Expression and diagnostic values of MIAT, H19, and NRON long non-coding RNAs in multiple sclerosis patients

Mehroosh Amiri1, Mohammad Javad Mokhtari1*, Mahnaz Bayat2*, Anahid Safari3, Mehdi Dianatpuor3, Reza Tabrizi4 and Afshin Borhani-Haghighi2

Abstract

Background: Multiple sclerosis (MS) is a chronic inflammatory disease. Various long non-coding RNAs (lncRNAs) appear to have an important role in the pathophysiology of MS. This study aimed at evaluating the expression levels of lncRNAs, MIAT, H19, and NRON in peripheral blood of MS cases to a healthy control group. We collected blood samples of 95 MS cases (76 relapsing-remitting (RR) and 19 secondary progressive (SP) MS) and 95 controls. We used quantitative real-time PCR for the evaluation of gene expression. The correlation between expression with clinical parameters was analyzed by a multiple linear regression model. Receiver operating characteristic (ROC) curve analysis was carried out to detect the diagnostic potential of lncRNAs levels according to the area under the curve (AUC).

Results: MIAT, H19, and NRON were significantly increased in the RRMS and SPMS subgroups compared to the controls. We found that the H19 and MIAT expression significantly were higher in SPMS compared with RRMS. Patients with RRMS had a greater level of the average NRON expression is compared with SPMS patients. The expression level of H19 significantly was higher in females relative to male patients. Based on the area under curve (AUC) values, NRON had the best performance in the differentiation of MS patients from controls (AUC = 0.95, P < 0.0001). A combination of MIAT, H19, and NRON expression levels could be useful in differentiating MS patients with 93.6% sensitivity, 98.9% specificity, and diagnostic power of 0.96 (P < 0.0001).

Conclusions: The levels of MIAT, H19, and NRON in peripheral blood could be important biomarkers for MS diagnosis.

Keywords: LncRNA, Multiple sclerosis, Biomarkers, Real-time PCR, Gene expression

Background

Multiple sclerosis (MS) is a chronic complex autoimmune disease that causes the demyelination of neurons in the central nervous system (CNS) [1]. MS is especially common in young female adults [2]. A broad range of symptoms may appear in MS patients, such as pain, visual sensory disturbance, motor impairments, cognitive deficits, and fatigue. The different factors such as autoimmunity, genetic predisposition, and environmental influenced the pathophysiology of MS diseases. Evidence indicates that the reaction between abnormal T cells and CNS autoantigens can cause inflammation, demyelination, and neurodegeneration [3]. In addition, B cells act as an important source of plasma cells that generate antibodies while also regulating T cell production and autoimmune processes. B cells may affect the pro-inflammatory process of other immune cells [4].

Many genomic studies have demonstrated the presence of a large portion of DNA that doesn’t code for proteins, but is transcribed to the different types of RNAs. The
lncRNAs are a type of non-coding RNAs known to possess the ability to regulate cell proliferation through different mechanisms [5]. The dysregulation of lncRNAs is considered in the cause of many neurological disorders [6–8]. Research works have indicated that the expression of lncRNAs is significantly dysregulated in MS patients [9–13].

Immune regulation was affected by the function of different lncRNAs. A previous study revealed an important connection between the lncRNA MIAT (Myocardial infarction-associated transcript) and inflammatory response [14]. MIAT regulates the differentiation of neural stem cells into oligodendrocytes in the CNS [15]. The NRON (Non-Coding Repressor Of NFAT) lncRNA is an endogenous ribonucleic acid that is detected in human T cells and can regulate IL-2 expression and NFAT Ca$^{2+}$-activated transcription factor in activated T cells [16].

The lncRNA H19 is a lncRNA with a critical role in immune response regulation. It is also an imprinted gene that is expressed only from the maternal allele [17, 18]. Although the role of MIAT, H19, and NRON in cellular pathways and different cancers has been assessed, only one study has been conducted to evaluate the expression level of NRON lncRNA in 10 MS cases. Because there is evidence showing that lncRNAs are stable in human serum, circulating serum RNA levels could serve as a biomarker in non-invasive diagnostic applications [19, 20]. Research on the role of lncRNAs in MS pathogenesis is just beginning. The significance of the present study becomes more apparent due to high incidence and prevalence of MS in Iran [21]. Therefore, we aimed to assess the effectiveness of using known lncRNAs as potential biomarkers for the diagnosis of MS.

**Methods**

**Study subjects**

The present case and control study was carried out to assess the expression levels of three lncRNAs (MIAT, H19, and NRON) in 95 MS cases, who were compared to 95 controls from August 2018 to August 2020 at Namazi Hospital in Shiraz. Among all patients, 76 individuals were relapsing–remitting (RR) and 19 were secondary progressive (SP). Controls comprised a representative sample of Shiraz’s population; sex- and age-matched controls were randomly selected from neighbor controls at the nearest case’s place matched.

All participants in the present study were clinically diagnosed by an expert neurologist according to McDonald’s criteria [22]. All cases included in this study showed EDSS progression with no evidence of a relapse in the last 24 months. In the present study, we considered the following characteristics as exclusion criteria: recent infection, smoking history, malignancy, alcohol abuse, and the presence of any other inflammatory or autoimmune diseases. The examined subjects were aged 20–56 years.

Ethics approval for this research was obtained by the local ethics committee of the Kazeroon Branch, Islamic Azad University, Iran (IR.IAU.KAU.REC.1398.034). The subjects completed a written informed consent and voluntarily agreed to participate in the present study as a part of a large prospective project.

**Collection of blood samples**

After obtaining an informed consent form, the peripheral venous blood samples were collected from MS cases and control individuals in ethylene diamine tetra acetate acid-coated (EDTA) tubes as an anticoagulant.

**RNA extraction and cDNA synthesis**

A total RNA extraction kit (Favorgen, Taiwan) was used to isolate Total RNA from peripheral blood according to the manufacturer’s instructions. cDNA synthesis Kit (Yektatajhiz, Iran) was employed for cDNA synthesis from RNA samples. For cDNA synthesis, RNA samples with theA260/A230 and A260/A280 ratios greater than 1.7 were selected.

**Quantitative RT-PCR measurement of the lncRNA levels**

Primer Express and Gene Runner software v.3.0 were applied to design and analyze the oligonucleotide primers. By searching the BLAST website, the primers were confirmed to avoid non-specific PCR product formation. Specific primers’ evaluations of MIAT, H19, NRON, and the TBP gene (used as a reference gene) are shown in Table 1. Gene expression was normalized relative to the housekeeping genes GAPDH and TBP. TBP was selected as the preferential reference gene given its low variance in Ct between the different samples (data not shown).

| Gene name | Primer sequences | Primer length (bp) | Product length (bp) |
|-----------|------------------|--------------------|---------------------|
| MIAT      | TCCCCATCCCGGAAGCTAGA | 20                 | 274                |
| H19       | GAGGCCATGAAATCACCCTCA | 20                 | 91                 |
| NRON      | GCAGACAGCTAGCACTCCA | 19                 | 91                 |
| TBP       | CCCGAACACCCCGAACATTATGCTATC | 21                 | 184                |

Table 1: The nucleotide sequence of primers
The relative abundance of the IncRNAs' levels was calculated by real-time RT-PCR using Real Q Plus 2 × Master Mix Green Low ROX™ (Ampliqon, Denmark). We used the thermal-cycling settings of 10 min at 95 °C (one repeat) accompanied by 40 cycles for 15 s at 95 °C and 1 min at 60 °C. Every complete amplification phase was accompanied by a melting phase (15 s at 95 °C, 30 s at 60 °C, and 15 s at 95 °C). Cycle threshold (Ct) values were accompanied by a melting phase (15 s at 95 °C, 30 s at 60 °C, and 15 s at 95 °C). Cycle threshold (Ct) values were used to express the differences in relative levels. ΔCt indicates the difference between the Ct values of TBP and IncRNAs. The relative IncRNAs expression levels for each subject were determined using $2^{-\Delta Ct}$ [23].

Statistical analysis

The IncRNAs relative expression levels were reported as mean ± SE. Student's t test was employed to compare the mean expression of each IncRNA gene between case and control groups. The Beta coefficient of IncRNAs expression levels was estimated in univariate and multiple linear regression analyses. Using a multiple linear regression model, we adjusted for the most important clinical factors. Receiver operating characteristic (ROC) curve analysis was carried out to detect IncRNAs levels as a diagnostic biomarker for MS according to the area under the curve (AUC). We used the multiple logistic regressions to combine the age and sex variables in ROC analysis. The analyses were performed using the GraphPad Prism 5.0 and the SPSS software (version 19.0). The $P$ value of < 0.05 was regarded as statistically significant.

Results

Clinical and demographic characteristics

Clinical and demographic characteristics of MS cases and healthy controls are summarized in Table 2. No significant difference was demonstrated in sex and age ratio ($P > 0.05$).

| IncRNAs (MIAT, H19 and NRON) expression levels |
|-----------------------------------------------|
| Figure 1 demonstrates that MIAT, H19, and NRON levels were significantly higher in MS cases than in healthy controls ($P < 0.0001$), the median log2 fold changes were as follows, respectively 1.10, 2.79, and 1.30. The significantly high expression of MIAT, H19, and NRON can distinguish MS cases from healthy cases (Fig. 1A). Then, IncRNAs expression levels were compared in RRMS and SPMS subtypes with healthy controls separately. MIAT, H19, and NRON expression levels were significantly increased in the RRMS and SPMS subgroups when compared to the controls separately ($P < 0.001$). One-way ANOVA analysis showed that the MIAT and H19 expression in SPMS significantly was higher than RRMS, while NRON level in RRMS was higher relative to SPMS (Fig. 1B).

Association between clinical variables and IncRNAs expression

Multiple linear regression analysis was done to detect the association between RRMS and SPMS patients and IncRNAs expression levels after adjusting for several clinical confounders (Table 3). The IncRNAs expression levels were significantly associated with RRMS and SPMS. Patients with SPMS had a greater level of the average MIAT expression compared with RRMS patients [Beta = 1.48, 95% CI (0.62–2.35) $P = 0.001$]. Compared with RRMS patients, patients with SPMS have a higher levels of H19 expression [Beta = 1.92, 95% CI (0.54–3.29) $P = 0.007$]. Also, H19 expression in female patients was significantly higher than male patients [Beta = 2.76 95% CI (1.25–4.28) $P = 0.001$]. Patients with RRMS had a greater level of the average NRON expression compared with SPMS patients [Beta = 1.49, 95% CI (0.48–2.51) $P = 0.004$].

Predictive power of MIAT, H19, and NRON in MS diagnosis

ROC curve analyses were done to assess the diagnostic values of MIAT, H19, NRON, and whole IncRNAs expression levels and their usefulness in predicting MS. The results of these analyses are presented in Fig. 2 and Table 4. Three IncRNAs were shown to be effective in differentiating MS cases from controls. MIAT had an AUC of 0.861 (95% CI: 0.804–0.918;
hypothesis, the sensitivity = 89.47%, specificity = 80.00%), H19 had an AUC of 0.941 (95% CI: 0.903–0.979; sensitivity = 91.58%, specificity = 91.58%), NRON had an AUC of 0.951 (95% CI: 0.922–0.981; sensitivity = 93.68%, specificity = 91.58%), and whole lncRNAs expression had an AUC of 0.966 (95% CI: 0.935–0.997; sensitivity = 93.68%, specificity = 98.95%). These findings

Fig. 1 The lncRNAs of MIAT, H19, and NRON versus the TBP gene levels in the MS cases and control, (I) the levels of MIAT (A), H19 (B), and NRON (C) in controls were significantly lower compared to MS patients, (II) the relative expression of MIAT (A), H19 (B), and NRON (C) (with log2 fold change) in MS subgroups in comparison with normal healthy controls. Data were shown as mean ± SE. The data were analyzed using Student’s t test or one-way ANOVA. 2−ΔΔCt values for each individual were used to create each figure. ***P < 0.001 significant differences vs. control subjects. Abbreviations: RR, relapsing–remitting; SP, secondary progressive.
| Characteristics                  | Univariate |                  | Multiplevariate * |                  |
|---------------------------------|------------|------------------|-------------------|------------------|
|                                 | Beta       | 95% CI           | P Value           | Beta             |
|                                 |            |                  |                   |                  |
| **MIAT relative expression (log$_2$)** |            |                  |                   |                  |
| Female sex                      | 0.21       | $-0.78, 1.19$    | 0.681             | 0.38             |
| > 30 age-old                    | 0.16       | $-0.744, 1.06$   | 0.731             | 0.28             |
| Type MS (SP vs. RR)             | 1.45       | $0.60, 2.29$     | 0.001             | 1.48             |
| Severity MS (≥ 0.5 vs. < 0.5)   | $-0.28$    | $-1.02, 0.46$    | 0.462             | $-0.16$          |

| **H19 relative expression (log$_2$)** |            |                  |                   |                  |
| Female sex                      | 2.41       | $0.86, 3.96$     | 0.003             | 2.76             |
| > 30 age-old                    | 0.79       | $-0.69, 2.26$    | 0.291             | 1.34             |
| Type MS (SP vs. RR)             | 1.62       | $0.17, 3.07$     | 0.028             | 1.92             |
| Severity MS (≥ 0.5 vs. < 0.5)   | $-0.11$    | $-1.33, 1.12$    | 0.846             | 0.14             |

| **NRON relative expression (log$_2$)** |            |                  |                   |                  |
| Female sex                      | 0.63       | $-0.51, 1.77$    | 0.276             | 0.57             |
| > 30 age-old                    | 0.39       | $-0.66, 1.43$    | 0.463             | 0.28             |
| Type MS (RR vs. SP)             | 1.53       | $0.53, 2.53$     | 0.003             | 1.49             |
| Severity MS (≥ 0.5 vs. < 0.5)   | $-0.20$    | $-1.06, 0.66$    | 0.640             | $-0.25$          |

**Fig. 2** ROC curves. A ROC curve analysis of MIAT for diagnosis MS patients from the controls, B ROC curve analysis of H19 for diagnosis MS patients from the controls, C ROC curve analysis of NRON for diagnosis MS patients from the controls, D ROC curve analysis of whole expression for diagnosis MS patients from the controls.
follows, respectively, 1.10, 2.79, and 1.30. The previous study showed that \( \text{NEAT1} \) and \( \text{MALAT1} \) found a significant increase in the expression levels of \( \text{NRON} \) in 10 MS cases in comparison with controls. They showed that \( \text{NRON} \) expression levels in RR patients showed significantly higher than in RR patients. However, the present case–control study was performed on southwest Iranian individuals. We reported an upregulation of \( \text{MIAT} \), \( \text{H19} \), and \( \text{NRON} \) lncRNAs in the peripheral blood of MS patients in comparison with healthy subjects, the median log2 fold changes were as follows, respectively, 1.10, 2.79, and 1.30. The previous studies reported the dysregulation of various lncRNAs in MS cases [9–11]. For example, Shaker et al. found a significant increase in the expression levels of \( \text{Inc-DC} \) and \( \text{MALAT1} \) in MS patients [10]. Dastmalchi et al. showed that \( \text{NEAT1}, \text{PANDA}, \) and \( \text{TUG1} \) lncRNAs were over-expressed in MS cases when compared with controls [11]. One previous study [13] showed that three lncRNAs \( \text{(NEAT1, TUG1, and RN7SKRNA)} \) were up-regulated in RR patients. The downregulation and upregulation of six lncRNAs in MS cases were investigated in another study [24]. We found that the expression levels of \( \text{MIAT} \) and \( \text{H19} \) in SP patients were significantly higher than in RR patients. However, \( \text{NRON} \) expression levels in RR patients showed significantly higher expression relative to SP patients.

\( \text{NRON} \) is a lncRNA repressor that acts as a specific regulator of NFAT nuclear trafficking and interacts with the importin-beta superfamily [25]. NFATs regulate the transcriptional induction of genes that encode for immune activators/modulators such as GM-CSF, IL-2, IL-3, IL-4, IL-5, IL-13, L-8, IFNy,CD5, CD25, CD28, and CD40 [26]. In T cells, NFAT proteins govern gene expression, thereby regulating their activation, differentiation, and development in addition to the maintenance and induction of T cell tolerance [27].

The expression level of the \( \text{NRON} \) lncRNA was significantly higher in MS patients than in controls. This outcome is contrary to that of Fenoglio et al., who found that \( \text{NRON} \) was downregulated in peripheral blood of 10 MS cases in comparison with controls. They showed that \( \text{NRON} \) correlates with MS duration [12].

In Fenoglio study, the downregulation of \( \text{NRON} \) in 10 cases (5 RRMS and 5 PPMS) was evaluated, while in our study, lncRNAs expression was evaluated in 95 MS patients (76 RRMS and 19 SPMS). We also reported the significant upregulation of \( \text{NRON} \) in two subtypes of MS relative to control. Additionally, our results showed no significant correlation between the expression levels of \( \text{NRON} \) with disease duration, EDSS score, and age among MS patients. The mean disease duration in our study was 7.82 ± 6.00 years it seems that the difference in duration of the MS disease in the two studies has led to this controversy. \( \text{NRON} \) expression is likely to be high in the early years of the MS disease and then gradually decreases.

The \( \text{H19} \) lncRNA has an important role in the regulation of immune response [17]. \( \text{H19} \) is an imprinted gene that is expressed from its maternal allele [18]. The deletion of \( \text{H19} \) genes facilitates cell cycle progression in hematopoietic stem cells through the induction of maternal IGF2 [28]. \( \text{H19} \) might contribute to the pathogenesis of MS based on its role in the IGF system and the importance of hematopoietic stem cells in the regulation of immune response [29, 30]. One previous investigation demonstrated the participation of \( \text{H19} \) in rheumatoid arthritis (RA) pathology according to its overexpression in RA cases and its hypersensitivity to cytokine regulation/starvation in these cases [31]. The upregulation of \( \text{H19} \) has been reported in ischemic stroke patients with a potential for the early diagnosis of IS [32]. Zeis et al. demonstrated that over-expression of the IGF1 and IGF2 genes was found in inactive demyelinated lesions [33]. We demonstrated that the expression level of \( \text{H19} \) was significantly up-regulated in the blood of MS cases in comparison with controls. We also observed that \( \text{H19} \) expression levels correlated with sex so that the higher expression in females was detected compared with male patients. The female-specific elevated expression of \( \text{H19} \) was reported in a previous study [34]. Adriaenssens et al. reported that 17-beta-estradiol stimulated the endogenous H19 gene in MCF-7 cells [35]. On the other hand, the estrogen–ERα–H19 signaling axis may also be important in the development of breast cancer [36].

Table 4 Prediction of diagnostic value by selected biomarkers (log2 fold change)

| Parameter     | AUC ± SE | 95% CI         | P      | Se (%) | Sp (%) |
|---------------|----------|----------------|--------|--------|--------|
| Expression MIAT | 0.861 ± 0.029 | 0.804–0.918 | <0.0001 | 89.47  | 80.00  |
| Expression H19 | 0.941 ± 0.019 | 0.903–0.979 | <0.0001 | 91.58  | 91.58  |
| Expression NRON | 0.951 ± 0.015 | 0.922–0.981 | <0.0001 | 93.68  | 91.58  |
| Combination of all genes | 0.966 ± 0.016 | 0.935–0.997 | <0.0001 | 93.68  | 98.95  |

Abbreviations: AUC, area under the curve; CI, confidence interval; Se, sensitivity; Sp, specificity.

The diagnostic analyses were conducted after combining with age and sex, using multiple logistic regression. P < 0.05 was considered statistically significant.
acts as an estrogen receptor modulator [37]. As many articles have suggested the importance of sex hormones in determining the onset and outcome of multiple sclerosis in females [38, 39]. These findings have raised the challenging question of whether estrogen may be present in the pathophysiology of MS disease by overexpression in H19 IncRNA? Further studies with a larger sample size are needed to confirm the strong correlation between sex and H19 expression level in MS patients. Our results also showed that the H19 expression in SPMS patients was 5.56 times higher than that in RRMS patients. SPMS patients are usually older than RRMS with higher disability and hospitalization rates relative to RR [40]. But in our study, patients with SPMS (n = 19) had not have higher EDSS scores and age compared with RRMS (76). Thus, we can suppose that a larger sample size for SPMS patients is needed to clarify the significant difference between age and EDSS with RRMS. This result indirectly suggests that H19 expression in MS disease may have a positive correlation with the disability score of patients but larger sample size is needed to reach this correlation.

MIAT is a well-characterized lncRNA that affects cellular functions such as apoptosis, invasion, and proliferation in many human diseases. The regulatory mechanism of MIAT is very complex [41]. In patients with coronary artery disease (CAD) compared with controls, the serum level of MIAT was significantly increased and positively correlated with serum IL-6 and TNF-α [42]. Previous research works have shown that the MIAT level can be used as a biomarker for the diagnosis and prognosis of different diseases such as CAD [42, 43], various cancers [44, 45], and ischemic stroke [46]. Zhu et al. demonstrated that the expression levels of MIAT were significantly up-regulated in the leukocytes of ischemic stroke (IS) patients in comparison with controls [46]. We demonstrated that the expression level of MIAT was significantly higher in MS cases than in controls. Also, in the present study, we showed that MIAT expression was 5.93 times higher in SP cases than in RR cases. We could not detect the positive correlation between MIAT expression and EDSS score but significantly high expression of MIAT in SPMS relative to RRMS indirectly may show the positive association between MIAT and MS severity.

A valuable method for the early screening of MS is developing a high-sensitive non-invasive blood biomarker. Evidence shows that circulating lncRNAs could be considered MS biomarkers [10, 11, 47]. Elevated MIAT, H19, and NRON expression levels have been reported as characteristics of various disorders (48–53). Therefore, they could also be used as a non-specific biomarker for MS. Among the three lncRNAs investigated in the present study, NRON showed the best efficiency as a biomarker-based on its sensitivity, specificity, and AUC values.

In the present study, all patients were chosen consecutively from hospitals during the same period. The participants represented a variety of ethnic groups, though selection bias could not be avoided. More accurate findings could be observed with larger sample sizes to verify our results. Also, in the current investigation, we evaluated the expression levels of IncRNAs without performing a full transcriptome analysis. Furthermore, more investigations are needed to detect the exact molecular mechanisms by which MIAT, H19, and NRON participate in MS pathology. Although the current study indicates that circulating MIAT, H19, and NRON can be used as diagnostic biomarkers for MS, further research is required to find reliable blood biomarkers for clinical use.

**Conclusions**

This research showed the upregulation of three specific lncRNAs (MIAT, H19, and NRON) in the peripheral blood of 95 MS cases. Additional research with larger sample size is needed to confirm the present findings and clarify the molecular mechanisms of these lncRNAs in the pathogenesis of MS. Their dysregulation profiles indicate that they could be considered potential biomarkers for predicting the course of MS or patients’ responses to treatment.

**Abbreviations**

MS: Multiple sclerosis; lncRNAs: Long non‑coding RNAs; RR: Relapsing–remitting; SP: Secondary progressive; AUC: Area under the curve; CNS: Central nervous system; EDSS: Expanded disability status scale; RA: Rheumatoid arthritis; PR: Progressive-relapsing; PP: Primary-progressing; Ct: Cycle threshold; OR: Odds ratio; ROC: Receiver operating characteristic; IS: Ischemic stroke.

**Acknowledgements**

The present study was derived from a thesis submitted by Mehrnoosh Amiri in partial fulfillment of the requirement for the degree of Master of Science in Genetics. This work was supported by Islamic Azad University, Zarghan Branch, Iran.

**Authors’ contributions**

MA contributed to study concept and design, acquisition of data. MJ contributed to study concept and design, acquisition of data, analysis and interpretation of data, study supervision, drafting/revising the manuscript for content. MB contributed to study concept and design, acquisition of data drafting/revising the manuscript for content. AS contributed to acquisition of data and materials will be available if needed.

**Availability of data and materials**

Data and materials will be available if needed.

**Funding**

Not applicable.
Declarations

Ethics approval and consent to participate
Ethics approval for this research was obtained by the local ethics committee of the Kazeroon Branch, Islamic Azad University, Iran (IR.IAU.KAU.REC.1398.034). The subjects completed a written informed consent and voluntarily agreed to participate in the present study as a part of a large prospective project.

Consent for publication
Not applicable.

Competing interests
All authors declare that they have no competing interests.

Author details
1 Department of Biology, Zarghan Branch, Islamic Azad University, Zarghan, Iran. 2 Clinical Neurology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. 3 Stem Cells Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran. 4 Non-Communicable Diseases Research Center, Fasa University of Medical Sciences, Fasa, Iran.

Received: 10 September 2021 Accepted: 14 February 2022
Published online: 07 March 2022

References

1. Abolhasani Foroughi A, Salahi R, Nikseresht A, Heidari H, Nazemi M, Khorzand S (2017) Comparison of diffusion-weighted imaging and enhanced T1-weighted sequencing in patients with multiple sclerosis. Neuroradiol J 30(4):347–351
2. Foroughi AA, Saeedi-Moghadam M, Zeinali-Rafsanjani B, Nazemi M (2019) Comparison between T2, STIR and PSIR sequences, for detection of cerebral cord MS plaques. Iran J Radiol 16(3):e82134
3. Münz C, Lünenmann JD, Getts MT, Miller SD (2009) Antiviral immune responses: triggers of or triggered by autoimmune? Nat Rev Immunol 9(4):246–258
4. Arneth BM (2019) Impact of B cells to the pathophysiology of multiple sclerosis. J Neuroinflammation 16(1):1–9
5. Fang Y, Fullwood MJ (2016) Roles, functions, and mechanisms of long non-coding RNAs in nervous system function and disease. Brain Res 1338:20–35
6. Qureshi IA, Mattick JS, Mehler MF (2010) Long non-coding RNAs in nervous system function and disease. Brain Res 1388:20–35
7. Pastori C, Wahlstedt C (2012) Involvement of long noncoding RNAs in diseases affecting the central nervous system. RNA Biol 9(6):860–870
8. Ghafouri-Fard S, Taheri M (2020) A comprehensive review of non-coding RNAs functions in multiple sclerosis. Eur J Pharmacol 879:173127
9. Efftekharian MM, Ghafouri-Fard S, Soudaby M, Omrani MD, Rahimi M, Sayad A et al. (2017) Expression analysis of long non-coding RNAs in the blood of multiple sclerosis patients. J Mol Neurosci 63(3):333–341
10. Shaker OG, Mahmoud RB, Abdelaleem GO, Ibrahim EG, Mohamed AA, Zaki OM et al (2019) lncRNAs, MALAT1 and Inc-DC as potential biomarkers for multiple sclerosis diagnosis. Biosci Rep 39(1):1–11
11. Dastmalchi R, Ghafouri-Fard S, Omrani MD, Mazdeh M, Sayad A, Taheri M (2018) Dysregulation of long non-coding RNA profile in peripheral blood of multiple sclerosis patients. Mult Scler Relat Disord 25:219–226
12. Fenoglio C, Oldoni E, Serpente M, Milena A, Arcaro M, D'Anca M et al. (2018) LncRNAs expression profile in peripheral blood mononuclear cells from multiple sclerosis patients. J Neuroimmunol 324:129–135
13. Santoro M, Nociti V, Lucchini M, De Fino C, Losavio FA, Mirabella M (2016) Expression profile of long non-coding RNAs in serum of patients with multiple sclerosis. J Mol Neurosci 59(5):18–23
14. Li C, Pan S, Song Y, Li Y, Qu J (2019) Silence of IncRNA MIAT protects ATDC5 cells against lipopolysaccharides challenge via up-regulating miR-132. Artif Cells Nanomed Biotechnol 47(1):2521–2527
15. Mercer TR, Dinger ME, Sunkin SM, Mehler MF, Mattick JS (2008) Specific expression of long noncoding RNAs in the mouse brain. Proc Natl Acad Sci 105(2):716–721
16. Chen J, Ao L, Yang J (2019) Long non-coding RNAs in diseases related to inflammation and immunity. Ann Transl Med 7(18):494
17. Safari MR, Rezaei FM, Dehgani A, Noroozi R, Taheri M, Ghafouri-Fard S (2019) Genomic variants within the long non-coding RNA H19 confer risk of breast cancer in Iranian population. Gene 701:121–124
18. Anel I, de Groot N, Hochberg A (2000) Imprinted H19 gene expression in embryogenesis and human cancer: the oncofetal connection. Am J Med Genet 91(1):46–50
19. Shaker OG, Senousy MA, Elbaz EM (2017) Association of rs6983267 at 8q24, HULC rs7763881 polymorphisms and serum IncRNAs CATAT2 and HULC with colorectal cancer in Egyptian patients. Sci Rep 7(1):1–11
20. Duan W, Du L, Jiang X, Wang R, Yan S, Xie Y et al. (2016) Identification of a serum circulating IncRNA panel for the diagnosis and recurrence prediction of bladder cancer. Oncotarget 7(48):78850
21. Azam M, Yektakoooshal MH, Shohani M, Khorshidi A, Mahmudi L (2019) Epidemiology of multiple sclerosis in Iran: a systematic review and meta-analysis. PLoS ONE 14(4):e0214738
22. Thompson AJ, Banwell BL, Barkhof F, Carroll WM, Coetzee T, Comi G et al. (2018) Diagnosis of multiple sclerosis: 2017 revisions of the McDonald criteria. Lancet Neurol 17(2):162–173
23. Schmittgen TD, Livak KJ (2008) Analyzing real-time PCR data by the comparative CT method. Nat Protoc 3(6):1101
24. Zhang F, Liu G, Bu Y, Ma X, Hao J (2016) Expression profile of long non-coding RNAs and miRNAs in peripheral blood mononuclear cells from myasthenia gravis patients. J Neuroimmunol 299:124–129
25. Turner M, Galloway A, Vigonto E (2014) Noncoding RNA and its associated proteins as regulatory elements of the immune system. Nat Immunol 15(6):484
26. Kipanyula MJ, Kimaro WH, Etet PF (2016) The emerging roles of the calcineurin-nuclear factor of activated T-lymphocytes pathway in nervous system functions and diseases. J Aging Res 2016:5081021
27. Dietz L, Frommer F, Vogel AL, Vaeth M, Serfling E, Waisman A et al. (2015) NFAT1 deficit and NFAT2 deficit attenuate EAE via different mechanisms. Eur J Immunol 45(S):1377–1389
28. Venkatraman A, He XC, Thorvaldsen JLN, Sugimura R, Perry JM, Tao F et al. (2013) Maternal imprinting at the H19–Igf2 locus maintains adult haematopoietic stem cell quiescence. Nature 503(7462):345–349
29. Chesik D, Wilczak N, De Keyser J (2007) The insulin-like growth factor system in multiple sclerosis. Int Rev Neurobiol 79:203–226
30. Granick JL, Simon SI, Borjesson DL (2012) Hematopoietic stem and progenitor cells as effectors in innate immunity. Bone Marrow Res 2012:165107
31. Stuhlmüller B, Kunisch E, Franz J, Martinez-Gamboa L, Hernandez MM, Pruss A et al. (2003) Detection of oncotelar h19 RNA in rheumatoid arthritis synovial tissue. Am J Pathol 163(3):901–911
32. Rezaei M, Mokhtari MJ, Bayat M, Safari A, Dianatpour MB, Tabrizi R et al. (2021) Long non-coding RNA H19 expression and functional polymorphism rs217727 are linked to increased ischemic stroke risk. BMC Neuro 21(1):54
33. Zesi T, Howell O, Reynolds R, Schaeren-Wiemers N (2018) Molecular pathology of multiple sclerosis lesion suggests a heterogeneous expression pattern of genes involved in oligodendroglioncogesis. Exp Neurol 305:76–88
34. Reineis B, Kanduri C (2013) Elevated expression of H19 and Igf2 in the female mouse eye. PLoS ONE 8(2):e56611
35. Adriaenssens E, Lottin S, Dugimont T, Faqueta W, Coll J, Dupuy JP et al. (1999) Steroid hormones modulate H19 gene expression in both mammary gland and uterus. Oncogene 18(31):4460–4473
36. Basak P, Charterjee S, Weger S, Bruce MC, Murphy LC, Raouf A (2015) Estrogen regulates luminal progenitor cell differentiation through H19 gene expression. Endocr Relat Cancer 22(4):S05–S17
37. Basak P, Charterjee S, Bhavt V, Su A, Jin H, Lee-Wing V et al. (2018) Long non-coding RNA H19 acts as an estrogen receptor modulator that is required for endurance therapy resistance in ER+ breast cancer cells. Cell Physiol Biochem 51(4):1518–1532
38. Avila M, Bansal A, Culberson J, Peiris AN (2018) The role of sex hormones in multiple sclerosis. Eur Neurol 80(1–2):93–99
39. Bove R, Chitnis T (2014) The role of gender and sex hormones in inflammation and immunity. Ann Transl Med 2(18):494
progressive multiple sclerosis: a cross-sectional US survey. Neuropsychiatr Dis Treat 13:1349–1357
41. Sun C, Huang L, Li Z, Leng K, Xu Y, Jiang X et al (2018) Long non-coding RNA MIAT in development and disease: a new player in an old game. J Biomed Sci 25(1):1–7
42. Tan J, Liu S, Jiang Q, Yu T, Huang K (2019) LncRNA-MIAT increased in patients with coronary atherosclerotic heart disease. Cardiol Res Pract 2019:6280194
43. Azat M, Huojiahemaiti X, Gao R, Peng P (2019) Long noncoding RNA MIAT: a potential role in the diagnosis and mediation of acute myocardial infarction. Mol Med Rep 20(6):5216–5222
44. Zhou S, Xu A, Song T, Gao F, Sun H, Kong X (2020) IncRNA MIAT regulates cell growth, migration, and invasion through sponging miR-150-5p in ovarian cancer. Cancer Biother Radiopharm 35(9):650–660
45. Ye T, Feng J, Cui M, Yang J, Wan X, Xie D et al (2021) LncRNA MIAT services as a noninvasive biomarker for diagnosis and correlated with immune infiltrates in breast cancer. Int J Womens Health 13:991–1004
46. Zhu M, Li N, Luo P, Jing W, Wen X, Liang C et al (2019) Peripheral blood leukocyte expression of lncRNA MIAT and its diagnostic and prognostic value in ischemic stroke. J Stroke Cerebrovasc Dis 27(2):326–337
47. Pahlevan Kakhki M, Nikravesh A, Shirvani Farsani Z, Sahraian MA, Behmanesh M (2018) HOTAIR but not ANRIL long non-coding RNA contributes to the pathogenesis of multiple sclerosis. Immunology 153(4):479–487
48. Sun W, Yang Y, Xu C, Xie Y, Guo J (2016) Roles of long noncoding RNAs in gastric cancer and their clinical applications. J Cancer Res Clin Oncol 142(11):2231–2237
49. Abdollahzadeh S, Ghorbani S (2019) Association of the study between LncRNA-H19 gene polymorphisms with the risk of breast cancer. J Clin Lab Anal 33(3):e22826
50. Blackshaw S, Harpavat S, Trimarchi J, Cai L, Huang H, Kuo WP et al (2004) Genomic analysis of mouse retinal development. PLoS Biol 2(9):E247
51. Sone M, Hayashi T, Tarui H, Agata K, Takeichi M, Nakagawa S (2007) The mRNA-like noncoding RNA Gomafu constitutes a novel nuclear domain in a subset of neurons. J Cell Sci 120(15):2498–2506
52. Ishii N, Ozaki K, Sato H, Mizuno H, Saito S, Takahashi A et al (2006) Identification of a novel non-coding RNA, MIAT, that confers risk of myocardial infarction. J Hum Genet 51(12):1087–1099
53. Xiong T, Huang C, Li J, Yu S, Chen F, Zhang Z et al (2020) LncRNA NRON promotes the proliferation, metastasis and EMT process in bladder cancer. J Cancer 11(7):1751

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.