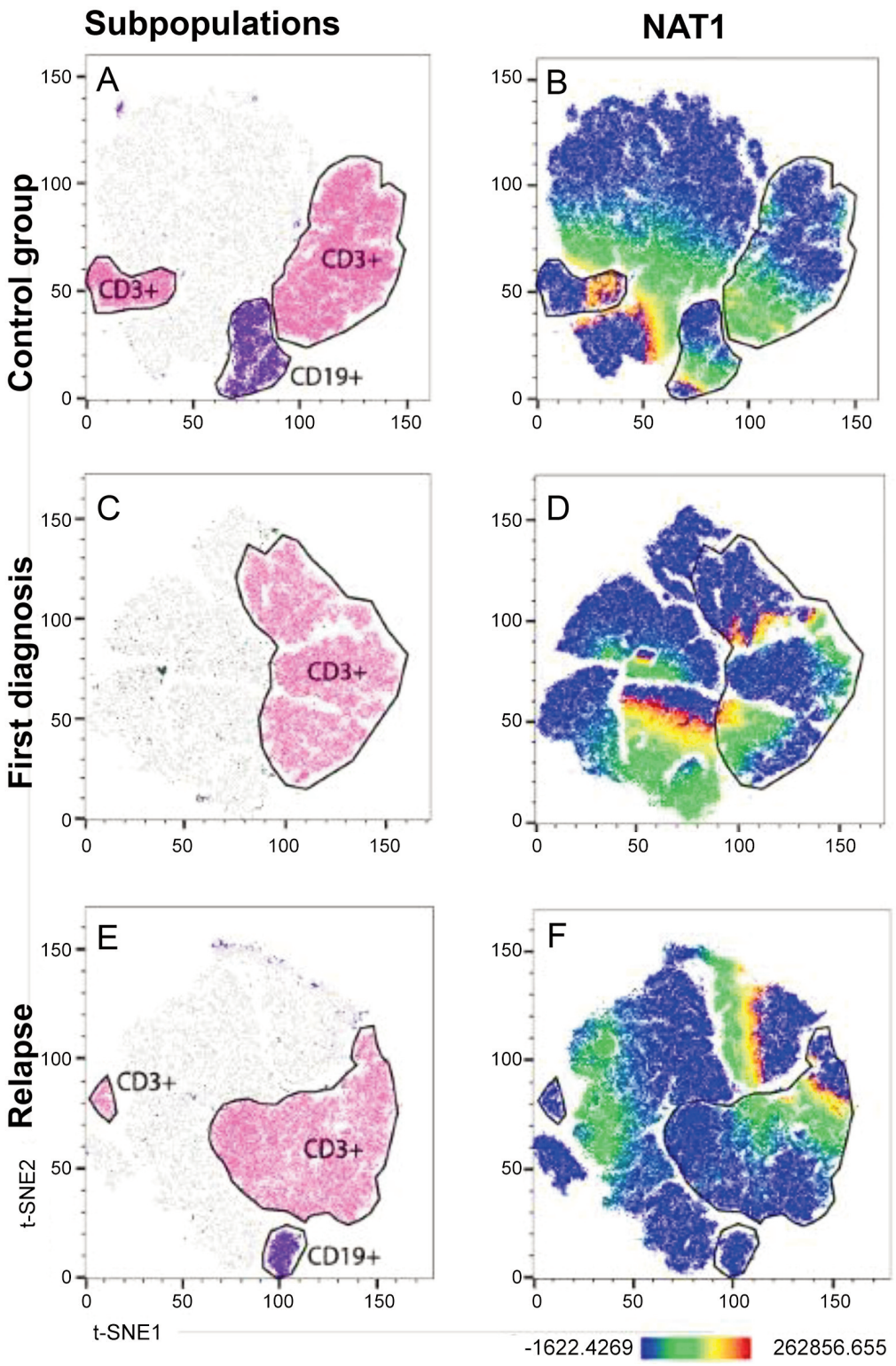

Figure 2. *Visualization of NAT1 in the t-SNE map of CD3⁺ and CD19⁺ lymphocytes from patients with acute lymphoblastic leukemia (ALL) and control subjects. t-SNE projections of six samples of lymphocytes with NAT1 from each group, namely Control, ALL diagnosed for the first time, and relapsed ALL. Lymphocytes have been coded according to fluorochrome staining (CD19⁺=purple; CD3⁺=pink) (A, C, and E). The heatmap is color-coded according to the expression level of the marker, as indicated (B, D, and F) (blue=low; red=high). The t-SNE parameters were perplexity=50, θ=0.5, iterations=1,000, and Euclidean distance.*

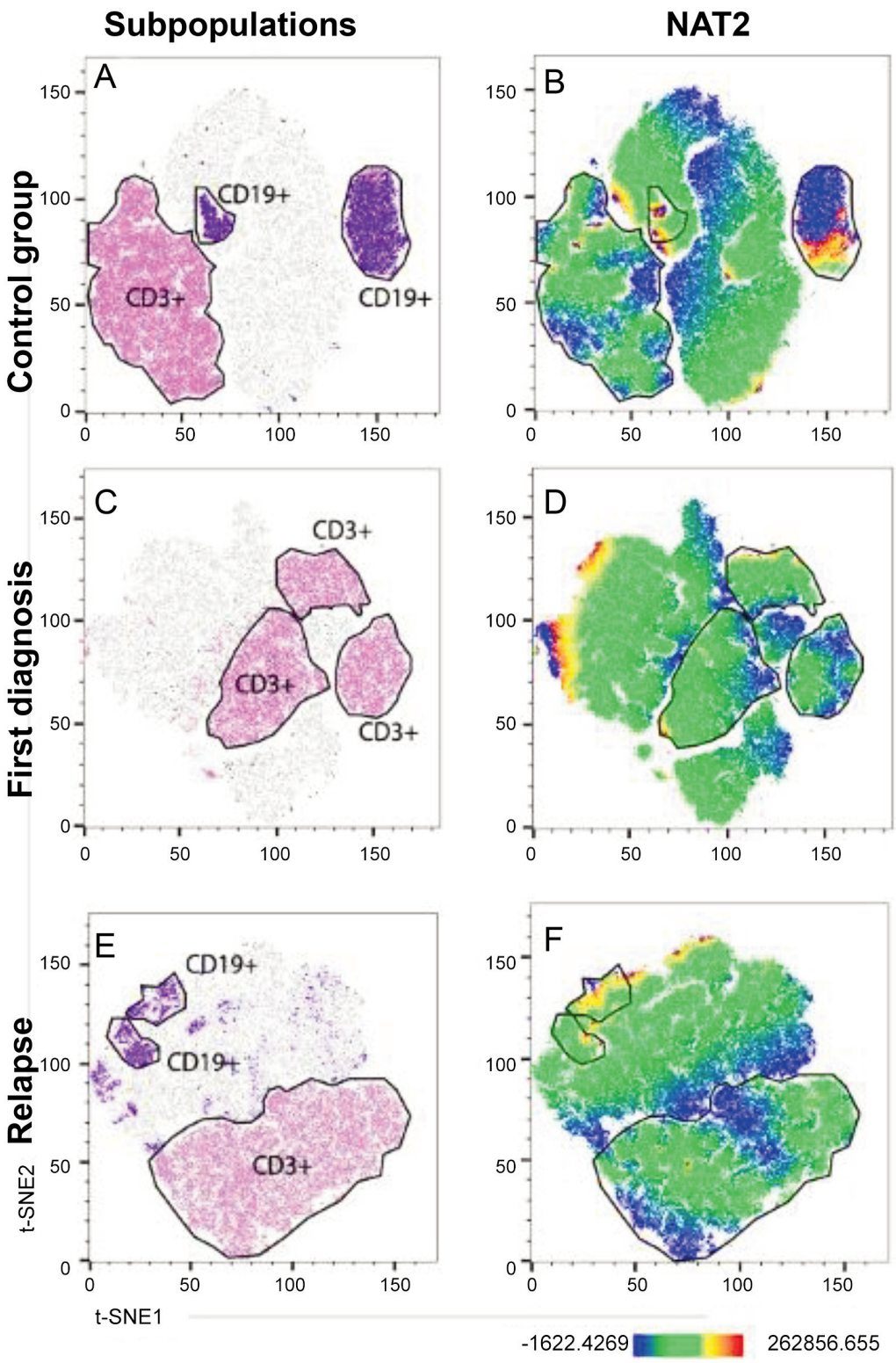

Figure 3. *Visualization of NAT2 in the t-SNE map of CD3⁺ and CD19⁺ lymphocytes from controls and patients with acute lymphoblastic leukemia (ALL). t-SNE projections of six samples of lymphocytes with NAT2 from each group, namely Control, ALL diagnosed for the first time, and relapsed ALL. Lymphocytes have been coded according to fluorochrome staining (CD19⁺=purple; CD3⁺=pink) (A, C, and E). The heatmap is color-coded according to the expression level of the marker, as indicated (B, D, and F) (blue=low; red=high). The t-SNE parameters were perplexity=50, θ=0.5, iterations=1,000, and Euclidean distance.*

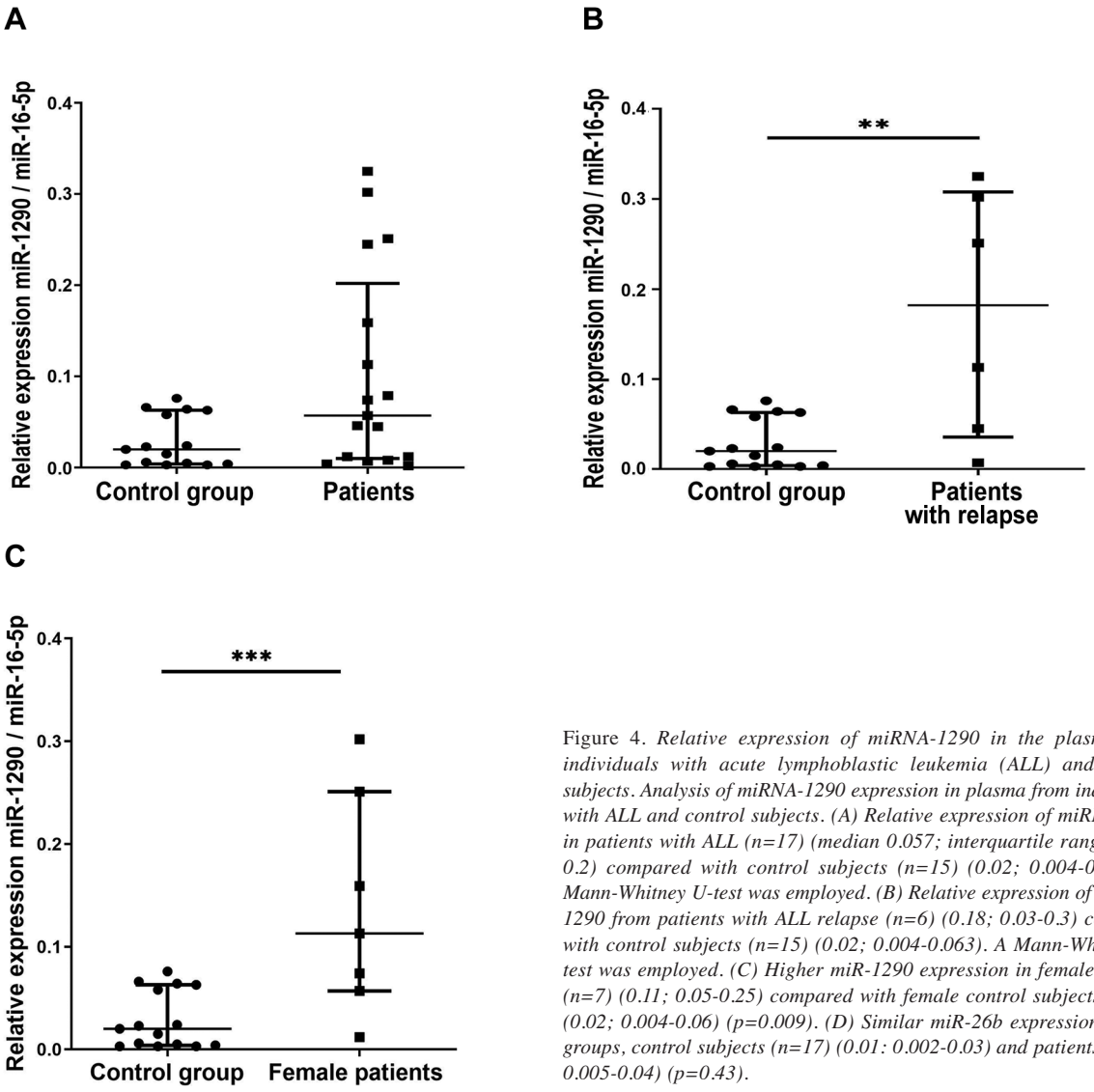

Figure 4. *Relative expression of miRNA-1290 in the plasma from individuals with acute lymphoblastic leukemia (ALL) and control subjects. Analysis of miRNA-1290 expression in plasma from individuals with ALL and control subjects. (A) Relative expression of miRNA-1290 in patients with ALL (n=17) (median 0.057; interquartile range=0.01-0.2) compared with control subjects (n=15) (0.02; 0.004-0.063). A Mann-Whitney U-test was employed. (B) Relative expression of miRNA–1290 from patients with ALL relapse (n=6) (0.18; 0.03-0.3) compared with control subjects (n=15) (0.02; 0.004-0.063). A Mann-Whitney U-test was employed. (C) Higher miR-1290 expression in female patients (n=7) (0.11; 0.05-0.25) compared with female control subjects (n=15) (0.02; 0.004-0.06) (p=0.009). (D) Similar miR-26b expression in both groups, control subjects (n=17) (0.01: 0.002-0.03) and patients (0.017; 0.005-0.04) (p=0.43).*

regulator miR-1290 (Figure 4). We found that patients with ALL express higher levels of miR-1290 compared with control subjects. However, due to the small number of patients included and the heterogeneity of the results, statistical significance was not obtained (Figure 4A, p=0.067). Interestingly, there were significantly higher miR-1290 levels in the plasma from patients with relapse and female patients compared with control subjects (Figure 4B, p=0.017) (Figure 4C, p=0.009). In contrast, miR-26b showed similar levels between the groups (Data not shown).

PBMC from patients with ALL present lower NAT1 activity. We measured the basal NAT1 and NAT2 enzymatic activity using HPLC in extracts of PBMC cultures to determine whether the low *NAT1* mRNA expression was consistent with its enzymatic activity. There was a significant decrease in *NAT1* activity in the majority of PBMC patients with ALL compared with control subjects (Figure 5A, p=0.03). This result suggests that lower NAT1 activity is due, at least in part, to the decreased *NAT1* gene transcription described above. As expected, there was no difference in NAT2 activity between the groups; however, a population of patients tended to present higher NAT2 activity than control subjects (Figure 5B). In addition, to investigate whether this lower NAT1 activity is related to the presence of ALL relapse, we analyzed this activity in PBMC from patients with relapse. However, there were no differences between patients with relapse and control subjects (Figure 5C). Likewise, for NAT2 activity, there were no differences between patients with relapse and control subjects (Figure 5D).

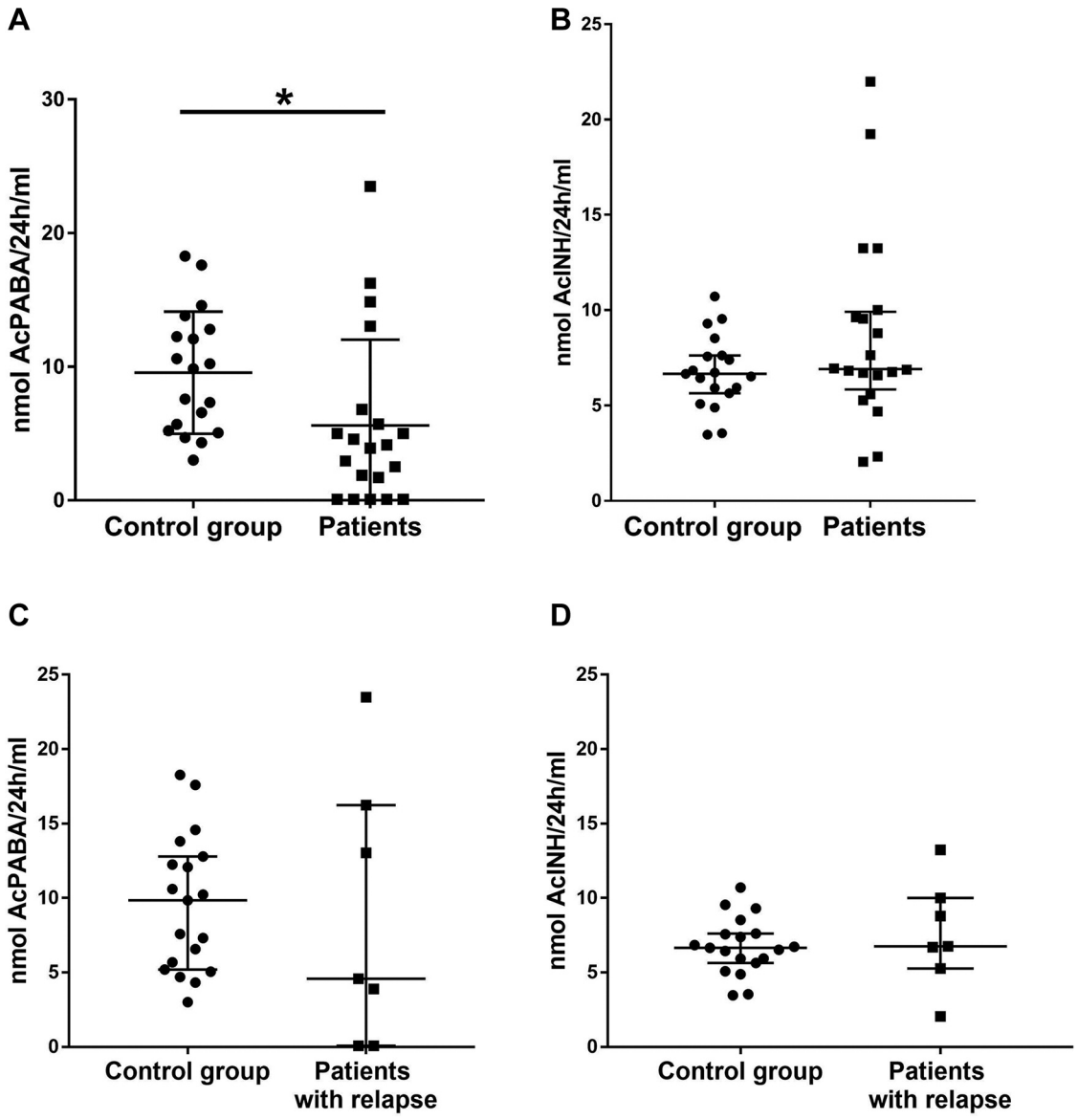

Figure 5. *NAT1 and NAT2 activities in the control group, patients with ALL and patients with acute lymphoblastic leukemia (ALL) relapse. (A) NAT1 activity in patients with ALL (n=20) (mean±standard deviation, 5.6±6.4) and control subjects (n=19) (9.5±4.5). Student's t-test was conducted. (B) NAT2 activity in patients with ALL (n=20) (median 6.9; interquartile range=5.84-9.91) and control subjects (n=19) (6.65; 5.63-7.61). The Mann-Whitney U-test was conducted. (C) NAT1 activity in patients with ALL relapse (n=7) (8.7±8.9) and control subjects (n=19) (9.5±4.5). (D) NAT2 activity in patients with ALL relapse (n=7) (6.75; 5.26-10) and control subjects (n=19) (6.65; 5.63-7.61).*

There is no relation between SNP 559 C>T or 560 G>A in the NAT1 gene and its low enzymatic activity. The low or lack of NAT1 enzymatic activity detected using HPLC in some patients led us to examine the possible presence of SNPs in the *NAT1* gene in these subjects. We evaluated the presence of the SNP 559 C>T (haplotype *NAT1*15*) and 560 G>A (haplotype *NAT1*14B*) using sequencing. We did not detect SNP 559 or 560 in any of the five patients with low NAT1 enzymatic activity. This group of patients was wild type homozygous (C,C for 559 and G,G for 560) (Figure 6). Therefore, we ruled out the influence of these SNPs on NAT1 activity in the patients studied.

Correlation analysis between NAT levels, enzymatic activity, and BMI. We next performed a PCA with the study variables, namely NAT1 and NAT2 mRNA and protein expression and enzymatic activity, and miR-1290 and miR-26b expression (Figure 7A). We identified a positive

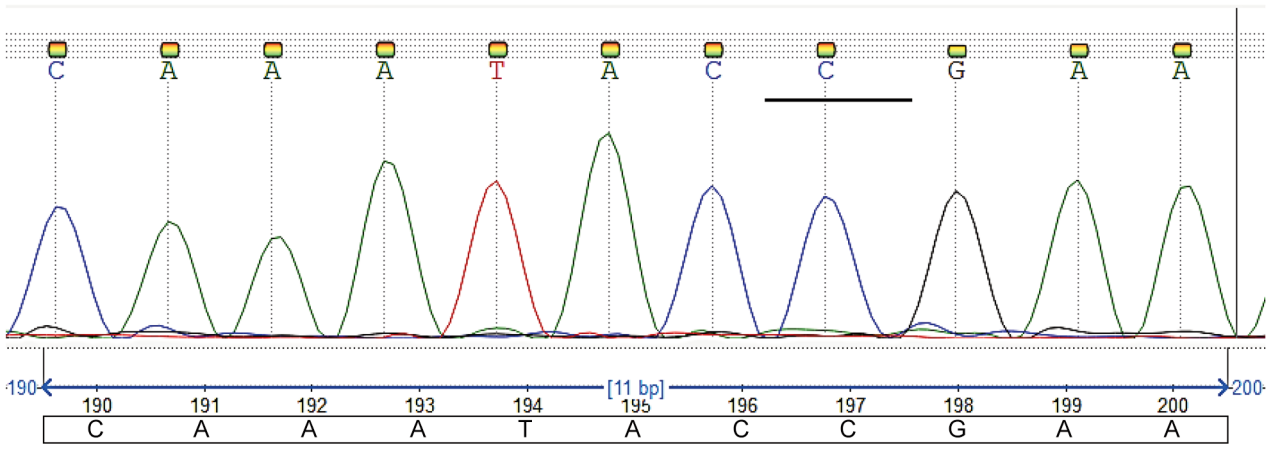

Figure 6. *Sequencing analysis of the NAT1 gene. Example of direct sequencing chromatogram. The SNPs 559 C>T (haplotype NAT1*15, truncated protein/no enzymatic activity) and 560 G>A (haplotype NAT1*14B, slow activity) were not observed in the NAT1 gene.*

correlation between miR-1290 and NAT1 mRNA (Figure 7B, p=0.016), but no correlations for NAT2 (data not shown). In addition, we evaluated the relationship between NAT expression and BMI. There was a significant correlation between BMI and the absolute values of NAT1 expression in control subjects (Spearman, r=-0.48, p=0.047) but not in patients with ALL (Pearson, r=0.15, p=0.62).

Decreased H3K14Ac in the promoters of NAT1 and NAT2 and H3K27me3 enrichment in the promoter of NAT2 in PBMC from patients with ALL. Given that patients with ALL had low NAT1 mRNA and protein expression and enzymatic activity, but there were no SNPs in the *NAT1* gene that could explain the low activity, we evaluated some epigenetic marks in the promoters of *NAT1* and *NAT2*. We found that lysine 14 acetylation in histone H3 (H3K14Ac) was decreased in the promoter of the *NAT1* gene in PBMC of patients with ALL compared with control subjects (Figure 8A and 8B). It is important to mention that *NAT1* mRNA has two isoforms; we evaluated isoform A. We also found an H3K14Ac decrease and an H3K27me3 increase in the *NAT2* promoter in patients with ALL compared with the control group (Figure 8C and D).

Discussion

Accumulating active carcinogenic metabolites due to continuous exposure to chemical agents play an essential role in the development of ALL. Genetic association studies have shown the presence of several SNPs in the phase 2 xenobiotic-metabolizing enzymes *NAT1* and *NAT2* and their impact on the likelihood of developing ALL (3, 10, 16, 17, 30). Hence, in-depth studies of NAT proteins could help us better understand their role in leukemogenesis.

In the present study, we demonstrated the altered expression and function of NAT1 in immune cells from patients with ALL for the first time. PBMC from patients with ALL displayed lower NAT1 mRNA and protein expression as well as enzymatic activity compared with control subjects. Human NAT1 has been widely studied, particularly its relationship with the development of certain types of cancer. In this sense, the global consensus is that NAT1 mRNA and protein levels and activity are typically elevated in cancer, especially breast, prostate, and liver (31). However, it is important to highlight that the tumor microenvironment and molecular signatures are different between various cancers. In this regard, a bioinformatic analysis carried out using data from The Cancer Genome Atlas and validated with Gene Expression Omnibus showed that in the kidney chromophobe, rectum, and colon adenocarcinoma *NAT1* mRNA is decreased compared with the corresponding normal tissues (31). Additionally, SW116 colon cancer cells express lower levels of NAT1 compared with NCM460 cells (normal colon cells) through modulation of the phosphoinositide 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) signaling pathway (32). Consistently, The Human Protein Atlas database reported very low or no NAT1 expression in colorectal, gastric, and renal cancer and lymphoma. Furthermore, when comparing NAT1 activity among cancer cell lines, it was found to be markedly lower in leukemia (THP-1, Jurkat, or CEM) as well as in liver (HepG2) and colon cancer (HT-29) cell lines than in other types of cancer cell lines (33).

Furthermore, when NAT1 activity was compared between cancer cell lines, it was undoubtedly lower in leukemia (THP-1, Jurkat, or CEM) compared to breast (ZR-751; T-47D), or prostate cell lines (LNCaP; 22RV1). These findings support

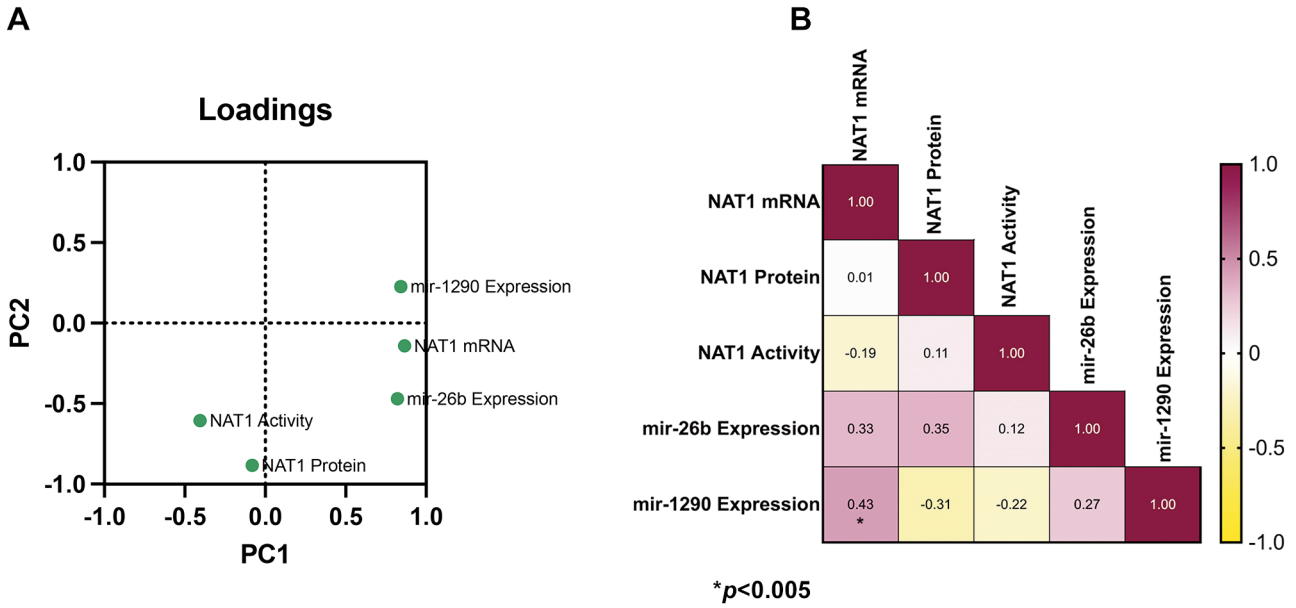

Figure 7. *Principal component analysis and Graph matrix. (A) This plot shows that the expression of NAT1 at the mRNA level is related to the expression of miR-1290 and miR-26b. (B) This graph matrix shows the correlations between the study variables, including a correlation between NAT1 mRNA expression and miR-1290 expression (p=0.016).*

our results and suggest that NAT1 is regulated by different molecular mechanisms depending on the cancer type.

It is essential to mention that for this study, PBMC from the two study groups were used since it is not possible to perform bone marrow aspiration in clinically healthy children to analyze and compare immune cells from this tissue. Furthermore, since this is a pilot and exploratory study, it is interesting first to investigate whether any abnormalities in cells from children with ALL are related to NATs. For this reason, T and B lymphocytes were analyzed when obtaining PBMC from the study groups.

We observed lower NAT1 expression in patients with ALL and lower number $CD3^+/NAT1^+$ cells compared with control subjects, and we decided to gain further insight into the behavior of NAT1 among lymphocyte subpopulations. The t-SNE map revealed important changes in the size and complexity of immune cells from patients compared to controls. Interestingly, control subjects had a group of CD3+ cells that showed heterogenous NAT1 expression. Notably, patients had no $CD19^+$ cells at their first ALL diagnosis. This can be because the patients were undergoing chemotherapy when the sample was taken, and the cytotoxic activity of the drugs decreased this cell subpopulation (20). $CD19^+$ cells reappeared in patients with relapse, but only the cluster of cells with low NAT1 expression. It disappeared in patients at initial diagnosis but reappeared in patients with relapse, but the cells only had low NAT1 expression. We collected a sample from a relapsed individual before the patient started chemotherapy to confirm this tendency. Under these conditions, we found a high number of $CD19^+$ lymphocytes with no NAT1 expression and no NAT1 activity. These results suggest that NAT1 might be decreased at the early stages of the disease, and this downregulation is a critical factor in the development of leukemia. Thus, overall low expression of NAT1 in $CD3^+$ or $CD19^+$ lymphocytes could be a prognostic factor. We cannot rule out the possibility that NAT1 expression is low at the beginning of the disease and increases during the later stages. This hypothesis must be confirmed by determining NAT1 expression at different stages of leukemia.

NAT2 is another phase 2 drug-metabolizing enzyme, and genotype association studies have shown that alterations in this enzyme are related to the risk of developing ALL (3, 10, 16, 17, 30). Our results indicate that the percentage of $NAT1^+$ cells is higher than that $NAT2^+$ cells in control subjects and patients with ALL, which is opposite to the results of a previous study in PBMC (23), although the population in both studies was different (children *vs*. adults). This result suggests that NAT1 and NAT2 expression can vary during aging. We found that $CD3^+$ lymphocytes from some patients with ALL presented high NAT2 expression compared with control subjects, although NAT2 activity was not different between the groups. Consistently with this, a study carried out in the Mexican population reported that the phenotype provided by the haplotypes *NAT2*11A* and *NAT2*12C* (rapid phenotype) was associated with the

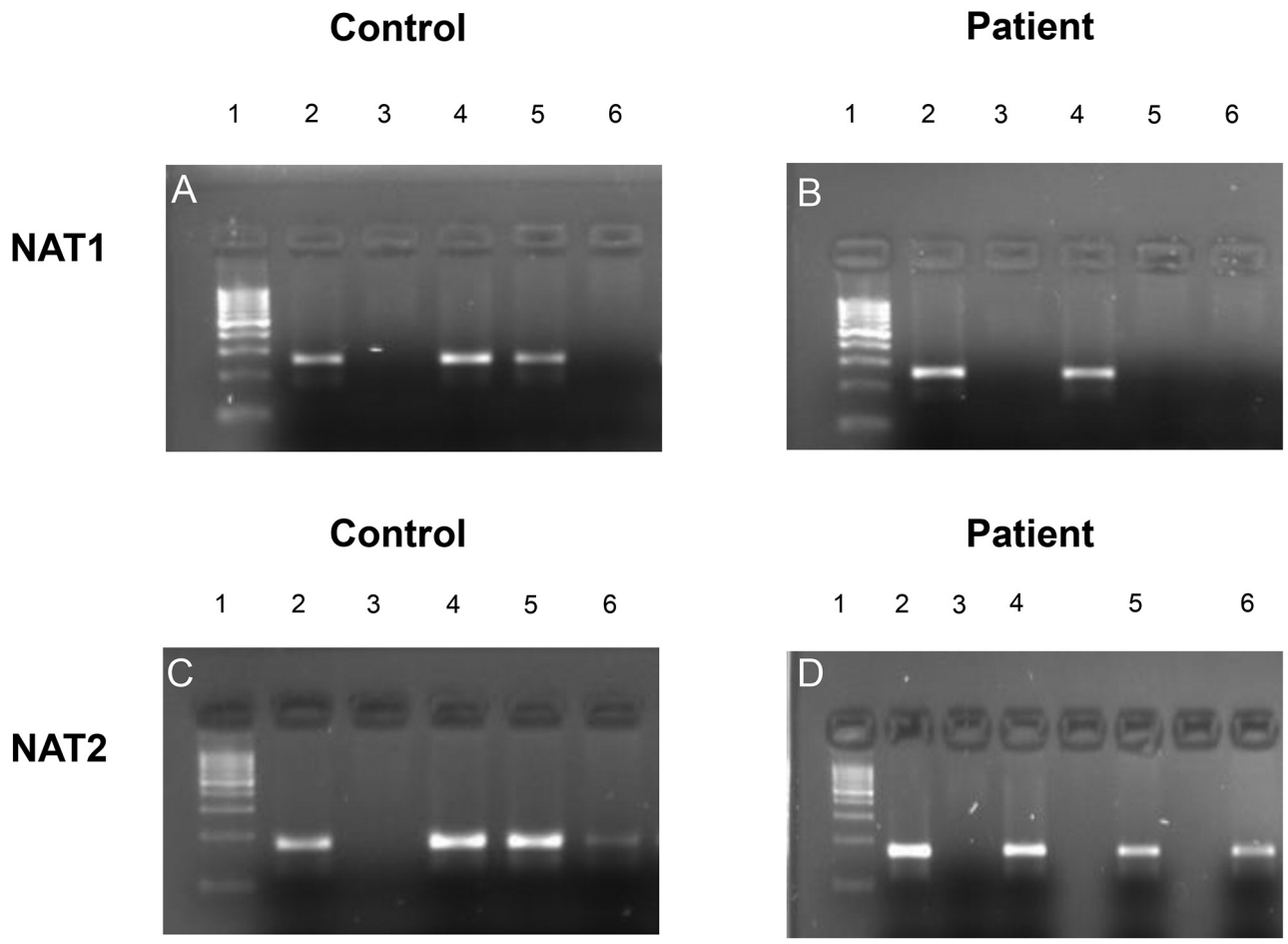

Figure 8. *Histone H3 acetylation and methylation in the NAT1 and NAT2 promoter region peripheral blood mononuclear cells (PBMC) from patients with ALL. Agarose gels (3%) of the amplicons of immunoprecipitated chromatin (CHiP) of NAT1 (202 bp) and NAT2 (171 bp) in a control subject (A and C) and a patient with ALL (B and D). Agarose gels were stained with ethidium bromide and run at 100 V for 30 min at room temperature. Lane 1 100-1000 bp DNA ladder (Jena Bioscience®), lane 2 Input, lane 3 negative, lane 4 anti-Histone H3 control, lane 5 anti-Histone H3 acetyl K14, lane 6 anti-Histone H3 trimethyl K27.*

probability of developing ALL (34). The t-SNE analysis showed that compared with patients at the initial ALL diagnosis and control subjects, patients with relapse had CD19+ cells with high levels of NAT2.

It is possible that we did not observe a statistical association between the study variables because NAT2 is expressed mainly in the liver, unlike NAT1, which has a higher expression in other organs. Therefore, only genetic association studies may be indicative of an abnormality in the cells; the results at the protein level do not indicate involvement of NAT2 in carcinogenesis. We propose performing additional studies to determine whether NAT2 expression is involved in relapse and whether it could be used as a factor to help detect minimal residual disease in ALL.

The fact that patients with ALL presented low NAT1 enzymatic activity, mainly in those who relapsed, prompted us to investigate the possible molecular mechanisms. Thus, we evaluated the SNPs 559 C>T (haplotype *NAT1*15*) and 560 G>A (haplotype *NAT1*14B*) in patients who showed differences in enzymatic activity. The presence of the first one generates a truncated protein, and the second one causes a slow acetylator phenotype (http://nat.mbg.duth.gr/). However, the patients did not carry any of the evaluated SNPs. Other SNPs, such as 97 C>T (haplotype *NAT1*19A*) and 190 C>T (haplotype *NAT1*19B*, with SNP 97), could be responsible for the low NAT1 enzymatic activity and need to be evaluated.

It is well known that post-translational modifications of NAT1, particularly lysine acetylation, play an important role in regulating its activation and function (35, 36). Researchers recently identified K100 and K188 as major sites for NAT1 post-translational modification. NAT1 is acetylated at these

sites by the acetyltransferase p300/CBP and is deacetylated by the sirtuins SIRT1 and SIRT2. The authors demonstrated that a p300/CBP inhibitor decreases NAT1 acetylation in HeLa cells. This modification was enhanced when cells were treated with nicotinamide, a sirtuin inhibitor (37). These proteins are altered in ALL; for example, there is a high frequency of CREB-binding protein (CBP) mutations in patients with recent diagnosis and relapse (38, 39), and SIRT1 expression is also elevated in ALL (40, 41). These data suggest that low NAT1 expression and activity could be due to alterations in its regulators and NAT1 itself. We have previously shown that SIRT1 is expressed in lymphocytes at levels above 42% (23); hence, it is necessary to explore the expression of proteins such p300/CBP, SIRT1, SIRT2, and other regulators of NAT1 and NAT2 in lymphocyte subpopulations and their relationship with NATs proteins in ALL. We are currently conducting these experiments.

Epigenetic mechanisms control the expression of genes in eukaryotic cells, and aberrations in these mechanisms can contribute to cancer development (42, 43). Abnormal epigenetic modifications are common in ALL (44, 45). miRNAs are part of epigenetic mechanisms and act as negative regulators of gene expression (46). Indeed, an altered miRNA profile has been detected in patients with ALL (47) and has been related to their chemoresistance (48). NAT1 is a direct target of miR-1290 and is differentially expressed in breast cancer (18). We found high miR-1290 expression in the plasma from patients with ALL and those with relapse and a correlation between *NAT1* mRNA levels and miR-1290 in patients with ALL. These findings are consistent with studies in breast cancer.

Although previous studies have shown the association between altered expression of certain miRNAs and ALL, we chose to evaluate miRNAs based on bioinformatic analysis. These miRNAs are possible regulators of the *NAT1* gene, and its expression may be affected by deregulation of these miRNAs. While most of the work on miRNA expression profiles has been carried out using PBMC, we examined the plasma expression profile of miR-1290 and miR-26b in patients with ALL. This analysis is very important because circulating miRNAs have great potential as non-invasive biomarkers for diagnosis and prognosis and new therapeutic targets that can benefit the pediatric population with ALL.

We hypothesized that up-regulation of miR-1290 during ALL progression decreases NAT1 expression. Researchers have also found a correlation between miR-26b and *NAT1* mRNA; although, miRNA-26b has also been characterized as a regulator of tumor suppression in healthy subjects (18, 49-51). Thus, we expected to find a positive correlation in control subjects; however, we did not find a negative correlation in patients with ALL. Therefore, circulating miR-1290 might be a potential biomarker for relapse and disease progression in patients with ALL. However, further studies are needed to demonstrate the possible role of miRNAs and NAT1 levels in childhood cancer.

We next investigated whether epigenetic alterations occur precisely at the promoter regions of the *NAT1* and *NAT2* genes. This information could provide a better understanding of the observed expression differences. We performed a computer analysis using the Encode® database through the UCSC Genome Browser and Washu epigenome Browser to determine which regions of the *NAT1* and *NAT2* promoters show the highest interaction with histone H3. Then, we performed a ChIP assay and observed a loss of H3K14Ac in the promoter of *NAT1* in patients with ALL compared with control subjects. This modification may play a key role in the adequate activation of the transcription of the *NAT1* gene in PBMC and may be the cause of low *NAT1* mRNA expression found in these cells. In agreement with our results, it has been reported that the acetylation of histone H3 and H4 in PBMC from patients with ALL and acute myeloid leukemia (AML) is deficient compared with healthy adults (44). Hence, if NAT1 plays a primary role in cell cycle regulation, promoting re-expression of the gene through H3K14Ac would perhaps help to decrease the proliferation of cancer cells. Unlike the control group, we found that in patients with ALL there was a minimal increase in methylation of histone 3 on lysine 27 (H3K27me3) in the promoter of *NAT2*. Our results concur with those reported by Yong Zou *et al*. (45), who observed aberrant methylation of histone H3 in PBMC from patients with ALL and AML compared with healthy adults. However, we do not know the influence of these modifications on this promoter because there were no changes at the mRNA level. It is possible that performing this analysis at the subpopulation level of lymphocytes would allow us to find a correlation between the cell subpopulation and enzymatic activity and deduce that NATs impact the progression of the disease. These variables could be used as a biomarker for relapse or pathogenic conditions.

To the best of our knowledge, this is the first study that has explored whether there are differences in NATs mRNA and protein expression in subpopulations of immune cells from patients with ALL. However, we are aware that our work has certain limitations. First, the sample size needs to be increased to confirm our results. Second, we need to validate the data obtained in other PBMC subpopulations. Finally, a deeper understanding of the underlying mechanisms that regulate NAT1 and NAT2 in ALL is required.

A limitation of our study is that it is monocentric, and we only relied on a single hospital to recruit patients into our study protocol. In addition, due to the distances from the places of origin of the patients, the Central Hospital "Dr. Ignacio Morones Prieto" is the main concentration center for patients in our State.

Conclusion

The low NAT1 mRNA and protein expression with low/null enzymatic activity in PBMC from patients with ALL may be partly due to the low expression of the transcript and the upregulation of miR-1290 which is a direct regulator of this protein. This phenomenon can influence carcinogenesis and relapses of ALL. We observed alterations in the post-translational modifications of histones in the promoters of *NAT1* (isoform A) and *NAT2*. However, the relevance of *NAT2* in the abnormalities present in this condition remains unclear. These determinations indicate that the expression of NAT1 and miR-1290 could be involved in this disease, however, more studies are needed to determine their role in this neoplasia.

Conflicts of Interest

The Authors report no conflicts of interest, and they declare did not receive support from any organization for the submitted work.

Authors' Contributions

DPPP: Conceived and designed the study; funding acquisition; supervision; analyzed and interpreted the results; writing—original draft. UREE: Supervision; analyzed and interpreted the results; writing—original draft. OHG: Methodology; Designed the study; sample collection and interpreted the results; writing—original draft. DZR: Methodology; writing—review & editing. DMAZ: Methodology; writing—review & editing. JJOZ: Data and sample collection; writing—review & editing. LCCG: Data and sample collection; writing—review & editing. JMVM: Funding acquisition; writing—review & editing. RCMS: Writing—review & editing. All Authors have read and approved the final report.

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References

1 Mejía-Aranguré JM: Etiology of acute leukemias in children. Cham, Switzerland, Springer, 2016.

2 Pui CH, Robison LL and Look AT: Acute lymphoblastic leukaemia. Lancet *371(9617)*: 1030-1043, 2008. PMID: 18358930. DOI: 10.1016/S0140-6736(08)60457-2

3 Kamel AM, Ebid GT and Moussa HS: N-Acetyltransferase 2 (NAT2) polymorphism as a risk modifier of susceptibility to pediatric acute lymphoblastic leukemia. Tumour Biol *36(8)*: 6341-6348, 2015. PMID: 25804798. DOI: 10.1007/s13277-015-3320-7

4 Jiménez-Morales S, Hidalgo-Miranda A and Ramírez-Bello J: [Acute lymphoblastic leukemia: a genomic perspective]. Bol Med Hosp Infant Mex *74(1)*: 13-26, 2017. PMID: 29364809. DOI: 10.1016/j.bmhimx.2016.07.007

5 Secretaría de Salud, Subsecretaría de Prevención y Promoción de la Salud and Dirección General de Epidemiología: Perfil epidemiológico del cáncer en niños y adolescentes en México. Available at: https://epidemiologiatlax.files.wordpress.com/2012/10/p_epi_del_cancer_en_nic3b1osyadolescentes_mexico.pdf [Last accessed on March 11, 2023]

6 Quiroz E, Aldoss I, Pullarkat V, Rego E, Marcucci G and Douer D: The emerging story of acute lymphoblastic leukemia among the Latin American population - biological and clinical implications. Blood Rev *33*: 98-105, 2019. PMID: 30126753. DOI: 10.1016/j.blre.2018.08.002

7 Navarrete-Meneses MDP and Pérez-Vera P: [Epigenetic alterations in acute lymphoblastic leukemia]. Bol Med Hosp Infant Mex *74(4)*: 243-264, 2017. PMID: 29382514. DOI: 10.1016/j.bmhimx.2017.02.005

8 Krajinovic M, Ghadirian P, Richer C, Sinnett H, Gandini S, Perret C, Lacroix A, Labuda D and Sinnett D: Genetic susceptibility to breast cancer in French-Canadians: role of carcinogen-metabolizing enzymes and gene-environment interactions. Int J Cancer *92(2)*: 220-225, 2001. PMID: 11291049. DOI: 10.1002/1097-0215(200102)9999:9999<::aid-ijc1184>3.0.co;2-h

9 Whyatt RM and Perera FP: Application of biologic markers to studies of environmental risks in children and the developing fetus. Environ Health Perspect *103 Suppl 6(Suppl 6)*: 105-110, 1995. PMID: 8549455. DOI: 10.1289/ehp.95103s6105

10 Krajinovic M, Richer C, Sinnett H, Labuda D and Sinnett D: Genetic polymorphisms of N-acetyltransferases 1 and 2 and gene-gene interaction in the susceptibility to childhood acute lymphoblastic leukemia. Cancer Epidemiol Biomarkers Prev *9(6)*: 557-562, 2000. PMID: 10868688.

11 Sinnett D, Labuda D and Krajinovic M: Challenges identifying genetic determinants of pediatric cancers—the childhood leukemia experience. Fam Cancer *5(1)*: 35-47, 2006. PMID: 16528607. DOI: 10.1007/s10689-005-2574-4

12 Buffler PA, Kwan ML, Reynolds P and Urayama KY: Environmental and genetic risk factors for childhood leukemia: appraising the evidence. Cancer Invest *23(1)*: 60-75, 2005. PMID: 15779869.

13 Sanderson S, Salanti G and Higgins J: Joint effects of the N-acetyltransferase 1 and 2 (NAT1 and NAT2) genes and smoking on bladder carcinogenesis: a literature-based systematic HuGE review and evidence synthesis. Am J Epidemiol *166(7)*: 741-751, 2007. PMID: 17675654. DOI: 10.1093/aje/kwm167

14 Jančová P and Šiller M: Phase II drug metabolism. Topics on Drug Metabolism. InTechOpen 35-60, 2012. DOI: 10.5772/29996

15 Wang L, Minchin RF and Butcher NJ: Arylamine N-acetyltransferase 1 protects against reactive oxygen species during glucose starvation: Role in the regulation of p53 stability. PLoS One *13(3)*: e0193560, 2018. PMID: 29518119. DOI: 10.1371/journal.pone.0193560

16 Zanrosso CW, Emerenciano M, Faro A, Gonçalves BA, Mansur MB and Pombo-de-Oliveira MS: Genetic variability in N-acetyltransferase 2 gene determines susceptibility to childhood lymphoid or myeloid leukemia in Brazil. Leuk Lymphoma *53(2)*: 323-327, 2012. PMID: 21888617. DOI: 10.3109/10428194.2011.619605

17 Hernández-González O, Ortiz-Zamudio JJ, Rodríguez-Pinal CJ, Alvarado-Morales I, Martínez-Jiménez VDC, Salazar-González

RA, Correa-González LC, Gómez R, Portales-Pérez DP and Milán-Segovia RDC: Genetic polymorphisms of arylamine N-acetyltransferases 1 and 2 and the likelihood of developing pediatric acute lymphoblastic leukemia. Leuk Lymphoma *59(8)*: 1968-1975, 2018. PMID: 29214875. DOI: 10.1080/10428194.2017.1406090

18 Endo Y, Yamashita H, Takahashi S, Sato S, Yoshimoto N, Asano T, Hato Y, Dong Y, Fujii Y and Toyama T: Immunohistochemical determination of the miR-1290 target arylamine N-acetyltransferase 1 (NAT1) as a prognostic biomarker in breast cancer. BMC Cancer *14*: 990, 2014. PMID: 25528056. DOI: 10.1186/1471-2407-14-990

19 Hernández-Bernal Y, Pantiga-Rosines IS, Organista-Nava J, Rivera-Ramírez AB, Campos-Olguin LM, Saavedra-Herrera MV, Jiménez-López MA, Illades-Aguiar B, Leyva-Vázquez MA and Gómez-Gómez Y: Expresión de mir-26b en leucemia linfoblástica aguda. Available at: https://speckle.inaoep.mx/~tecnologia_salud/2018/Resumenes/MyT2018-116_C.pdf [Last accessed on March 11, 2023]

20 Diagnóstico y Tratamiento Leucemia Linfoblástica Aguda en el Adulto. Guía de Referencia Rápida Guía de Práctica Clínica. México, Instituto Mexicano del Seguro Social; 2018. Available at: http://www.imss.gob.mx/sites/all/statics/guiasclinicas/142GER.pdf [Last accessed on March 14, 2023]

21 Barker DF, Husain A, Neale JR, Martini BD, Zhang X, Doll MA, States JC and Hein DW: Functional properties of an alternative, tissue-specific promoter for human arylamine N-acetyltransferase 1. Pharmacogenet Genomics *16(7)*: 515-525, 2006. PMID: 16788383. DOI: 10.1097/01.fpc.0000215066.29342.26

22 Husain A, Zhang X, Doll MA, States JC, Barker DF and Hein DW: Functional analysis of the human N-acetyltransferase 1 major promoter: quantitation of tissue expression and identification of critical sequence elements. Drug Metab Dispos *35(9)*: 1649-1656, 2007. PMID: 17591675. DOI: 10.1124/dmd.107.016485

23 Turiján-Espinoza E, Salazar-González RA, Uresti-Rivera EE, Hernández-Hernández GE, Ortega-Juárez M, Milán R and Portales-Pérez D: A pilot study of the modulation of sirtuins on arylamine N-acetyltransferase 1 and 2 enzymatic activity. Acta Pharm Sin B *8(2)*: 188-199, 2018. PMID: 29719779. DOI: 10.1016/j.apsb.2017.11.008

24 Salazar-González RA, Turiján-Espinoza E, Hein DW, Niño-Moreno PC, Romano-Moreno S, Milán-Segovia RC and Portales-Pérez DP: Arylamine N-acetyltransferase 1 in situ N-acetylation on CD3+ peripheral blood mononuclear cells correlate with NATb mRNA and NAT1 haplotype. Arch Toxicol *92(2)*: 661-668, 2018. PMID: 29043425. DOI: 10.1007/s00204-017-2082-y

25 Validation of analytical procedures: Text and methodology q2 (r1). Geneva, Switzerland, International conference on harmonization, 11-12, 2005. Available at: https://www.ema.europa.eu/en/documents/scientific-guideline/ich-q-2-r1-validation-analytical-procedures-text-methodology-step-5_en.pdf [Last accessed on March 11, 2023]

26 Van Der Maaten L: Accelerating t-sne using tree-based algorithms. J Mach Learn Res *15(1)*: 3221-3245, 2014.

27 Belkina AC, Ciccolella CO, Anno R, Halpert R, Spidlen J and Snyder-Cappione JE: Automated optimized parameters for T-distributed stochastic neighbor embedding improve visualization and analysis of large datasets. Nat Commun *10(1)*: 5415, 2019. PMID: 31780669. DOI: 10.1038/s41467-019-13055-y

28 Pieters R and Carroll WL: Biology and treatment of acute lymphoblastic leukemia. Pediatr Clin North Am *55(1)*: 1-20, ix, 2008. PMID: 18242313. DOI: 10.1016/j.pcl.2007.11.002

29 Tovar L and Fabian C: Factores de pronóstico en leucemia linfoblástica aguda pediátrica: Posibles marcadores moleculares. Universidad Autónoma del Estado de México, 2015. DOI: 10.1016/j.mei.2015.02.008

30 Gra OA, Glotov AS, Kozhekbaeva Zhm, Makarova OV and Nasedkina TV: [Genetic polymorphism in GST, NAT2, and MTRR and susceptibility to childhood acute leukemia]. Mol Biol (Mosk) *42(2)*: 214-225, 2008. PMID: 18610829.

31 Shi C, Xie LY, Tang YP, Long L, Li JL, Hu BL and Li KZ: Hypermethylation of N-Acetyltransferase 1 is a prognostic biomarker in colon adenocarcinoma. Front Genet *10*: 1097, 2019. PMID: 31781164. DOI: 10.3389/fgene.2019.01097

32 Cai J, Sun H, Chen L, Xie M, Zhuang J, Gao L and Wei XX: NAT1 is a critical prognostic biomarker and inhibits proliferation of colorectal cancer through modulation of PI3K/Akt/mTOR. Future Oncol *17(19)*: 2489-2498, 2021. PMID: 33906370. DOI: 10.2217/fon-2020-0992

33 Butcher NJ and Minchin RF: Arylamine N-acetyltransferase 1: a novel drug target in cancer development. Pharmacol Rev *64(1)*: 147-165, 2012. PMID: 22090474. DOI: 10.1124/pr.110.004275

34 Medina-Sanson A, Núñez-Enríquez JC, Hurtado-Cordova E, Pérez-Saldivar ML, Martínez-García A, Jiménez-Hernández E, Fernández-López JC, Martín-Trejo JA, Pérez-Lorenzana H, Flores-Lujano J, Amador-Sánchez R, Mora-Ríos FG, Peñaloza-González JG, Duarte-Rodríguez DA, Torres-Nava JR, Flores-Bautista JE, Espinosa-Elizondo RM, Román-Zepeda PF, Flores-Villegas LV, González-Ulivarri JE, Martínez-Silva SI, Espinoza-Anrubio G, Almeida-Hernández C, Ramírez-Colorado R, Hernández-Mora L, García-López LR, Cruz-Ojeda GA, Godoy-Esquivel AE, Contreras-Hernández I, Medina-Hernández A, López-Caballero MG, Hernández-Pineda NA, Granados-Kraulles J, Rodríguez-Vázquez MA, Torres-Valle D, Cortés-Reyes C, Medrano-López F, Pérez-Gómez JA, Martínez-Ríos A, Aguilar-De Los Santos A, Serafin-Díaz B, Bekker-Méndez VC, Mata-Rocha M, Morales-Castillo BA, Sepúlveda-Robles OA, Ramírez-Bello J, Rosas-Vargas H, Hidalgo-Miranda A, Mejía-Aranguré JM and Jiménez-Morales S: Genotype-environment interaction analysis of NQO1, CYP2E1, and NAT2 polymorphisms and the risk of childhood acute lymphoblastic leukemia: a report from the Mexican Interinstitutional Group for the Identification of the Causes of Childhood Leukemia. Front Oncol *10*: 571869, 2020. PMID: 33072605. DOI: 10.3389/fonc.2020.571869

35 Minchin RF, Rosengren KJ, Burow R and Butcher NJ: Allosteric regulation of arylamine N-acetyltransferase 1 by adenosine triphosphate. Biochem Pharmacol *158*: 153-160, 2018. PMID: 30342020. DOI: 10.1016/j.bcp.2018.10.013

36 Minchin RF and Butcher NJ: The role of lysine(100) in the binding of acetylcoenzyme A to human arylamine N-acetyltransferase 1: implications for other acetyltransferases. Biochem Pharmacol *94(3)*: 195-202, 2015. PMID: 25660616. DOI: 10.1016/j.bcp.2015.01.015

37 Butcher NJ, Burow R and Minchin RF: Modulation of human arylamine N-Acetyltransferase 1 activity by lysine acetylation:

Role of p300/CREB-binding protein and Sirtuins 1 and 2. Mol Pharmacol *98(2)*: 88-95, 2020. PMID: 32487734. DOI: 10.1124/mol.119.119008

38 Mullighan CG, Zhang J, Kasper LH, Lerach S, Payne-Turner D, Phillips LA, Heatley SL, Holmfeldt L, Collins-Underwood JR, Ma J, Buetow KH, Pui CH, Baker SD, Brindle PK and Downing JR: CREBBP mutations in relapsed acute lymphoblastic leukaemia. Nature *471(7337)*: 235-239, 2011. PMID: 21390130. DOI: 10.1038/nature09727

39 Oshima K, Zhao J, Pérez-Durán P, Brown JA, Patiño-Galindo JA, Chu T, Quinn A, Gunning T, Belver L and Ambesi-Impiombato A: Mutational and functional genetics mapping of chemotherapy resistance mechanisms in relapsed acute lymphoblastic leukemia. Nat Cancer 1(11): 1113-1127, 2020. PMID: 33796864. DOI: 10.1038/s43018-020-00124-1

40 Jin Y, Cao Q, Chen C, Du X, Jin B and Pan J: Tenovin-6-mediated inhibition of SIRT1/2 induces apoptosis in acute lymphoblastic leukemia (ALL) cells and eliminates ALL stem/progenitor cells. BMC Cancer *15*: 226, 2015. PMID: 25884180. DOI: 10.1186/s12885-015-1282-1

41 Li L, Ye S, Yang M, Yu W, Fan Z, Zhang H, Hu J, Liang A and Zhang W: SIRT1 downregulation enhances chemosensitivity and survival of adult T-cell leukemia-lymphoma cells by reducing DNA double-strand repair. Oncol Rep *34(6)*: 2935-2942, 2015. PMID: 26398583. DOI: 10.3892/or.2015.4287

42 Taylor KH, Bennett LB, Arthur GL, Shi H and Caldwell CW: The epigenetics of age-related cancers. In: Epigenetics of aging. Tollefsbol TO (ed.). New York, NY, USA, Springer, pp. 285-313, 2010. DOI: 10.1007/978-1-4419-0639-7

43 Kanwal R and Gupta S: Epigenetic modifications in cancer. Clin Genet *81(4)*: 303-311, 2012. PMID: 22082348. DOI: 10.1111/j.1399-0004.2011.01809.x

44 Xiao L, Huang Y, Zhen R, Chiao JW, Liu D and Ma X: Deficient histone acetylation in acute leukemia and the correction by an isothiocyanate. Acta Haematol *123(2)*: 71-76, 2010. PMID: 20051681. DOI: 10.1159/000264628

45 Zou Y, Ma X, Huang Y, Hong L and Chiao JW: Effect of phenylhexyl isothiocyanate on aberrant histone H3 methylation in primary human acute leukemia. J Hematol Oncol *5*: 36, 2012. PMID: 22747680. DOI: 10.1186/1756-8722-5-36

46 Arif KMT, Elliott EK, Haupt LM and Griffiths LR: Regulatory mechanisms of epigenetic miRNA relationships in human cancer and potential as therapeutic targets. Cancers (Basel) *12(10)*: 2922, 2020. PMID: 33050637. DOI: 10.3390/cancers12102922

47 Chatterton Z, Morenos L, Saffery R, Craig JM, Ashley D and Wong NC: DNA methylation and miRNA expression profiling in childhood B-cell acute lymphoblastic leukemia. Epigenomics *2(5)*: 697-708, 2010. PMID: 22122053. DOI: 10.2217/epi.10.39

48 Schotte D, De Menezes RX, Akbari Moqadam F, Khankahdani LM, Lange-Turenhout E, Chen C, Pieters R and Den Boer ML: MicroRNA characterize genetic diversity and drug resistance in pediatric acute lymphoblastic leukemia. Haematologica *96(5)*: 703-711, 2011. PMID: 21242186. DOI: 10.3324/haematol.2010.026138

49 Gao Z, Ye X, Bordeaux A, Hettich S, Lin S, Han F and Jia Y: miR-26b regulates cell proliferation and apoptosis of CD117+CD44+ ovarian cancer stem cells by targeting PTEN. Eur J Histochem *65(1)*: 3186, 2021. PMID: 33634678. DOI: 10.4081/ejh.2021.3186

50 Ding Q, Wang Y, Zuo Z, Gong Y, Krishnamurthy S, Li CW, Lai YJ, Wei W, Wang J, Manyam GC, Diao L, Zhang X, Lin F, Symmans WF, Sun L, Liu CG, Liu X, Debeb BG, Ueno NT, Harano K, Alvarez RH, Wu Y, Cristofanilli M and Huo L: Decreased expression of microRNA-26b in locally advanced and inflammatory breast cancer. Hum Pathol *77*: 121-129, 2018. PMID: 29689244. DOI: 10.1016/j.humpath.2018.04.002

51 Endo Y, Toyama T, Takahashi S, Yoshimoto N, Iwasa M, Asano T, Fujii Y and Yamashita H: miR-1290 and its potential targets are associated with characteristics of estrogen receptor α-positive breast cancer. Endocr Relat Cancer *20(1)*: 91-102, 2013. PMID: 23183268. DOI: 10.1530/ERC-12-0207

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