Comprehensive Expression of Long non-coding RNAs and association with the Iron and Erythropoiesis Regulatory Proteins in Transfusion-dependent β-Thalassemia

Keywords
ferritin, LncRNA NEAT1, LncRNA GAS5, Transfusion dependent β-Thalassemia, HAMP

Abstract

Introduction
β-Thalassemia is a genetic disorder characterized by a quantitative defect in β-globin synthesis caused by genetic and epigenetic alterations. However, the expression patterns of long non-coding RNAs (LncRNAs) and their relationship with genes and proteins involved in iron metabolism and erythropoiesis remain largely unknown. We aimed to investigate the expression of LncRNAs and their correlation with iron and erythropoiesis regulatory proteins in patients with transfusion dependent-β-Thalassemia (TDβ-T).

Material and methods
Whole blood samples and clinical records were collected from 60 patients with TDβ-T and 20 healthy controls. Expression levels of selected LncRNAs were measured using qRT-PCR. Iron metabolism and erythropoiesis-related proteins were quantified using ELISA.

Results
TDβ-T patients exhibited significantly elevated levels of iron and erythropoiesis-regulating proteins, as well as increased expression of HAMP, GDF-15, FAM132B, and SLC40A1 compared to controls. Additionally, LncRNAs ANRIL, H9, LINCO133, MIAT, and NEAT1 were markedly upregulated, while LncRNA GAS5 was downregulated in patients with TDβ-T. Among these, LncRNAs NEAT1 and GAS5 showed the strongest diagnostic performance. A significant correlation was observed between the expression of HAMP and FAM132B and LncRNAs ANRIL, H19, LINCO133, and MIAT. Furthermore, LncRNA NEAT1 expression correlated positively with SLC40A1 and negatively with urea levels, whereas LncRNA GAS5 was inversely correlated with HAMP expression.

Conclusions
This study is the first to demonstrate altered LncRNA expression patterns and their associations with iron metabolism, erythropoiesis-regulating proteins, and urea levels in patients with TDβ-T. These findings provide new insights for future research and potential therapeutic targets.

Comprehensive Expression of *Long Non-Coding RNAs* and association with Iron and Erythropoiesis Regulatory Proteins in Transfusion-Dependent β-Thalassemia

Abstract

Introduction:

β-Thalassemia is a genetic disorder characterized by a quantitative defect in β-globin synthesis caused by genetic and epigenetic alterations. However, the expression patterns of *long non-coding RNAs (LncRNAs*) and their relationship with genes and proteins involved in iron metabolism and erythropoiesis remain largely unknown. We aimed to investigate the expression of *LncRNAs* and their correlation with iron and erythropoiesis regulatory proteins in patients with transfusion dependent-β-Thalassemia (TDβ-T).

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TDβ-T patients exhibited significantly elevated levels of iron and erythropoiesis-regulating proteins, as well as increased expression of *HAMP*, *GDF-15*, *FAM132B*, and *SLC40A1* compared to controls. Additionally, *LncRNAs ANRIL*, *H19*, *LINCO133*, *MIAT*, and *NEAT1* were markedly upregulated, while *LncRNA GAS5* was downregulated in patients with TDβ-T. Among these, *LncRNAs NEAT1* and *GAS5* showed the strongest diagnostic performance. A significant correlation was observed between the expression of *HAMP* and F*AM132B* and

LncRNAs ANRIL, *H19*, *LINCO133*, and *MIAT*. Furthermore, *LncRNA* N*EAT1* expression correlated positively with *SLC40A1* and negatively with urea levels, whereas *LncRNA GAS5* was inversely correlated with *HAMP* expression.

Conclusion:

This study is the first to demonstrate altered *LncRNA* expression patterns and their associations with iron metabolism, erythropoiesis-regulating proteins, and urea levels in patients with TDβ-T. These findings provide new insights for future research and potential therapeutic targets.

Key Words: *LncRNA NEAT1*; *LncRNA GAS5*; Transfusion dependent β-Thalassemia; Iron regulatory proteins; Erythropoiesis regulatory proteins.

Introduction

β-Thalassemia is an autosomal recessive disorder caused by a quantitative defect in β-globin synthesis, leading to impaired hemoglobin production and ineffective erythropoiesis with varying degrees of anemia [1]. Globally, over 30,000 new cases of β-Thalassemia cases are reported each year, with the majority occurring in developing countries [2]. Classification of β-Thalassemia is based on either the level of β-globin reduction [3] or the necessity for regular blood transfusions [1]. Gene expression and epigenetic regulation play crucial roles in the production of hemoglobin chains, with distinct regulatory mechanisms influencing the pathogenesis of β-Thalassemia which exacerbate thalassemia severity and associated complications such as ineffective erythropoiesis and iron overload [1, 4]. Additional

contributors to these complications include abnormal regulation of iron metabolism markers like ferritin [5] and hepcidin [6], and erythropoietic regulators such as erythropoietin (EPO) [7], growth differentiation factor 15 (GDF-15) [8], and erythroferrone (ERFE) [9, 10].

Long non-coding RNAs (*LncRNAs*) are emerging as critical regulators in various biological processes, including hematopoiesis. Disruption in *LncRNA* expression has been linked to impaired hemoglobin synthesis and anemia [11]. Recent studies have identified several *lncRNAs* as potential biomarkers or contributors to the pathology of cardiovascular, metabolic, thalassemia and neoplastic diseases. These include Antisense Non-coding RNA in the INK4 Locus (*ANRIL)* [12, 13], Growth Arrest-Specific 5 (*GAS5)* [14], *H19* [15], Metastasis Associated Lung Adenocarcinoma Transcript 1 (*MALAT1)* [16], Nuclear Enriched Abundant Transcript 1 *(NEAT1) [17]*, and Maternally Expressed Gene 3 *(MEG3)* [18]. Additional *lncRNAs* including *LINC0133*, *SNGH20*, and urothelial carcinoma associated 1(*UCA1)* are implicated in gene expression regulation in hematological disorders [19]. Despite this growing body of research, the role of lncRNAs in regulating iron metabolism and erythropoiesis in β-thalassemia remains underexplored. To the best of our knowledge, this is the first study to investigate the expression of *lncRNAs* and their correlation with iron and erythropoiesis regulatory proteins in patients with transfusion dependent-β-Thalassemia (TDβ-T). These findings may offer novel insights into disease mechanisms and uncover potential therapeutic targets.

Materials and Methods

Study design and sample collection

This study was conducted from September 2022 to December 2023 and included 80 participants: 60 patients with TDβ-T and 20 healthy controls. Participants, aged 7–35 years and of both sexes, were recruited from the Department of Thalassemia and Hemophilia at Al-Zarqa Public Hospital, Jordan. Written informed consent was obtained from all participants, and the study was approved by the Institutional Review Board (IRB) of the Ministry of Health, Amman, Jordan.

From each participant, 10 mL of whole blood was collected and divided equally into EDTA and plain tubes. Clinical records were also obtained for all patients with TDβ-T. Blood samples from patients with thalassemia were collected immediately prior to their scheduled blood transfusions.

Quantitation serum levels of hepcidin, GDF-15, erythropoietin and erythroferrone

Serum levels of hepcidin (Cat#MBS2700551, MyBioSource, USA), GDF-15 (Cat# BMS2258, ThermoFisher Scientific, USA), erythropoietin (Cat# BMS2035-2, Thermofisher Scientific, USA), and erythroferrone (Cat# EH1681-HS, FineTest Biotch Inc., USA) were quantified using ELISA kits, following the manufacturer's instructions.

Total RNA extraction and cDNA synthesis

Total RNA was extracted from the collected whole blood samples using the Direct-zol RNA Purification Kit (Zymo Research, USA) according to the manufacturer's protocol. RNA purity and concentration were assessed using a NABI spectrophotometer (MicroDigital, Korea).

Complementary DNA (cDNA) was synthesized using the PrimeScript™ RT Master Mix Kit (Takara, Japan) following the manufacturer's instructions.

Quantitive real-time polymerase chain reaction (qRT-PCR)

Gene expression was quantified using a QuantGene 9600 thermal cycler (Bioer Technology, Japan) and TB Green® Premix Ex Taq™ _II (Tli RNase H Plus, Japan). Primers were sourced from Integrated DNA Technologies (IDT, Coravillle, IA, USA) (Table 1). Each qRT-PCR reaction was performed in a 20 µL final volume, containing 2 µL (60 ng) of cDNA, 10 µL of master TB green mix, 2 µL of (10 pmole/µL) primers, and 6 µL of nuclease free water. The thermal cycling conditions consisted of an initial denaturation at 95ºC for 10 minutes, followed by 40 cycles of denaturation at 95ºC for 30 seconds, annealing at 60ºC for 30 seconds, and extension at 72 ºC for 30 seconds.

Statistical analysis

Continuous variables were expressed as mean ± standard deviation or median (min-max), depending on data distribution. Categorical variables were presented as frequencies (percentages). Fold changes relative to control mean were calculated using the delta-delta C_t method ($2^{-\Delta\Delta Ct}$) and subsequently $\log^2$-transformed to get $\log^2$ fold changes. Differences between patients and controls were assessed using Welch's two-sample t-test to account for unequal variances and sample sizes, or the Wilcoxon rank sum test for non-normal distributions. Correlation between $\log_2$ fold changes and other patient clinical parameters was assessed using Spearman correlation. The ability of the differentially expressed *LncRNAs* to distinguish between patients and controls was evaluated using receiver operating

characteristic (ROC) curves. Additionally, DeLong's test was used to assess if the area under the ROC curve was significantly different from 0.5. All analyses were conducted in R version 4.3.3 (2024-02-29 ucrt).

Results

Demographic characteristics, clinical parameters, and iron metabolism indices of the study population

The mean age and body mass index (BMI) were comparable between groups. Hematological parameters showed significant differences in mean values between TD-βT and controls, except for MCHC (Table II). Furthermore, mean liver enzyme levels were significantly increased in TDβ-T compared to controls ($p<0.0001$). Additionally, the mean urea level was significantly higher ($p=0.004$), while the mean creatinine level was significantly lower ($p<0.0001$) in the TD-βT group (Table II).

Iron-related parameters, including ferritin and serum iron levels, were markedly elevated ($p<0.001$) in TDβ-T compared to controls. No significant difference was observed in the Log^2 fold change of *HAMP* expression ($p=0.16$). In contrast, *GDF-15* expression was significantly upregulated ($p<0.01$) in TDβ-T, as were *FAM132B* and *SLC40A1* ($p<0.05$). Hepcidin protein levels did not differ significantly ($p=0.645$) between patients with TDβ-T and controls. However, the median protein concentrations of GDF-15, ERFF, and EPO were significantly elevated ($p<0.001$) in TDβ-T (Table II).

Differential expression of *long non-coding RNAs* of the study population

LncRNAs; *ANRIL* (p=0.044), *H19* (p=0.049), *LINC0133* (p=0.047), *MIAT* (p=0.046), and *NEAT1* (p<0.001) were upregulated on average in patients with TDβ-T compared to controls except for *LncRNA GAS5*, which had a significant (p<0.001) fold downregulation (Table III).

Receiver operation curve Analysis

LncRNA NEAT1 and *GAS5* exhibited the highest diagnostic performance with area under the receiver operating characteristic (ROC) curve of 93.5%, and 80.6% respectively, comapred to other *LncRNAs* Table (IV) and (Fig 1).

Association between *long non-coding RNAs* and iron regulatory proteins in transfusion-dependent β-Thalassemia

Log_2 fold changes in *HAMP* and F*AM132B* were strongly and significantly correlated (p<0.0001) with those of *LncRNA ANRIL*, *LncRNA H19*, *LncRNA LINCO133*, and *LncRNA MIAT* (Table V) and (Fig. 2A, C-I). In contrast, *GAS5* expression exhibited a significant negative correlation (p<0.05) with *HAMP* expression (Table V) (Fig. 2B). Furthermore, log_2 fold change in *SLC40A1* (encoding ferroportin) positively and markedly (p<0.001) correlated with log_2 fold change in *LncRNA* N*EAT1* (Table V) (Fig. 2J). No significant correlations were observed between the differentially expressed *LncRNAs* and the iron/erythropoiesis-regulating proteins in the control group (data not shown).

Correlation between *long non-coding RNAs* expression, ferritin, and liver/kidney function parameters in transfusion-dependent β-Thalassemia

Serum AST and ALT were strongly (rho of 0.55) correlated with ferritin. However, they were not correlated with *LncRNAGAS5* and *LncRNA NEAT1* $\log_2$ fold change except for urea, which was negatively (rho of -0.3) correlated with log2 fold change of *LncRNA NEAT1* (Table VI).

Discussion

β-Thalassemia is a quantitative impairment of β-globin chain biosynthesis caused by genetic and epigenetic aberrations, characterized by ineffective erythropoiesis and a high susceptibility to iron overload [20]. Although few studies have investigated *LncRNAs* expression in β-Thalassemia [21, 22], none have directly linked these RNAs to their distinguishing clinical features. Our study identified a notable reduction in hematological parameters and a significant increase in biochemical markers in patients with TDβ-TM compared to controls, consistent with previous findings [23, 24].

We observed a significant ($p<0.05$) upregulation of genes involved in iron level regulation–*GDF-15*, *ERFE*, and *SLC40A1*–in TDβ-T. Protein levels of GDF-15, ERFF, EPO, ferritin, and serum iron were also significantly ($p<0.0001$) elevated. Elevated EPO levels stimulate erythropoiesis by promoting the production of ERFE and GDF-15, which subsequently suppresses hepcidin, thereby enhancing iron availability for erythropoiesis [25, 26]. However, we observed no significant difference in hepcidin levels or *HAMP* expression between TDβ-T and controls, consistent with some studies [27, 28] but contrasting with findings by Chamaschella *et al.* [26]. This discrepancy may be attributed to variability in transfusion frequency [29], use and type of iron-chelators [30], genetic factors, or

178 inflammatory states. Genetic mutations can worsen or mitigate iron overload, influencing
179 disease severity [31].

180 *LncRNAs* are known to disrupt hematopoiesis [19, 32] and hemoglobin production in
181 thalassemia [33]. We observed significant upregulation of *LncRNAs ANRIL, MIAT1,* and
182 *NEAT1,* consistent with Fakhr-Eldeen (2019) [34], and for the first time, we report the
183 downregulation of *LncRNA GAS5* and upregulation of *LICN0133 and H19* in TD-βT. ROC
184 analysis identified *LncRNAs NEAT1* and *GAS5* as strong diagnostic biomarkers.
185 Interestingly, none of the measured proteins–ferritin, hepcidin, GDF-15, ERFE, EPO, or
186 serum iron–correlated significantly with *LncRNA* expression in TDβ-T. However, we found
187 strong correlations between TD-βT *HAMP* and F*AM132B* and *LncRNAs ANRIL, H19,*
188 *LINCO133,* and *MIAT,* representing novel findings. ANRIL is known to influence gene
189 expression involved in metabolic pathways [35], and limited empirical data regarding the
190 roles of *LncRNA H19, LncRNA LINC0133,* and *LncRNA MIAT* hampers firm conclusions
191 regarding their roles. Nevertheless, we are at the forefront of research in this area, as we are
192 documenting compelling evidence that indicates a robust significant ($p<0.001$) correlation
193 between *HAMP* and *FAM132B* and *LncRNAs: ANRIL, H19, LINC0133,* and *MIAT.*
194

195 Another pioneering result for this current study was the significant ($p<0.001$) positive
196 correlation between *LncRNA* N*EAT1* and *SLC40A1* (encoding for ferroportin). While the
197 current body of literature does not explicitly establish a direct association between *LncRNA*
198 *NEAT1* and *SLC40A1,* the evidenced regulatory functions of *LncRNA NEAT1* within the
199 contexts of oncogenesis and immune system modulation imply that it may exert influence

over, or exhibit correlation with various genes that participate in interconnected biological pathways [36, 37]. Additionally, we are the first to document a significant ($p<0.05$) negative correlation between *LncRNA GAS5* and *HAMP* expression. This observed inverse relationship can be contextualized within the broader framework of gene expression regulation, while also reflecting the underlying biological implications that negative correlations may signify in various molecular interactions across different pathological conditions–for example, its relationship with HMGB1 in sepsis [38] and with IL-18 in rheumatoid arthritis [39].

Our results showed a robust and significant ($p<0.001$) correlation between serum AST/ALT and ferritin, aligning with other reports [40, 41]. This reinforces the notion that increased ferritin levels may serve as a biomarker for liver dysfunction specifically among patients with TD-βT. While GAS5 and NEAT1 expression showed no significant correlation with liver/kidney function overall, a noteworthy exception was the negative correlation between NEAT1 and urea levels ($p < 0.05$), which may suggest metabolic regulation, as discussed by Moreno *et al.* [42].

This study had some limitations including a small sample size, lack of analysis across different β-Thalassemia phenotypes, and absence of post-transfusion *LncRNAs* expression data. Nevertheless, our findings offer novel insights into the molecular landscape of TD-βT.

Conclusion

To the best of our knowledge, this is the first study to report *LncRNAs* alterations in TD-βT, with *NEAT1* and *GAS5* emerging as promising diagnostic biomarkers. Moreover, the

observed association between *LncRNAs* and iron-regulatory and erythropoiesis-related genes warrants further investigation.

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Competing of interests

The authors declare no conflict of interests.

Abbreviations

The following abbreviations are used in this manuscript:

LncRNAs	*long non-coding RNAs*
TDβ-T	transfusion-dependent β-Thalassemia
EPO	Erythropoietin
GDF-15	growth differentiation factor 15
ERFF	erythroferrone
ANRIL	Antisense Non-coding RNA in the INK4 Locus
GAS5	Growth Arrest-Specific 5
MALAT1	Metastasis Associated Lung Adenocarcinoma Transcript 1
NEAT1	Nuclear Enriched Abundant Transcript 1
MEG3	Maternally Expressed Gene 3
UCA1	urothelial carcinoma associated 1
qRT-PCR	Quantitive real-time polymerase chain reaction
BMI	Body Mass Index
ROC	Receiver Operating Curve

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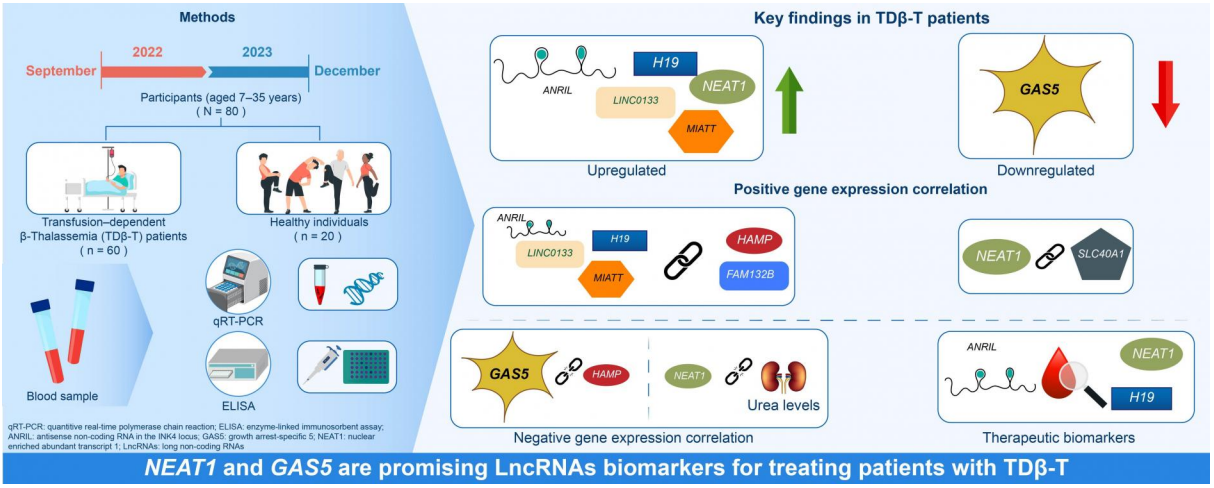
Methods
2022
2023
September
December
Participants (aged 7–35 years)
(N = 80)
Transfusion–dependent
β-Thalassemia (TDβ-T) patients
(n = 60)
Healthy individuals
(n = 20)
qRT-PCR
Blood sample
ELISA
qRT-PCR: quantitive real-time polymerase chain reaction; ELISA: enzyme-linked immunosorbent assay; ANRIL: antisense non-coding RNA in the INK4 locus; GAS5: growth arrest-specific 5; NEAT1: nuclear enriched abundant transcript 1; LncRNAs: long non-coding RNAs
Key findings in TDβ-T patients
ANRIL
H19
NEAT1
LINC0133
MIATT
Upregulated
GAS5
Downregulated
Positive gene expression correlation
HAMP
FAM132B
SLC40A1
Negative gene expression correlation
Urea levels
Therapeutic biomarkers
NEAT1 and GAS5 are promising LncRNAs biomarkers for treating patients with TDβ-T

Table I

Gene	Forward 3`-5`	Reverse 3`-5`
LncRNA NEAT1	CTTCCTCCCTTTAACTTATCCATTCAC	CTCTTCCTCCACCATTACCAACAATAC
LncRNA LASER	AAGGTGCCACAGATGCTCAA	GGGAGGTATCCCGGAGAAGT
LncRNA MALAT1	GAAGGAAGGAGCGCTAACGA	TACCAACCACTCGCTTTCCC
LncRNA MIAT	TCCCATTCCCGGAAGCTAGA	GAGGCATGAAATCACCCCCA
LncRNA UCA1	ATTAGGCCGAGAGCCGATCA	CCAGAGGAACGGATGAAGCC
LncRNA SNHG20	AGCAACCACTATTTTCTTCC	CCTTGGCGTGTATCTATTTAT
LncRNA H19	TCAGCTCTGGGATGATGTGGT	CTCAGGAATCGGCTCTGGAAG
LncRNA ANRIL	GCCGGACTAGGACTATTTGCC	TGGCATACCACACCCTAAC
LncRNA LINC01133	CCTAATCTCACCACAGCCTGG	TCAGAGGCACTGATGTTGGG
LncRNA MEG3	CTCCCCTTCTAGCGCTCACG	CTAGCCGCCGTCTATACTACCGGCT
LncRNA GAS5	TGTGTCCCCAAGGAAGGATG	TCCACACAGTGTAGTCAAGCC
HAMP	CCTGACCAGTGGCTCTGTTT	CACATCCCACACTTTGATCG
GDF-15	TCAGATGCTCCTGGTGTTGC	GATCCCAGCCGCACTTCTG
FAM132B	GTCCCAGAGTAGGTAGTGAAGA	TCCGGAGGCTAGTTAGTTAGAA
SLC40A1	TCCTTGGCCGACTACCTGAC	TCCCTTTGGATTGTGATTGC
GAPDH	AATGCCTCCTGCACCACCAAC	AAGGCCATGCCAGTGAGCTTC

Table II

Parameters	**Control (N=20)**	**TDβ-T (N=60)**	***p*-Value**
Demographic			
Age (years)	19.1±7	20.2±6.9	0.27
BMI (kg/m^2)	21.2±1.3	21.5±2.6	0.31
Gender: Female (n /%)	11 (55%)	30 (50%)	-
Male (n /%)	9 (45%)	30 (50%)	-
Hematological			
Hb (g/dL)	13.5±1.6	8.6±0.9	*** p<0.001
RBC (10^6/µL)	4.7±0.5	3.2±0.5	*** p<0.001
PCV (%)	38.4±3.7	24.6±2.6	*** p<0.001
RDW (%)	13.4±1.6	20.1±5.3	*** p<0.001
MCV (fL)	81.7±6.1	77.4±6.2	** p<0.01
MCH (pg/L)	28.7±3.0	27.0±2.8	* p<0.05
MCHC (g/dL)	35.0±1.5	34.9±1.7	0.672
WBC (10^3/µL)	7.5±2.5	15.4±6.6	**** p<0.0001
Platelets (10^3/µL)	291.1 ± 78.9	618.9 ± 78.9	**** p<0.0001
Biochemical			
ALT (U/L)	12.0±4.0	48.2±29.6	**** p<0.0001
AST (U/L)	18.8±6.4	49.7±25.3	**** p<0.0001
ALP (U/L)	83.6±24.1	163.5±79.5	**** p<0.0001
Urea (mg/L)	22±5.4	29.4±15.3	**0.004
Creatinine (mg/L)	0.6±0.2	0.4±0.3	**** p<0.0001
Iron Metabolism variable			
Ferritin (ng/mL)	36.1 (17.7-165)	2719 (160.0 -14658)	*** p<0.001

Iron (mg/dL)	84.3±22.2	222±58.8	*** p<0.001
log_2 Δ*HAMP*	-0.0±1.5	0.7±1.6	0.160
log_2 Δ*GDF-15*	0.0±0.9	4.4±2.1	** p<0.01
log_2 Δ*FAM132B*	0.0 ±1.2	0.9±1.5	*p<0.05
log_2 Δ*SLC40A1*	0.0±1.3	0.8±1.3	*p<0.05
Hepcidin (pg/mL)	133 (83.1-539)	135 (29.2-337)	0.645
Erythropoiesis regulatory proteins			
GDF-15 (pg/mL)	135 (31-350)	3907 (1276-7090)	*** p<0.001
ERFF (pg/mL)	317 (208-1108)	628 (216-1518)	*** p<0.001
EPO (mUI/mL)	5.6 (0-27.8)	156 (25.1-667)	*** p<0.001

Numeric variables are summarized as mean±standard deviation or median (min-max), depending normality.*p*-values indicate significance, as determined by Welch's two-sample t-test. Significance levels are represented as follows: *p<0.05, **p<0.01, ***p<0.001, and **** p<0.0001. The following abbreviations indicated for, TDβ-T: transfusion dependent β-Thalassemia, BMI: Body Mass Index, WBC: White Blood Cells, RBC: Red Blood Cells, PCV: Packed Cell Volume, MCV: Mean Corpuscular volume, MCH: Mean Corpuscular Hemoglobin, MCHC: Mean Corpuscular Hemoglobin Concentration, Hb: Hemoglobin, RDW: Red cell Distribution Width, ALT:Alanine Transaminase, AST: Aspartate Transaminase, ALP: Alkaline Phosphates, GDF-15: growth dependent factor-15, ERFF: erythroferrone, EPO: erythropoietin.

Table III

LncRNAs	**Mean log^2 fold change relative to control**		**Mean fold change relative to control**	***p*-Value**
	Control (N=20)	**TDβ-T (N=60)**		
ANRIL	$3.8x10^{-3}$±1.4	1±1.4	3.4±4.7	**0.044***
GAS5	$-2.7x10^{-3}$±1.0	-2.7±4.9	0.6±0.8	$2.5x10^{-4}$
H19	$6.1x10^{-11}$ ± 1.2	1.0±1.7	3.6±4.1	**0.049***
LASER	$-2.5x10^{-10}$±1.6	0.6±1.6	2.9±3.9	0.174
LINC0133	$-2.5x10^{-10}$±1.2	0.8±1.4	2.9±3.8	**0.047***
MALAT1	$-9.5x10^{-4}$±1.5	0.6±1.7	2.8±3.2	0.157
MEG3	$-9.7x10^{-4}$±1.2	0.7±1.3	2.5±3.5	0.095
MIAT	$-1.4x10^{-3}$±0.8	0.8±1.8	4.3±8.9	**0.046***
NEAT1	$-3.9x10^{-11}$±1.0	3.0±1.7	15.3±21.6	**<0.001***
SNGH20	$-2.9x10^{-10}$±1.3	1.1±1.6	3.5±3.9	0.093
UCA1	0.02±1.3	0.1±1.4	1.8±2.8	0.891

Numeric variables are summarized as mean±standard deviation.. *P*-values indicate significance, as determined by Welch's two-sample t-test. Significance levels are represented as follows: *p<0.05, **p<0.01, and ***p<0.001. On log scale, 0 represents no change, negative values represent down-regulation, and positive values represent up-regulation. TDβ-T indicates of transfusion dependent β-Thalassemia.

Table IV

$log_2\Delta$ Target *LncRNAs*	AUC (95% CI)	*p*-Value	Specificity	Sensitivity
ANRIL	67.1% (53-81.3%)	**0.020***	38.9%	93.2%
GAS5	80.6% (70.3-91%)	**<0.0001*****	80%	75%
H19	68.3% (53.3-83.3%)	**0.020***	82%	57%
LINC0133	66% (51.8-80.1%)	**0.030***	61%	73%
MIAT	67.1% (54-80.3%)	**0.013****	93.8%	46.2%
NEAT1	93.5% (87-100%)	**<0.0001*****	100%	76.9%

p-values indicates significant as determined by DeLong's test. Significance levels are represented as follows: **p*<0.05, ***p*<0.01, and ****p*<0.0001. The following abbreviations indicates as: AUC: area under curve, CI: Confidence Interval.

Table V

log2 Δ*LncRNAs*	*ANRIL*	*GAS5*	*H19*	*LINCO133*	*MIAT*	*NEAT1*
Measured Parameters						
Ferritin (ng/mL)	0.02	-0.12	0.06	0.08	-0.04	0.25
Iron (mg/dL)	0.08	-0.02	-0.07	0.16	0.05	0.04
log_2 Δ*HAMP*	**0.61******	**-0.38***	**0.56******	**0.58******	**0.59******	0.12
log_2 Δ*GDF-15*	-0.21	0.25	-0.19	-0.28	-0.13	0.27
log_2Δ*FAM132B*	**0.64******	-0.36	**0.75******	**0.54******	**0.57******	0.17
log_2 Δ*SLC40A1*	0.09	0.03	0.04	0.09	0.03	**0.47*****
Hepcidin (pg/mL)	-0.09	0.18	-0.02	-0.02	-0.10	-0.10
GDF-15 (pg/mL)	-0.22	0.20	-0.19	-0.13	-0.05	0.22
ERFF (pg/mL)	-0.12	0.11	0.00	-0.01	0.03	0.22
EPO (mUI/mL)	-0.06	0.06	-0.19	-0.04	-0.06	0.05

Bold font and stars represent significant correlations. Significance was based on Benjamini-Hochberg adjusted *p*-values from Spearman correlation. Significance levels are represented as follows: ***:** *p*<0.05, *****:** *p*<0.001, ******:** *p*<0.0001. The following abbreviations indicated for: TDβ-TM:transfusion dependent β-Thalassemia, GDF-15:growth dependent factor-15, ERFF:erythroferrone, EPO:erythropoietin.

Table VI

Biochemical parameters	log_2 Δ*LncRNA GAS5*	log_2 Δ*LncRNA NEAT1*	Ferritin (ng/mL)
AST (U/L)	-0.11	0.24	**0.55******
ALT (U/L)	-0.13	0.25	**0.55******
Creatinine (mg/L)	0.07	-0.18	-0.06
Urea (mg/L)	0.17	**-0.30***	-0.15

Bold font and stars represent significant correlations. Significance was based on Spearman correlation. Significance levels are represented as follows: ***:** *p*<0.05, ******:** *p*< 0.0001. The following abbreviations indicated for ALT: Alanine Transaminase, AST: Aspartate Transaminase.

Tables Illustrations

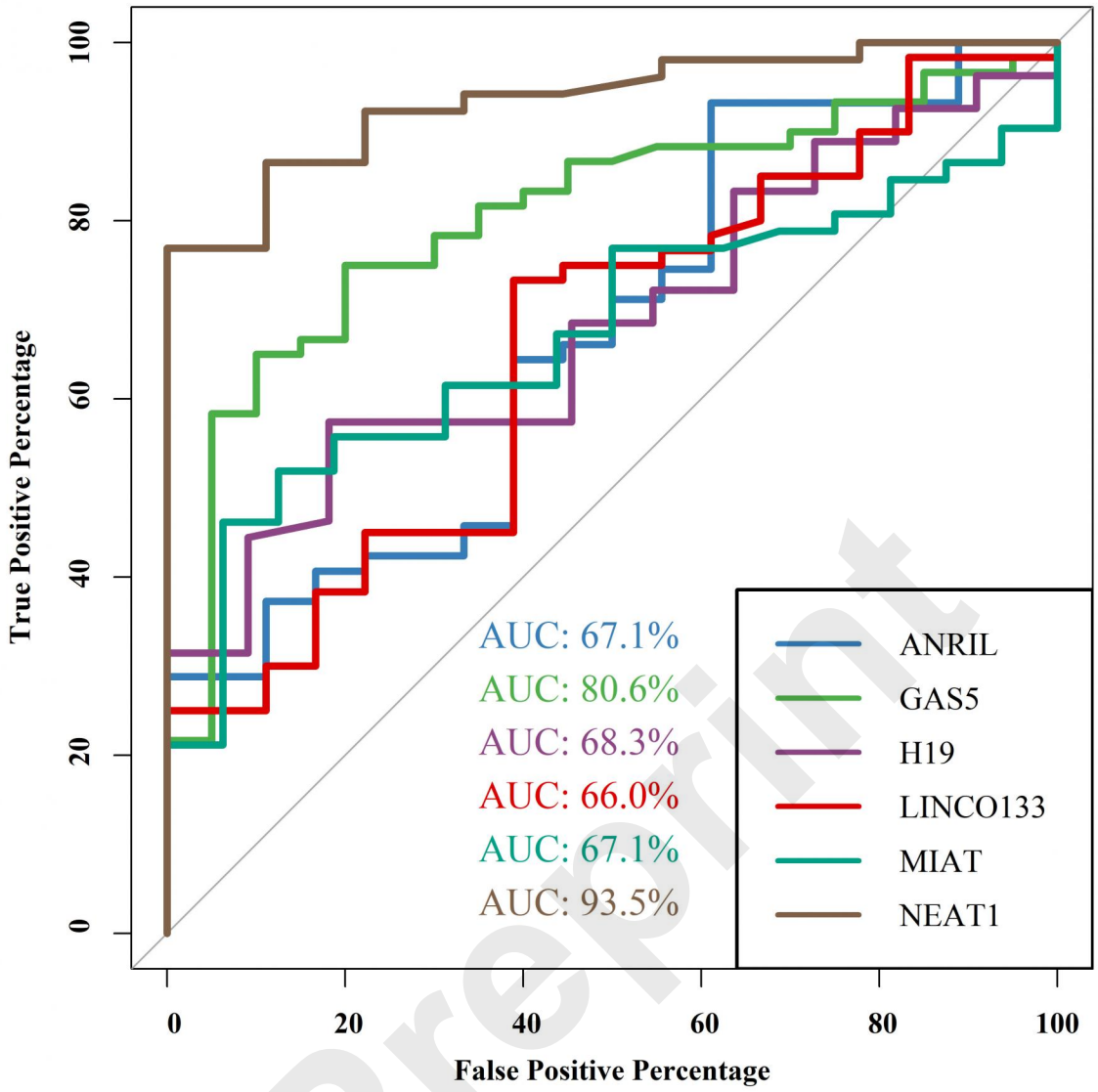

Fig. 1 Receiver operating characteristic curves analysis of long non-coding RNAs in thalassemia patients

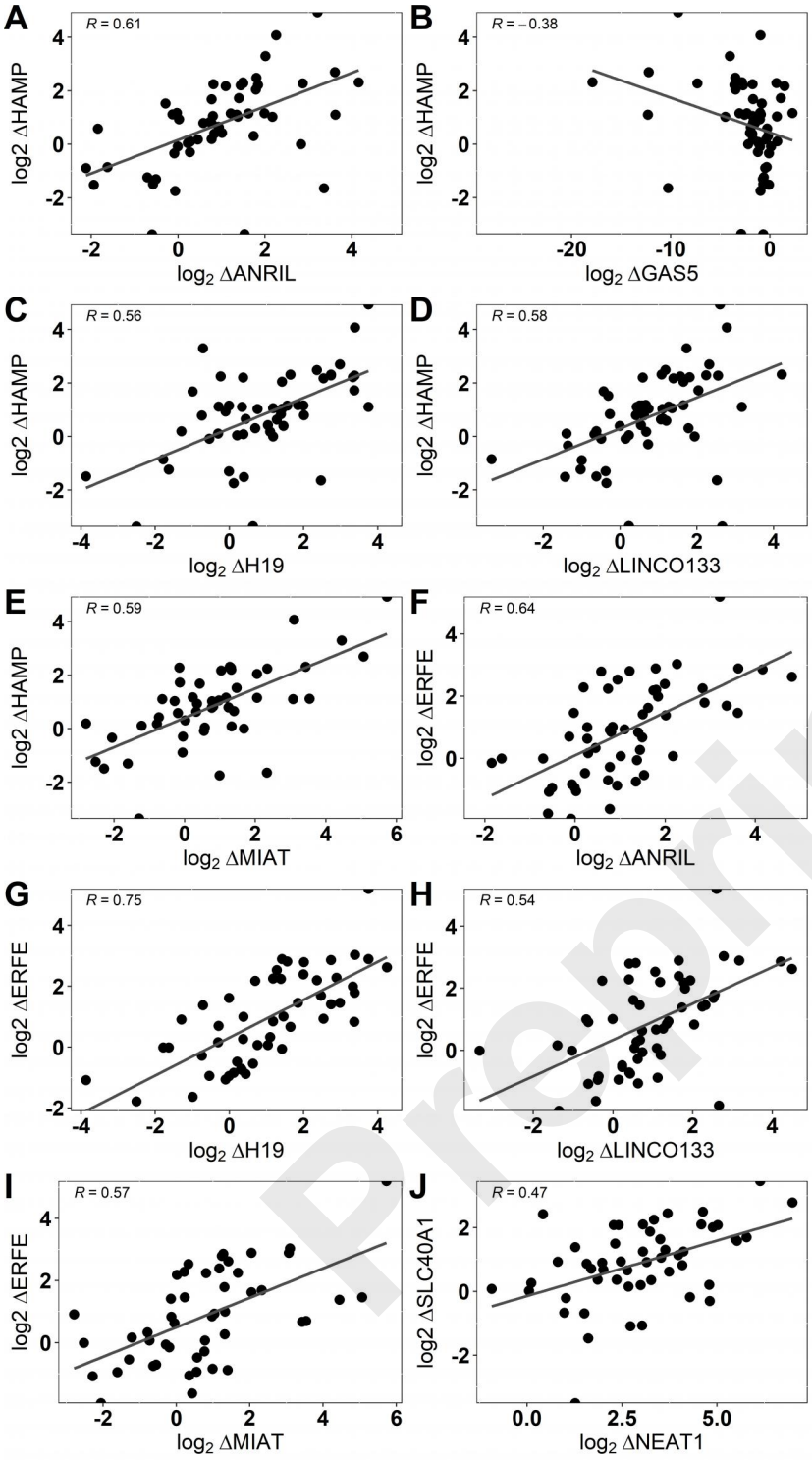

Fig. 2 Correlation between log2 fold changes in long non-coding RNA genes (x-axes) and HAMP, ERFE, and SLC40A1 genes (y-axes). R: Spearman correlation coefficient