Saudi Journal of Biological Sciences 29 (2022) 103344

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Association of long non-coding RNAs NEAT1, and MALAT1 expression and pathogenesis of Behçet's disease among Egyptian patients

Asmaa Mohammed [a], Olfat G. Shaker [b], Mahmoud A.F. Khalil [c,*], Yumn A. Elsabagh [d], Mohammed Gomaa [e], Azza M. Ahmed [f], Randa Erfan [b]

[a] Department of Biochemistry and Molecular Biology, Faculty of Medicine, Fayoum University, Fayoum, Egypt
[b] Department of Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University, Cairo, Egypt
[c] Department of Microbiology and Immunology, Faculty of Pharmacy, Fayoum University, Fayoum 63514, Egypt
[d] Department of Internal Medicine, Rheumatology and Clinical Immunology Unit, Faculty of Medicine, Cairo University, Cairo, Egypt
[e] Department of Neurology, Faculty of Medicine, Fayoum University, Fayoum, Egypt
[f] Department of Rheumatology, Faculty of Medicine, Fayoum University, Fayoum, Egypt

ARTICLE INFO

Article history:
Received 2 October 2021
Revised 12 May 2022
Accepted 10 June 2022
Available online 16 June 2022

Keywords:
Behçet's disease
Long non-coding RNAs
NEAT1
MALAT1

ABSTRACT

Behçet's disease (BD) is a chronic inflammatory disease. Immunological defects have been shown to play a significant role in the progression of BD. The serum levels of two long non-coding RNAs (lncRNAs), NEAT1 and MALAT1, were examined in patients with BD to identify their role in the disease pathogenesis. Both lncRNAs were mentioned as essential regulators of innate immune responses and have a crucial role in inflammatory diseases. Fifty patients with BD and a similar number of control individuals were involved in our study. At enrollment, data was collected from patients and controls, and the disease severity in active cases was determined using the Behçet's Disease Current Activity Form (BDCAF). Levels of the two studied biomarkers in the serum, NEAT1 and MALAT1, were investigated by quantitative RT-PCR (qRT-PCR). NEAT1 levels were significantly turned down in BD patients (fold changes = 0.77, $p = 0.0001$) and correlated negatively with the BDCAF ($r = -0.41$; $p = 0.003$). On the other hand, the MALAT1 levels were significantly up-regulated in BD patients (fold changes = 2.65, $p = 0.003$). Serum levels of NEAT1 were significantly decreased in patients with active states than in stationary cases (0.387 versus 1.99, respectively; $p = 0.01$) and compared with controls ($p = 0.001$). Also, NEAT1 levels were significantly increased in patients with stationary states compared to controls ($p = 0.03$). There was a positive association between NEAT1 and MALAT1 levels among BD patients ($r = 0.29$, $p = 0.04$). Our findings demonstrate a possible role of NEAT1 and MALAT1 in the pathogenesis of BD.

1. Introduction

Behçet's disease (BD) is a vasculitis condition marked by recurrent oral aphthous ulcers, genital ulcers, and uveitis (Davatchi et al., 2017). According to BD etiology, it is an inflammatory disorder caused by a variety of genetic and environmental factors (Scully et al., 2003). HLA-B51 and HLA-Cx14 alleles were frequently found in Turkish and Japanese patients with BD (Nakamura et al., 2019). A previous study discovered a relationship between BD and pro-inflammatory factors such as interleukin (IL)-1, IL-10, IL-21, IL-17, and tumor necrosis factor-α (TNF-α) (Kulaber et al., 2007). So, TNF-α, and ILs have been considered novel therapeutic targets in treating BD patients to avoid the side effects of prolonged use of corticosteroids. Besides, the T-helper 17 (Th17)/T-regulatory (Treg) balance was mentioned to play a crucial role in the immunological mechanisms of BD as well as its pathological features, which means that BD is dependent on the Th1/Th17 immune response (Tong et al., 2019). The accelerated development in research has contributed to the discovery of novel and promising biomarkers such as long non-coding RNAs (lncRNAs), which

* Corresponding author at: Department of Microbiology and Immunology, Faculty of Pharmacy, Fayoum University, City Fayoum 63514, Egypt.
E-mail addresses: amm18@fayoum.edu.eg (A. Mohammed), Olfat.shaker@kasralainy.edu.eg (O.G. Shaker), maf04@fayoum.edu.eg (M.A.F. Khalil), Dr_youm@yahoo.com (Y.A. Elsabagh), mgd00@fayoum.edu.com (M. Gomaa), ama56@fayoum.edu.eg (A.M. Ahmed), randa.erfan@cu.edu.eg (R. Erfan).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2022.103344
1319-562X/

are RNA transcripts that are not translated. Many biological processes, such as chromatin remodeling, gene transcription, and protein transport, are regulated by them (Mercer et al., 2009). However, lncRNAs are emerging as important regulators of an inflammatory immune response in various human diseases, such as tumors, neurological diseases, and autoimmune diseases (Yue et al., 2018). They are also in charge of controlling the transcription and synthesis of immune-response genes and inflammatory cytokines, respectively (Sigdel et al., 2015).

Nuclear paraspeckle assembly transcript 1 (NEAT1) is one of the lncRNAs that has been related to a variety of pathological and physiological processes, including the immune response (Huang et al., 2019), viral infection (Imamura et al., 2014), cancer (Li and Cheng, 2018), and neurodegeneration (Wang et al., 2019). As a result, the studies revealed that NEAT1 might be a novel therapeutic target for various diseases (Wang et al., 2020). Several studies have shown that NEAT1 is also involved in immunoregulation and plays a vital role in innate immune responses. NEAT1 has been linked to the innate immune response by increasing the expression of IL8. It was discovered to be up-regulated in autoimmune disorders such as Sjögren's syndrome (SS) and systemic lupus erythematosus (SLE) (Gao et al., 2018). NEAT1 improves CD4 + T cell differentiation into Th17 cells during the development of rheumatoid arthritis by increasing STAT3 expression, a vital agent for Th17 cell differentiation, through interaction with STAT3 and protecting it from destruction by decreasing its ubiquitination (Shui et al., 2019). MALAT1 is another long noncoding RNA, known as metastasis-associated lung adenocarcinoma transcript 1. It was primarily discovered in cancers and was recently mentioned to have a role in the innate immune response (Chen et al., 2017). The SLE patients expressed MALAT1 in monocytes and its expression levels were significantly elevated and positively related to sirtulin1 (SIRT1) signaling and IL-21 expression. These findings point to MALAT-1 as a potential treatment target in SLE (Yang et al., 2017).

Furthermore, according to West and co-workers report, the NEAT1 and MALAT1 loci are close to each other in the genome. This discloses that these lncRNAs may work together to control the expression of a large number of genes. There are some noteworthy discrepancies between these two lncRNAs. NEAT1 is found at transcriptional start sites (TSS) and termination sites (TTS), while MALAT1 is found primarily in gene bodies and near TTS, demonstrating that these RNAs play independent but complementary activities at co-bound locations. Transcription changes are mainly associated with changes in NEAT1 localization, indicating that certain components of the transcription process have been contacted (West et al., 2014).

Notably, multiple studies attempted to investigate the relationship between the synergistic function of both lncRNAs and the pathogenesis of different diseases, as type 2 diabetes mellitus (Alfaifi et al., 2021), rheumatoid arthritis (Chatterjee et al., 2020), atherosclerosis (Gast et al., 2019), lung cancer (Jen et al., 2017), breast cancer (Arshi et al., 2018), and acute lymphoblastic leukemia (Pouyanrad et al., 2019). Consequently, these two related lncRNAs were considered as promising noninvasive markers linked to the pathogenesis of different diseases. The current work aimed to clarify the role of both lncRNAs NEAT1 and MALAT1 among BD patients in disease pathogenesis and their correlation with the disease's clinical course.

2. Materials and methods

The local ethics committee (The Scientific Research Ethics Committee of the Faculty of Medicine, Fayoum University) approved the protocol for the current study. All procedures are run in compliance with the standards of the Declaration of Helsinki. Informed written consent to participate in the study has been obtained from participants upon enrollment. We prepared the following manuscript in accordance with Strengthening the Reporting of Observational studies in Epidemiology (STROBE) guidelines (Moher et al., 2012).

2.1. Study design and patients

This case-control study was conducted on 50 patients with BD and a similar number of age and sex-matched controls. Patients with BD were recruited from the Department of Rheumatology, Cairo University Hospital, Egypt, and diagnosed using the International Study Group Criteria for Behçet's Disease ISBD (1990). The study included a BD patients group and a healthy volunteers group.

2.1.1. BD patients group

2.1.1.1. Inclusion criteria. Patients had active BD, exhibiting at least one of the symptoms of BD, despite treatment, or had stable disease with well-controlled symptoms. The patients had different disease phenotypes such as uveitis, cutaneous lesions, genital ulcers, and neurological affections to determine the relation of the investigated biomarkers to different phenotypes.

2.1.1.2. Exclusion criteria. Those who had an autoimmune disease, cancer, a chronic infectious disease, or a new infection within a month of enrollment were exempted.

2.1.2. Healthy volunteers group

The control group consisted of 50 healthy volunteers who were age and sex-matched and had no family history of BD or other autoimmune disorders to compare them with the patient group who had the same criteria except for BD to exclude the affection of normal physiological conditions on the expression levels of the studied lncRNAs. Before being enrolled, all participants were asked to fill out an informed consent form. Those who had an autoimmune disease, cancer, a chronic infectious disease, or a new infection within a month of enrollment were exempted.

2.2. Data collection and laboratory investigation

At registration, the following data was collected from patients and controls: age, gender, clinical presentation, fundus and central nervous system examination, treatment modalities, disease activity as measured by Behçet's Disease Current Activity Form (BDCAF) (Lawton et al., 2004). The vacutainer device was used to collect (5 mL) of blood from each subject. Samples were collected in tubes with separator gels lodged between packed cells and the top serum layer, then allowed to clot for 15 min before centrifugation at 4000 × g for 10 min. The serum samples were isolated from clotted whole blood and kept at 80 °C until required (Bush et al., 2001).

2.3. RNA extraction

The serum samples were used for the estimation of the lncRNAs expression levels by real-time PCR. RNA extraction was performed using the miRNeasy extraction kit (Qiagen, Valencia, CA, United States) as instructed by the manufacturer. A NanoDrop (ND)-1000 spectrophotometer (NanoDrop Technologies, Inc. Wilmington, DE, USA) was used to estimate the RNA concentration. RNA was kept at −80 °C.

2.4. LncRNAs expression by quantitative RT-PCR

In the reverse transcription (RT) step, 60 ng of RNA was used. GAPDH (internal control), readymade primers, and RT2 SYBR Green

PCR kit (Qiagen, Germantown, MD, USA) were used to assess lncRNA serum expression (Duan et al., 2016). The RT2 lncRNA PCR assay for MALAT1 was dependent on predesigned primers catalog no: 330701 LPH18065A, Accession no: NR_002819.2. The RT2 lncRNA PCR assay for NEAT1 was dependent on predesigned primers catalog no: 330,701 LPH15809A, Accession no: NR_028272.1. We used GAPDH as an internal housekeeping gene (Adriaens et al., 2016) (Catalog no: 330,701 LPH31725A, Accession no: ENST00000496049.0). Twenty microliter reaction mixtures were used in RT-PCR by mixing 10 µl of master mix, 1 µl ready-made assay primer, 2.5 µl of dil. cDNA, and 5.5 µl RNAase-free water. PCR conditions were as follows: 95 °C for 10 min, after that, 45 cycles at 95 °C for 15 s and 60 °C for 60 s. Gene expression relative to internal control ($2^{-\Delta Ct}$) was determined. A melt curve analysis was done to ensure the specificity of the corresponding RT-PCR reactions. Fold change was calculated using $2^{-\Delta\Delta Ct}$ for relative quantification (Livak and Schmittgen, 2001). For the control sample, ΔΔCt equals zero, and 2^0 is equal to one (Shaker et al., 2019a,b).

2.5. Study outcomes

The present study's main parameters were fold changes in the two studied lncRNAs, NEAT1 and MALAT1, and their diagnostic utility in BD patients. The secondary outcomes included the association between the fold changes in the two studied biomarkers and the clinical activity of BD, clinical presentation, and treatment modality.

2.6. Statistical analysis

Data analysis was performed using the Statistical Package of Social Science (SPSS) software version 25. Simple descriptive analysis in the form of numbers and percentages for qualitative data, and arithmetic means as central tendency measurement and standard deviations as a measure of dispersion for quantitative parametric data. Quantitative data included in the study was first tested for normality by the One-Sample Kolmogorov-Smirnov test in each study group, then inferential statistical tests were selected. For quantitative parametric data, an In-dependent student T-test was used to compare measures of two independent groups of quantitative data. Regarding quantitative non-parametric data, the Mann-Whitney test was used for comparing two independent groups. For qualitative data, the Chi-square test can be used to compare two or more qualitative groups. The Spearman correlation test was used to determine the association between variables. The P-value < 0.05, was considered the cut-off value for significance.

3. Results

A total of 50 patients and a similar number of matched controls were recruited. The patients' and healthy controls' mean ages were 34.3 (26.8 – 38) and 33.6 (25 – 40.3), respectively (p = 0.7). Males made up the majority of subjects in both groups (82% versus 80%, p = 0.9). Forty eight percent (48%) of cases had an oral ulcer, 22% had a genital ulcer, and 34% had cutaneous lesions. The examination revealed the presence of uveitis in 26% of the patients, and 12% of the patients had neurological affections. Regarding medications, 72% were taking colchicine, 62% were taking steroids, and 30% were taking azathioprine at the time of the study. As regard to activity, 76% of our patients are in an active state (on double or triple treatment) and 24% of the patients are in a stationary state (on no treatment or on azathioprine only) (Table 1).

Table 1
Demographic and clinical characters of the studied groups.

Variables	BD Group (n = 50)	Control Group (n = 50)	P-value
Age, mean/SD	34.3 ± 8.7	33.6 ± 9.5	0.7
Male, No. (%)	41 (82%)	36 (80%)	0.9
Clinical Presentation, No. (%)			
Oral Ulcers	24 (48%)	–	–
Genital Ulcers	11 (22%)	–	–
Cutaneous Lesions	17 (34%)	–	–
Uveitis, No. (%)	13 (26%)	–	–
Neurological affection, No. (%)	6 (12%)	–	–
Treatment, No. (%)			
Azathioprine	15 (30%)	–	–
Steroids	31 (62%)	–	–
Colchicine	36 (72%)	–	–
Active disease, No. (%)	38 (76%)	–	–
BDCAF, mean/SD	2.3 ± 1.3	–	–

BDCAF = Behçet's disease Current Activity Form.
SD = standard deviation.

3.1. Serum levels of lncRNA NEAT1 among cases

The LncRNA NEAT1 was significantly down-regulated in BD patients compared to controls; the decrease was > 2 folds from controls. NEAT1 fold change was 0.77 (p = 0.0001) (Table 2). As regard to disease activity, we found that there was a statistically significant low mean of NEAT1 (with p = 0.01) among active cases compared to stationary cases (Table 3), (Figs. 1, 2).

3.2. Correlation between NEAT1 and BDCAF in BD patients

According to this study, there was a statistically significant negative correlation between NEAT1 and BDCAF levels (r = −0.41, p = 0.003) which indicated that a decrease in BDCAF would be associated with an increase in NEAT1 levels (Fig. 3).

3.3. Serum levels of lncRNA MALAT1 among cases

LncRNA MALAT1 was significantly up-regulated in BD patients compared to controls, with an increase ≥ of 2 folds from controls. The fold change was 2.65 (p = 0.003) (Table 2). As regards to disease activity, we found that there was no statistically significant difference between active and stationary cases (with p-value > 0.05) as regards the MALAT1 level (Table 3), (Figs. 4, 5).

3.4. Correlation between MALAT1, NEAT1, and BDCAF in BD patients

There was a positive association between NEAT1 and MALAT1 levels among BD patients (r = 0.29, *p = 0.04).* On the other hand, There was no statistically significant correlation with a p-value > 0.05 between MALAT1 and BDCAF.

3.5. Relationship between the expression levels of NEAT1 and MALAT1 markers and different disease phenotypes

There was no statistically significant difference with p-value > 0.05 in NEAT1 and MALAT1 levels in different disease phenotypes or different treatment types (Table 4).

3.6. Sensitivity and specificity test for NEAT1 and MALAT1 in BD diagnosis

The sensitivity and specificity tests for NEAT1 and MALAT1 illustrated that NEAT1 is more sensitive than MALAT1 in the diagnosis of cases with sensitivity (76%) and specificity (100%) at cut off (0.786) (AUC = 88.4%, 95% CI: 80.9–95.5) versus 60% and 100%

Table 2
Comparisons of NEAT1, and MALAT1 levels in different study groups. Table illustrates that there is statistically significant **low** mean of NEAT1 levels, and **high** mean of MALAT1 levels with p-value < 0.05 among cases.

Variables	Cases (n = 50)		Control (n = 45)		p-value
	Mean	SD	Mean	SD	
NEAT1	**0.77**	1.5	1.03	0.23	**0.0001***
MALAT1	**2.65**	8.9	1.03	0.23	**0.003***

Table 3
Comparisons of NEAT1, and MALAT1 levels in different disease activity among cases. Table illustrates that there is statistically significant **low** mean of NEAT1, with p-value < 0.05 in active cases than stationary, and low mean among active and stationary in comparison to control. On the other hand there is no statistically significant difference with p-value > 0.05 as regards MALAT1 level between active, stationary cases and controls.

Variables	Active (n = 38)		Stationary (n = 12)		Control (n = 45)		p-value
	Mean	SD	MEAN	SD	Mean	SD	
NEAT1	**0.38**	0.32	1.99	2.9	0.93	0.13	**0.01*[a] < 0.001*[b] 0.03*[c]**
MALAT1	**2.5**	9.7	3.22	5.3	0.95	0.14	0.4

a: statistical significance difference between active and stationary.
b: statistical significance difference between active and controls.
c: statistical significance difference between stationary and controls.

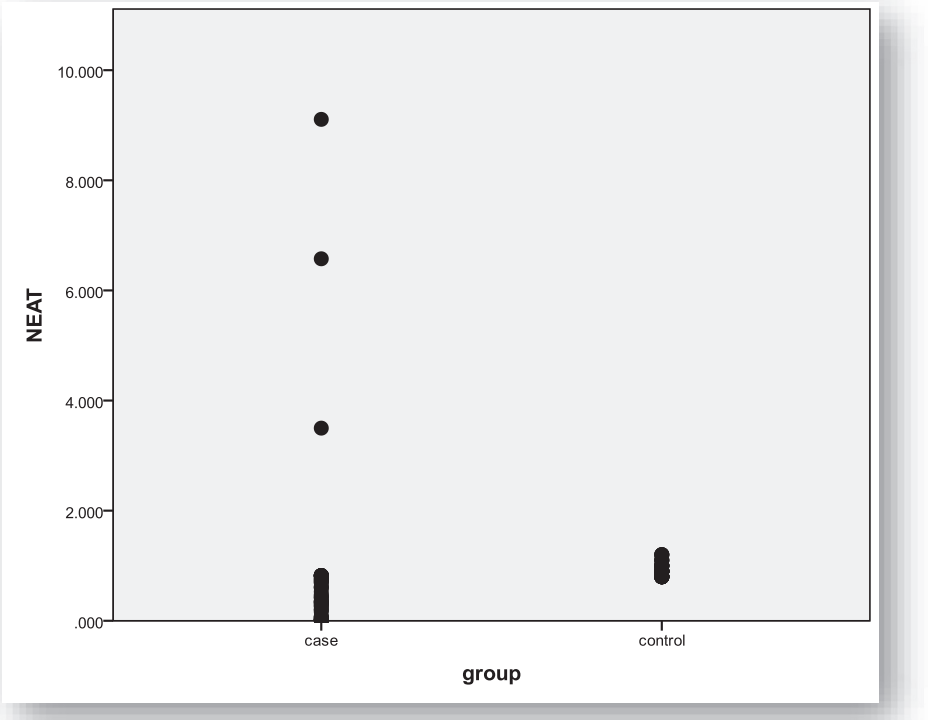

Fig. 1. Dot plots for relative expression levels of NEAT1 in Cases and Healthy Controls.

respectively for MALAT1 at cut off (0.744) (AUC = 67.1%, 95% CI: 54.7–79.4) (Table 5) (Fig. 6).

4. Discussion

The present report is the first study investigating the expression of NEAT1 and MALAT1 in patients with BD to the best of our knowledge. MALAT1, also known as NEAT2, is a lncRNA that has recently attracted a lot of attention. MALAT1 is located on the 11q13 human chromosome and is extensively expressed in many tissues, especially the lung and pancreas (Wang et al., 2015). There is growing evidence that NEAT1 and MALAT1 are co-enriched at specific gene loci, resulting in augmented transcription activity (Zhang et al., 2012). The current study, investigated these lncRNAs that play a crucial role in the innate immune response.

This study showed that NEAT1 levels were significantly down-regulated in BD patients compared to controls (fold change > 2,

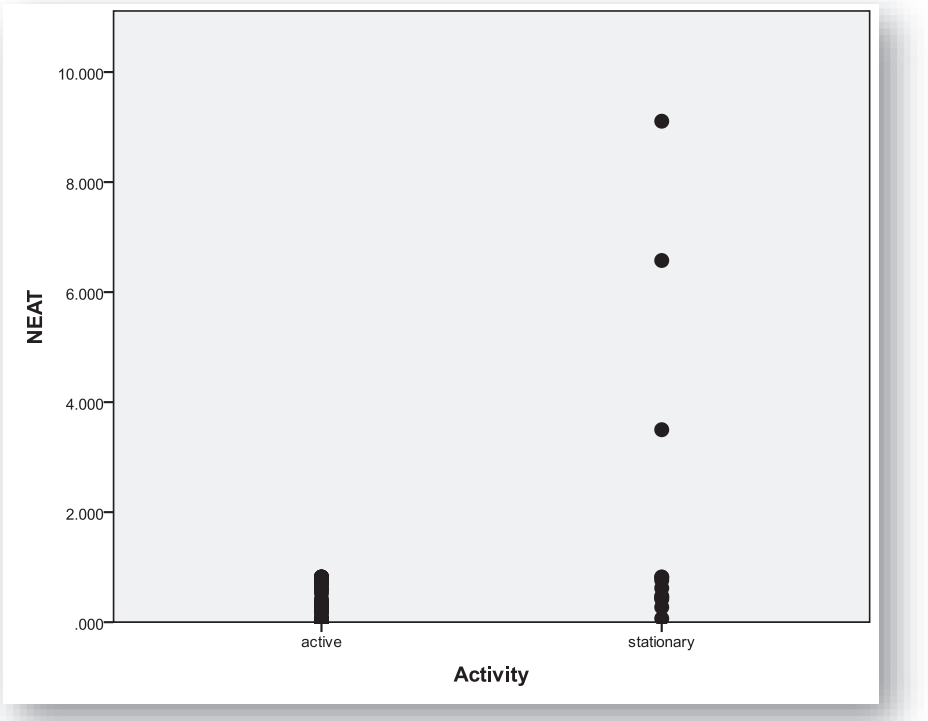

Fig. 2. Dot plots for relative expression levels of NEAT1 in the patients with active state versus the patients with stationary state.

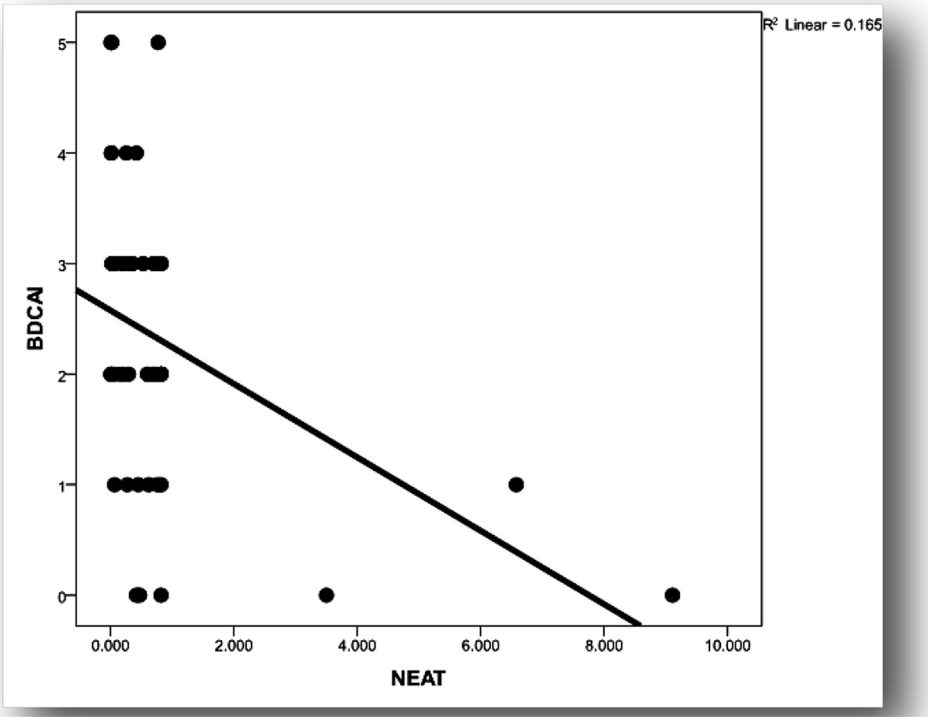

Fig. 3. Correlation between expression levels of NEAT1 and BDCAF of the patients.

p = 0.0001). The levels of NEAT1 were altered with the disease course. NEAT1 levels were increased in patients with the stationary course and significantly decreased in the active state (p = 0.01).

These findings could be attributed to the therapeutic effect of colchicine and steroids used in active cases. In line with our findings, Han et al. discovered that plasma levels of NEAT1, IL-2, IL-1,

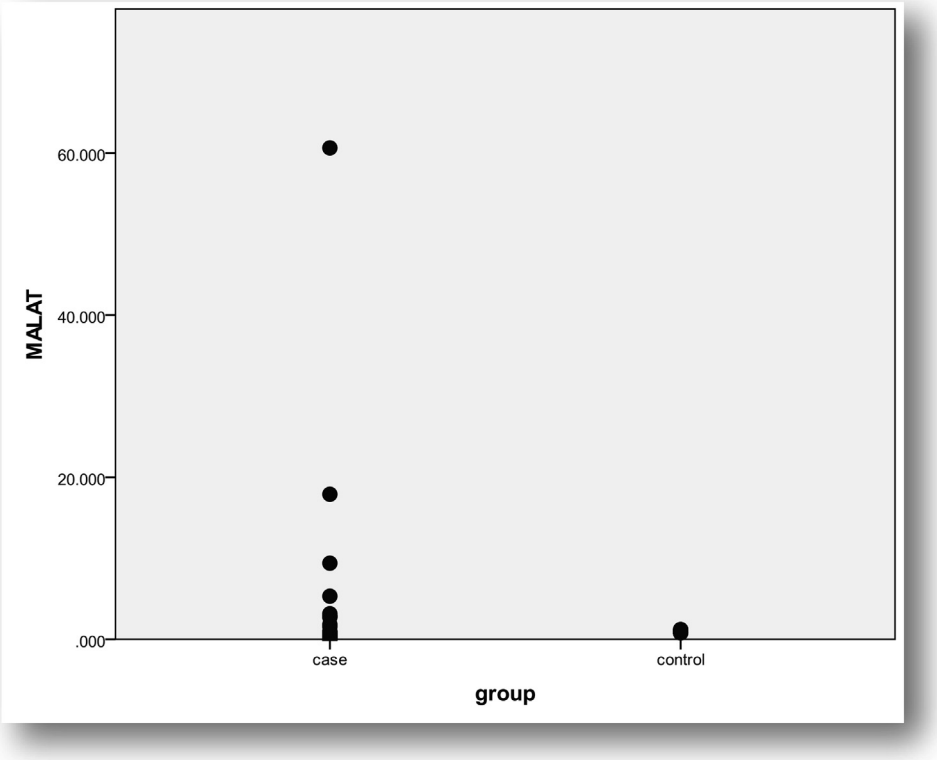

Fig. 4. Dot plots for relative expression levels of MALAT1 in Cases and Healthy Controls.

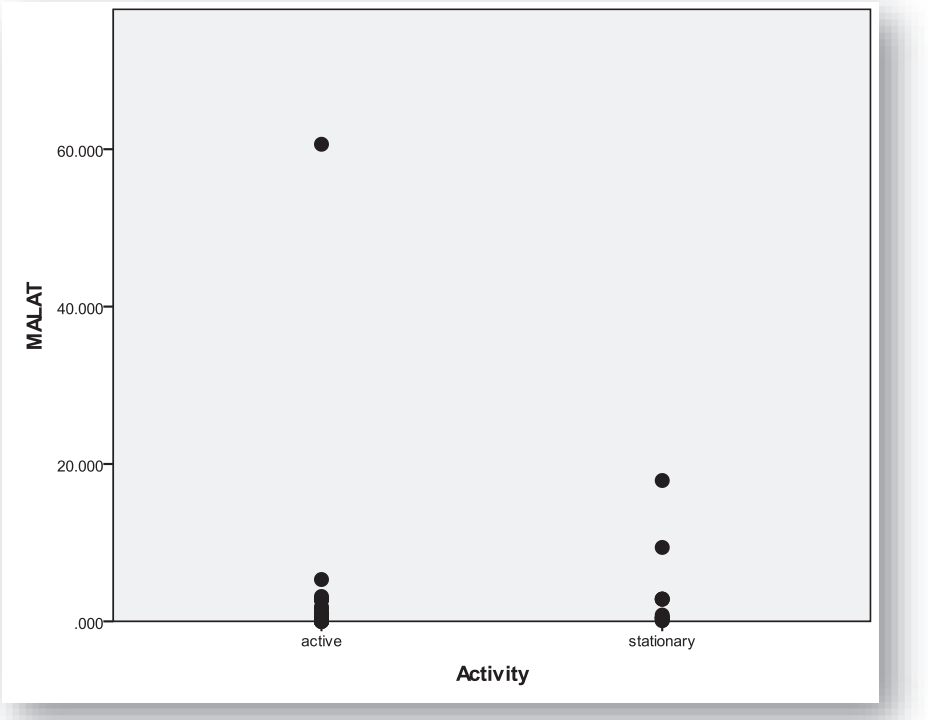

Fig. 5. Dot plots for relative expression levels of MALAT1 in the active and stationary cases.

Table 4
Comparisons of NEAT1, and MALAT1 relative expression levels in different disease phenotypes. Table illustrates that there was no statistically significant difference with p-value > 0.05 in NEAT1 and MALAT levels in different disease phenotypes, and in different types of treatment.

Variables		NEAT1			MALAT1		
		Mean	SD	p-value	Mean	SD	p-value
Fundus examination							
Normal		0.899	1.8	0.6	1.76	3.3	0.4
Uveitis		0.41	0.32		5.2	16.7	
Oral ulcer							
No		0.36	0.32	0.07	0.96	1.4	0.2
Yes		1.15	2.1		4.2	12.1	
Genital ulcer							
No		0.42	0.35	0.4	1.72	1.7	0.7
Yes		0.87	1.8		2.9	9.9	
Cutaneous lesion							
No		0.35	0.29	0.2	0.81	1.3	0.3
Yes		0.99	1.9		3.6	10.8	
Neurological affection							
No		0.80	1.7	0.7	2.91	9.4	0.6
Yes		0.56	0.24		0.68	1.1	
Treatment							
Steroids	Yes	0.91	1.9	0.7	3.53	11.2	0.8
	No	0.55	0.76		1.21	1.4	
Azathioprine	Yes	1.12	2.2	0.08	6.1	15.7	0.1
	No	0.62	1.2		1.2	1.9	
Colichicine	Yes	0.83	1.8	0.9	1.69	3.3	0.9
	No	0.63	0.87		5.1	16	

Table 5
Sensitivity and specificity of NEAT1 and MALAT1 in the diagnosis of BD patients. Table illustrated that NEAT1 is more sensitive than MALAT1 in the diagnosis of BD cases with sensitivity (76%) and specificity (100%) at cut off (0.786) (AUC = 88.4%, 95% CI: 80.9–95.5) versus 60% and 100% respectively for MALAT1 at cut off (0.744) (AUC = 67.1%, 95% CI: 54.7–79.4).

Variable	Sensitivity	Specificity	AUC	Cut off point	95% CI
NEAT1	**76%**	**100%**	**88.4%**	**0.786**	**80.9–95.5**
MALAT1	**60%**	**100%**	**67.1%**	**0.744**	**54.7–79.4**

AUC = area under curve.
CI = confidence interval.

and TNF-α were highest in symptomatic recurrent aphthous stomatitis patients before using colchicine in treatment. Patients with recurrent aphthous stomatitis treated with colchicine had significantly lower plasma levels of NEAT1, IL-2, IL-1, and TNF-α (Han et al., 2021). As a result, NEAT1 is linked to the occurrence of BD, and changes in the plasma level of NEAT1 may be used as a prognostic marker to monitor therapeutic outcomes and predict recurrence of BD. Elevated NEAT1 levels in stationary patients with no symptoms suggest the presence of subclinical inflammation. NEAT1 may be used to monitor and control subclinical inflammatory reactions of BD, thus, preventing the diseases progression and development. On the other hand, our results may disagree with the outcome of the study by Mohammed and coworkers (Mohammed et al., 2021). This could be attributed to the difference in the applied therapeutic options as well as disease state, patient's symptoms, and subgroups.

Moreover, the NEAT1 fold changes correlated negatively with the BDCAF ($r = -0.41$; $p = 0.003$). This finding indicated that the down-regulation of NEAT1 was associated with severe BD, and NEAT1 may have a prognostic value in the disease. The role of NEAT1 in regulating the innate immune response has been recognized recently. Pre-clinical studies have identified NEAT1 as a novel immunoregulator lncRNA that acts on Th17 cell differentiation and monocyte-macrophage functions (Chen et al., 2018). Deniz et al. (2017) mentioned that Th17 expresses IL17 and interferon γ (INF γ) in BD. IL17 and interferon γ are associated with elevated innate responses, neutrophil infiltrations in the tissues, and late adaptive immune responses in BD (Deniz et al., 2017).

In addition, Zhang and co-workers reported that NEAT1 expression in SLE patients' monocytes was positively associated with SLE disease activity. NEAT1 silencing also decreased the expression of chemokines and cytokine groups such as IL-6 and CXCL10 (C-X-C motif chemokine ligand 10). This is mainly attributed to its influence on the late mitogen activated protein kinase (MAPK) pathways (Zhang et al., 2016). Accordingly, Pandey et al. (2017) found that NEAT1 levels were significantly increased in a stable state of dengue fever while decreasing during disease activity. Also, NEAT1 levels were negatively correlated with dengue severity (Pandey et al., 2017). In addition, Bayyurt and his group found that NEAT1 was down-regulated in Crimean–Congo hemorrhagic fever (CCHF) patients and fatal groups compared with controls (Bayyurt et al., 2020).

Regarding MALAT1, we found that its fold change was significantly increased in BD patients (fold change was 2.65, $p = 0.003$) but did not correlate with the BDCAF ($r = -0.133$; $p = 0.36$). Due to its broad expression, a major association was described between MALAT1 and autoimmune diseases. Its expression was elevated in SLE patients and positively related to SIRT1 signaling and IL-21 expression (Yang et al., 2017). IL-21 has a critical role in enhancing inflammatory reactions in BD by increasing Th17 and decreasing Treg cells. IL-21 is considered a novel therapeutic target in BD (Geri et al., 2011). In addition, Shaker and co-workers found that MALAT1 was significantly increased in multiple sclerosis (MS) patients (Shaker et al., 2019a,b).

Our findings demonstrated the substantial role of MALAT1 expression in the pathogenesis of BD, and this lncRNA may have good predictive power as the MALAT1 levels did not change with the disease activity. On the other hand, detection of decreased NEAT1 levels in patients with active disease implies that this lncRNA may be useful for patients's follow-up, determination of

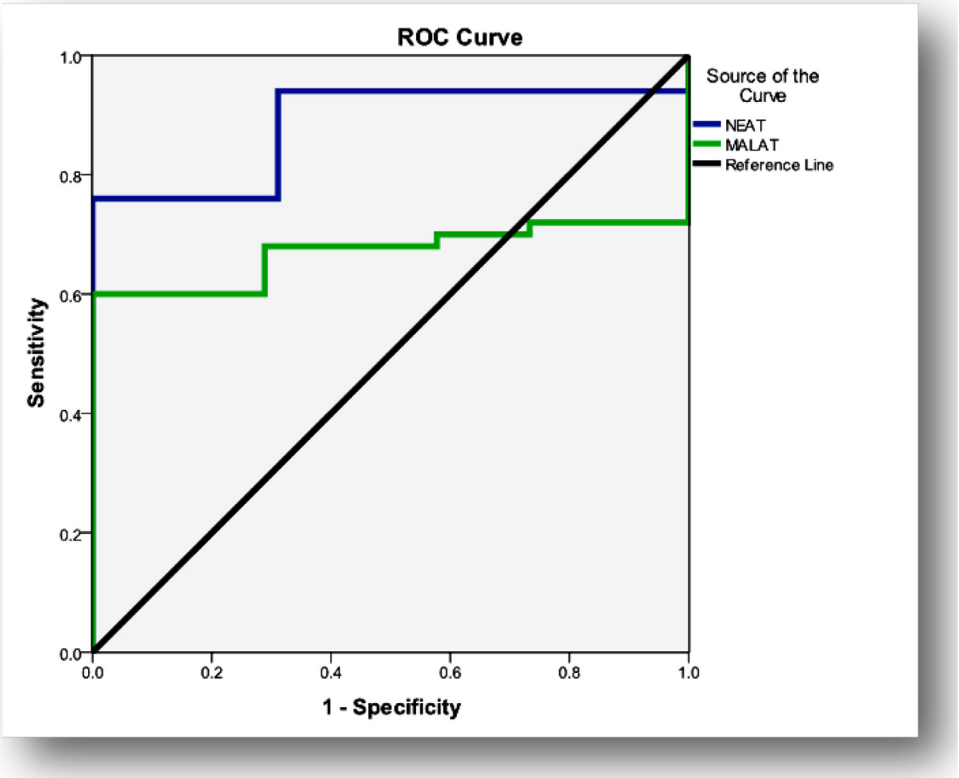

Fig. 6. ROC curve for detecting the predictive power of NEAT1 and MALAT1 in BD diagnosis.

subclinical inflammation, and predictability of attacks, so it can be considered as a good prognostic biomarker in BD. In accordance, Chatterjee and colleagues discovered that NEAT1 expression levels correlated with RA disease activity, detecting their possible effect on disease flare, but they did not find a correlation between disease activity and MALAT1 expression levels (Chatterjee et al., 2020). We found that there was a positive correlation between NEAT1 and MALAT1 levels among BD patients ($r = 0.29$, $p = 0.04$) and this result may refer to their synergistic effect.

NEAT1 demonstrated high sensitivity and specificity for BD (AUC = 0.884, 95% CI: 80.9–95.5). In patients with colorectal cancer, NEAT1 showed a significant predictive role in differentiating between patients and normal controls (AUC = 0.9471; $P < 0.01$) (Peng et al., 2017). A comparable finding was also observed in COPD cases (AUC = 0.869, 95% CI: 0.817–0.921) (Ming et al., 2019), myocardial infarction (AUC = 0.822; $P < 0.01$) (Chen et al., 2020), and malignant thyroid nodules (AUC = 0.9304, $P < 0.01$) (Zhao et al., 2020). With respect to MALAT1, many studies have indicated its role in the diagnosis of various diseases, like non-traumatic osteonecrosis of the femoral head (AUC = 0.681, $P = 0.009$) (Jin et al., 2020), diabetic retinopathy (AUC = 0.741, $P < 0.001$) (Shaker et al., 2019a,b), and diabetic nephropathy (AUC = 0.914, $P < 0.01$) (Zhou et al., 2020).

Moreover, the role of both lncRNAs in the pathogenesis of BD may refer to the possibility of using these biomarkers as targets for biological treatments. Even though the current study has limitations due to the small number of subjects studied, our findings highlight the importance of these biomarkers and their promising therapeutic role in combination with immunosuppressive therapy to control refractory cases and prevent irreversible multisystemic damage. However, further investigations into these biomarker cohorts will be required to confirm their importance.

5. Conclusion

In conclusion, our findings demonstrated an essential role of NEAT1 and MALAT1 in the pathogenesis of BD and the possibility of considering both lncRNAs as interesting diagnostic biomarkers. Moreover, NEAT1 findings refer to the possibility of considering it a promising prognostic biomarker. Further consideration is recommended to investigate their pathways affecting the disease. They may also have a promising role as therapeutic targets in combination with traditional treatment to control severe refractory cases.

CRediT authorship contribution statement

Asmaa Mohammed: Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Olfat G. Shaker:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Mahmoud A.F. Khalil:** Conceptualization, Methodology, Writing – original draft, Supervision. **Yumn A. Elsabagh:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing. **Mohammed Gomaa:** Conceptualization, Methodology, Writing – original draft, Supervision. **Azza M. Ahmed:** Conceptualization, Methodology, Writing – original draft, Writing – review & editing, Supervision. **Randa Erfan:** Conceptualization, Methodology, Writing – original draft, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

Not applicable.

Funding

None.

References

1990. Criteria for diagnosis of behcet's disease. International study group for behcet's disease. Lancet, 335(8697): 1078-1080. Available from http://www.ncbi.nlm.nih.gov/pubmed/1970380.

Alfaifi, M., Ali Beg, M.M., Alshahrani, M.Y., Ahmad, I., Alkhathami, A.G., Joshi, P.C., Alshehri, O.M., Alamri, A.M., Verma, A.K., 2021. Circulating long non-coding rnas nkila, neat1, malat1, and miat expression and their association in type 2 diabetes mellitus Available from BMJ Open Diabetes Res. Care 9 (1) http://www.ncbi.nlm.nih.gov/pubmed/33436407. https://doi.org/10.1136/bmjdrc-2020-001821.

Arshi, A., Sharifi, F.S., Khorramian Ghahfarokhi, M., Faghih, Z., Doosti, A., Ostovari, S., Mahmoudi Maymand, E., Ghahramani Seno, M.M., 2018. Expression analysis of malat1, gas5, sra, and neat1 lncrnas in breast cancer tissues from young women and women over 45 years of age. Molecular therapy Available from Nucleic Acids 12, 751–757 http://www.ncbi.nlm.nih.gov/pubmed/30126830. https://doi.org/10.1016/j.omtn.2018.07.014.

Bayyurt, B., Bakir, M., Engin, A., Oksuz, C., Arslan, S., 2020. Investigation of neat1, ifng-as1, and nrir expression in crimean-congo hemorrhagic fever. J. Medical Virol. http://wwwncbi.nlm.nih.gov/pubmed/33064349. https://doi.org/10.1002/jmv.26606.

Bush, V.J., Janu, M.R., Bathur, F., Wells, A., Dasgupta, A., 2001. Comparison of bd vacutainer sst plus tubes with bd sst ii plus tubes for common analytes Available from Clinica Chim. Acta; Int. J. Clin. Chem. 306 (1–2), 139–143 http://www.ncbi.nlm.nih.gov/pubmed/11282105. https://doi.org/10.1016/s0009-8981(01)00396-5.

Chatterjee, S., Bhattcharjee, D., Misra, S., Saha, A., Bhattacharyya, N.P., Ghosh, A., 2020. Increase in meg3, malat1, neat1 significantly predicts the clinical parameters in patients with rheumatoid arthritis Available from Personalized Med. 17 (6), 445–457 http://www.ncbi.nlm.nih.gov/pubmed/33026292. https://doi.org/10.2217/pme-2020-0009.

Chen, R., Liu, Y., Zhuang, H., Yang, B., Hei, K., Xiao, M., Hou, C., Gao, H., Zhang, X., Jia, C., Li, L., Li, Y., Zhang, N., 2017. Quantitative proteomics reveals that long non-coding rna malat1 interacts with dbc1 to regulate p53 acetylation Available from Nucleic Acids Res. 45 (17), 9947–9959 http://www.ncbi.nlm.nih.gov/pubmed/28973437. https://doi.org/10.1093/nar/gkx600.

Chen, Y., Qiu, J., Chen, B., Lin, Y., Chen, Y., Xie, G., Qiu, J., Tong, H., Jiang, D., 2018. Long non-coding rna neat1 plays an important role in sepsis-induced acute kidney injury by targeting mir-204 and modulating the nf-kappab pathway Available from Int. Immunopharmacol. 59, 252–260 http://www.ncbi.nlm.nih.gov/pubmed/29669307. https://doi.org/10.1016/j.intimp.2018.03.023.

Chen, Z., Yan, Y., Wu, J., Qi, C., Liu, J., Wang, J., 2020. Expression level and diagnostic value of exosomal neat1/mir-204/mmp-9 in acute st-segment elevation myocardial infarction Available from IUBMB Life 72 (11), 2499–2507 http://www.ncbi.nlm.nih.gov/pubmed/32916037. https://doi.org/10.1002/iub.2376.

Davatchi, F., Chams-Davatchi, C., Shams, H., Shahram, F., Nadji, A., Akhlaghi, M., Faezi, T., Ghodsi, Z., Sadeghi Abdollahi, B., Ashofteh, F., Mohtasham, N., Kavosi, H., Masoumi, M., 2017. Behcet's disease: Epidemiology, clinical manifestations, and diagnosis Available from Expert review of clinical immunology 13 (1), 57–65 http://www.ncbi.nlm.nih.gov/pubmed/27351485. https://doi.org/10.1080/1744666X.2016.1205486.

Deniz, R., Tulunay-Virlan, A., Ture Ozdemir, F., Unal, A.U., Ozen, G., Alibaz-Oner, F., Aydin-Tatli, I., Mumcu, G., Ergun, T., Direskeneli, H., 2017. Th17-inducing conditions lead to in vitro activation of both th17 and th1 responses in behcet's disease Available from Immunological investigations 46 (5), 518–525 http://www.ncbi.nlm.nih.gov/pubmed/28414558. https://doi.org/10.1080/08820139.2017.1306865.

Duan, W., Du, L., Jiang, X., Wang, R., Yan, S., Xie, Y., Yan, K., Wang, Q., Wang, L., Zhang, X., Pan, H., Yang, Y., Wang, C., 2016. Identification of a serum circulating lncrna panel for the diagnosis and recurrence prediction of bladder cancer Available from Oncotarget 7 (48), 78850–78858 http://www.ncbi.nlm.nih.gov/pubmed/27793008. https://doi.org/10.18632/oncotarget.12880.

Gao, Y., Li, S., Zhang, Z., Yu, X., Zheng, J., 2018. The role of long non-coding rnas in the pathogenesis of ra, sle, and ss Available from Front. Med. 5, 193 http://www.ncbi.nlm.nih.gov/pubmed/30018955. https://doi.org/10.3389/fmed.2018.00193.

Gast, M., Rauch, B.H., Nakagawa, S., Haghikia, A., Jasina, A., Haas, J., Nath, N., Jensen, L., Stroux, A., Bohm, A., Friebel, J., Rauch, U., Skurk, C., Blankenberg, S., Zeller, T., Prasanth, K.V., Meder, B., Kuss, A., Landmesser, U., Poller, W., 2019. Immune system-mediated atherosclerosis caused by deficiency of long non-coding rna malat1 in apoe-/-mice Available from Cardiovasc. Res. 115 (2), 302–314 http://www.ncbi.nlm.nih.gov/pubmed/30101304. https://doi.org/10.1093/cvr/cvy202.

Geri, G., Terrier, B., Rosenzwajg, M., Wechsler, B., Touzot, M., Seilhean, D., Tran, T.A., Bodaghi, B., Musset, L., Soumelis, V., Klatzmann, D., Cacoub, P., Saadoun, D., 2011. Critical role of il-21 in modulating th17 and regulatory t cells in behcet disease Available from The J. Allergy Clin. Immunol. 128 (3), 655–664 http://www.ncbi.nlm.nih.gov/pubmed/21724243. https://doi.org/10.1016/j.jaci.2011.05.029.

Han, Y., Wang, L., Li, Q., Chen, H., Ma, X., 2021. Lncrna neat1 is upregulated in recurrent aphthous stomatitis (ras) and has predictive values Available from BMC Oral Health 21 (1), 673 http://www.ncbi.nlm.nih.gov/pubmed/34972503. https://doi.org/10.1186/s12903-021-01909-1.

Huang, S., Dong, D., Zhang, Y., Chen, Z., Geng, J., Zhao, Y., 2019. Neat1 regulates th2 cell development by targeting stat6 for degradation Available from Cell Cycle 18 (3), 312–319 http://www.ncbi.nlm.nih.gov/pubmed/30654703. https://doi.org/10.1080/15384101.2018.1562285.

Imamura, K., Imamachi, N., Akizuki, G., Kumakura, M., Kawaguchi, A., Nagata, K., Kato, A., Kawaguchi, Y., Sato, H., Yoneda, M., Kai, C., Yada, T., Suzuki, Y., Yamada, T., Ozawa, T., Kaneki, K., Inoue, T., Kobayashi, M., Kodama, T., Wada, Y., Sekimizu, K., Akimitsu, N., 2014. Long noncoding rna neat1-dependent sfpq relocation from promoter region to paraspeckle mediates il8 expression upon immune stimuli Available from Mol. Cell 53 (3), 393–406 http://www.ncbi.nlm.nih.gov/pubmed/24507715. https://doi.org/10.1016/j.molcel.2014.01.009.

Jen, J., Tang, Y.A., Lu, Y.H., Lin, C.C., Lai, W.W., Wang, Y.C., 2017. Oct4 transcriptionally regulates the expression of long non-coding rnas neat1 and malat1 to promote lung cancer progression Available from Mol. Cancer 16 (1), 104 http://www.ncbi.nlm.nih.gov/pubmed/28615056. https://doi.org/10.1186/s12943-017-0674-z.

Jin, Y., Zhu, H.X., Wei, B.F., 2020. Reduced serum and local lncrna malat1 expressions are linked with disease severity in patients with non-traumatic osteonecrosis of the femoral head Available from Technol. Health Care : Off. J. Eur. Soc. Eng. Med. http://wwwncbi.nlm.nih.gov/pubmed/32716338. https://doi.org/10.3233/THC-202244.

Kulaber, A., Tugal-Tutkun, I., Yentur, S.P., Akman-Demir, G., Kaneko, F., Gul, A., Saruhan-Direskeneli, G., 2007. Pro-inflammatory cellular immune response in behcet's disease Available from Rheumatol. Int. 27 (12), 1113–1118 http://www.ncbi.nlm.nih.gov/pubmed/17549482. https://doi.org/10.1007/s00296-007-0367-9.

Lawton, G., Bhakta, B.B., Chamberlain, M.A., Tennant, A., 2004. The behcet's disease activity index Available from Rheumatology 43 (1), 73–78 http://www.ncbi.nlm.nih.gov/pubmed/12890862. https://doi.org/10.1093/rheumatology/keg453.

Li, Y., Cheng, C., 2018. Long noncoding rna neat1 promotes the metastasis of osteosarcoma via interaction with the g9a-dnmt1-snail complex Available from Am. J. Cancer Res. 8 (1), 81–90 http://www.ncbi.nlm.nih.gov/pubmed/29416922.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative pcr and the 2(-delta delta c(t)) method Available from Methods 25 (4), 402–408 http://www.ncbi.nlm.nih.gov/pubmed/11846609. https://doi.org/10.1006/meth.2001.1262.

Mercer, T.R., Dinger, M.E., Mattick, J.S., 2009. Long non-coding rnas: Insights into functions Available from Nature reviews. Genetics 10 (3), 155–159 http://www.ncbi.nlm.nih.gov/pubmed/19188922. https://doi.org/10.1038/nrg2521.

Ming, X., Duan, W., Yi, W., 2019. Long non-coding rna neat1 predicts elevated chronic obstructive pulmonary disease (copd) susceptibility and acute exacerbation risk, and correlates with higher disease severity, inflammation, and lower mir-193a in copd patients Available from Int. J. Clin. Exp. Path. 12 (8), 2837–2848 http://www.ncbi.nlm.nih.gov/pubmed/31934120.

Mohammed, S.R., Abdelaleem, O.O., Ahmed, F.A., Abdelaziz, A.A., Hussein, H.A., Eid, H.M., Kamal, M., Ezzat, M.A., Ali, M.A., 2021. Expression of lncrnas neat1 and lnc-dc in serum from patients with behcet's disease can be used as predictors of disease Available from Front. Mol. Biosci. 8, http://www.ncbi.nlm.nih.gov/pubmed/35127819. https://doi.org/10.3389/fmolb.2021.797689 797689.

Moher, D., Hopewell, S., Schulz, K.F., Montori, V., Gotzsche, P.C., Devereaux, P.J., Elbourne, D., Egger, M., Altman, D.G., Consort, 2012. Consort 2010 explanation and elaboration: Updated guidelines for reporting parallel group randomised trials Available from Int. J. Surgery 10 (1), 28–55 http://www.ncbi.nlm.nih.gov/pubmed/22036893. https://doi.org/10.1016/j.ijsu.2011.10.001.

Nakamura, J., Meguro, A., Ishii, G., Mihara, T., Takeuchi, M., Mizuki, Y., Yuda, K., Yamane, T., Kawagoe, T., Ota, M., Mizuki, N., 2019. The association analysis between hla-a*26 and behcet's disease Available from Sci. Rep. 9 (1), 4426 http://www.ncbi.nlm.nih.gov/pubmed/30872678. https://doi.org/10.1038/s41598-019-40824-y.

Pandey, A.D., Goswami, S., Shukla, S., Das, S., Ghosal, S., Pal, M., Bandyopadhyay, B., Ramachandran, V., Basu, N., Sood, V., Pandey, P., Chakrabarti, J., Vrati, S., Banerjee, A., 2017. Correlation of altered expression of a long non-coding rna, neat1, in peripheral blood mononuclear cells with dengue disease progression Available from J. Infect. 75 (6), 541–554 http://www.ncbi.nlm.nih.gov/pubmed/29031635. https://doi.org/10.1016/j.jinf.2017.09.016.

Peng, W., Wang, Z., Fan, H., 2017. Lncrna neat1 impacts cell proliferation and apoptosis of colorectal cancer via regulation of akt signaling Available from Pathol. Oncol. Res : POR 23 (3), 651–656 http://www.ncbi.nlm.nih.gov/pubmed/28013491. https://doi.org/10.1007/s12253-016-0172-4.

Pouyanrad, S., Rahgozar, S., Ghodousi, E.S., 2019. Dysregulation of mir-335-3p, targeted by neat1 and malat1 long non-coding rnas, is associated with poor prognosis in childhood acute lymphoblastic leukemia Available from Gene 692, 35–43 http://www.ncbi.nlm.nih.gov/pubmed/30639603. https://doi.org/10.1016/j.gene.2019.01.003.

Scully, C., Gorsky, M., Lozada-Nur, F., 2003. The diagnosis and management of recurrent aphthous stomatitis: A consensus approach Available from J. Am. Dental Assoc. 134 (2), 200–207 http://www.ncbi.nlm.nih.gov/pubmed/12636124. https://doi.org/10.14219/jada.archive.2003.0134.

Shaker, O.G., Abdelaleem, O.O., Mahmoud, R.H., Abdelghaffar, N.K., Ahmed, T.I., Said, O.M., Zaki, O.M., 2019a. Diagnostic and prognostic role of serum mir-20b, mir-17-3p, hotair, and malat1 in diabetic retinopathy Available from IUBMB life 71 (3), 310–320 http://www.ncbi.nlm.nih.gov/pubmed/30468285. https://doi.org/10.1002/iub.1970.

Shaker, O.G., Mahmoud, R.H., Abdelaleem, O.O., Ibrahem, E.G., Mohamed, A.A., Zaki, O.M., Abdelghaffar, N.K., Ahmed, T.I., Hemeda, N.F., Ahmed, N.A., Mansour, D.F., 2019b. Lncrnas, malat1 and lnc-dc as potential biomarkers for multiple sclerosis diagnosis Available from Biosci. Rep. 39 (1) http://www.ncbi.nlm.nih.gov/pubmed/30514825. https://doi.org/10.1042/BSR20181335.

Shui, X., Chen, S., Lin, J., Kong, J., Zhou, C., Wu, J., 2019. Knockdown of lncrna neat1 inhibits th17/cd4(+) t cell differentiation through reducing the stat3 protein level Available from J. Cellular Physiol. 234 (12), 22477–22484 http://www.ncbi.nlm.nih.gov/pubmed/31119756. https://doi.org/10.1002/jcp.28811.

Sigdel, K.R., Cheng, A., Wang, Y., Duan, L., Zhang, Y., 2015. The emerging functions of long noncoding rna in immune cells: Autoimmune diseases Available from J. Immunol. Res. 2015 http://www.ncbi.nlm.nih.gov/pubmed/26090502. https://doi.org/10.1155/2015/848790.

Tong, B., Liu, X., Xiao, J., Su, G., 2019. Immunopathogenesis of behcet's disease Available from Front. Immunol. 10, 665 http://www.ncbi.nlm.nih.gov/pubmed/30984205. https://doi.org/10.3389/fimmu.2019.00665.

Wang, Y., Zhong, H., Xie, X., Chen, C.Y., Huang, D., Shen, L., Zhang, H., Chen, Z.W., Zeng, G., 2015. Long noncoding rna derived from cd244 signaling epigenetically controls cd8+ t-cell immune responses in tuberculosis infection Available from Proc. National Acad. Sci. USA 112 (29), E3883–E3892 http://www.ncbi.nlm.nih.gov/pubmed/26150504. https://doi.org/10.1073/pnas.1501662112.

Wang, Z., Li, K., Huang, W., 2020. Long non-coding rna neat1-centric gene regulation Available from Cell. Mol. Life Sci. : CMLS 77 (19), 3769–3779 http://www.ncbi.nlm.nih.gov/pubmed/32219465. https://doi.org/10.1007/s00018-020-03503-0.

Wang, Z., Zhao, Y., Xu, N., Zhang, S., Wang, S., Mao, Y., Zhu, Y., Li, B., Jiang, Y., Tan, Y., Xie, W., Yang, B.B., Zhang, Y., 2019. Neat1 regulates neuroglial cell mediating abeta clearance via the epigenetic regulation of endocytosis-related genes expression Available from Cell. Mol. Life Sci. : CMLS 76 (15), 3005–3018 http://www.ncbi.nlm.nih.gov/pubmed/31006037. https://doi.org/10.1007/s00018-019-03074-9.

West, J.A., Davis, C.P., Sunwoo, H., Simon, M.D., Sadreyev, R.I., Wang, P.I., Tolstorukov, M.Y., Kingston, R.E., 2014. The long noncoding rnas neat1 and malat1 bind active chromatin sites Available from Mol. Cell 55 (5), 791–802 http://www.ncbi.nlm.nih.gov/pubmed/25155612. https://doi.org/10.1016/j.molcel.2014.07.012.

Yang, H., Liang, N., Wang, M., Fei, Y., Sun, J., Li, Z., Xu, Y., Guo, C., Cao, Z., Li, S., Jiao, Y., 2017. Long noncoding rna malat-1 is a novel inflammatory regulator in human systemic lupus erythematosus Available from Oncotarget 8 (44), 77400–77406 http://www.ncbi.nlm.nih.gov/pubmed/29100395. https://doi.org/10.18632/oncotarget.20490.

Yue, Y., Zhang, J., Yang, L., Liu, S., Qi, J., Cao, Q., Zhou, C., Wang, Y., Kijlstra, A., Yang, P., Hou, S., 2018. Association of long noncoding rnas polymorphisms with ankylosing spondylitis, vogt-koyanagi-harada disease, and behcet's disease Available from Invest. Ophthalmol. Visual Sci. 59 (2), 1158–1166 http://www.ncbi.nlm.nih.gov/pubmed/29490353. https://doi.org/10.1167/iovs.17-23247.

Zhang, B., Arun, G., Mao, Y.S., Lazar, Z., Hung, G., Bhattacharjee, G., Xiao, X., Booth, C. J., Wu, J., Zhang, C., Spector, D.L., 2012. The lncrna malat1 is dispensable for mouse development but its transcription plays a cis-regulatory role in the adult Available from Cell Reports 2 (1), 111–123 http://www.ncbi.nlm.nih.gov/pubmed/22840402. https://doi.org/10.1016/j.celrep.2012.06.003.

Zhang, F., Wu, L., Qian, J., Qu, B., Xia, S., La, T., Wu, Y., Ma, J., Zeng, J., Guo, Q., Cui, Y., Yang, W., Huang, J., Zhu, W., Yao, Y., Shen, N., Tang, Y., 2016. Identification of the long noncoding rna neat1 as a novel inflammatory regulator acting through mapk pathway in human lupus Available from Journal of autoimmunity 75, 96–104 http://www.ncbi.nlm.nih.gov/pubmed/27481557. https://doi.org/10.1016/j.jaut.2016.07.012.

Zhao, L., Zhou, N., Zhao, P., 2020. Expression level of neat1 differentiates benign and malignant thyroid nodules by regulating neat1/mir9/pten and neat1/mir124/pdcd6 signalling Available from Int. J. Mol. Med. 46 (5), 1661–1670 http://www.ncbi.nlm.nih.gov/pubmed/32901835. https://doi.org/10.3892/ijmm.2020.4721.

Zhou, L.J., Yang, D.W., Ou, L.N., Guo, X.R., Wu, B.L., 2020. Circulating expression level of lncrna malat1 in diabetic kidney disease patients and its clinical significance Available from J. Diabetes Res. 2020, 4729019 http://www.ncbi.nlm.nih.gov/pubmed/32832561. https://doi.org/10.1155/2020/4729019.