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Expression analysis of Treg-related lncRNAs in neuromyelitis optica spectrum disorder

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ABSTRACT

Neuromyelitis Optica Spectrum Disorder (NMOSD) is an autoimmune condition affecting the central nervous system, in which various kinds of immune cells, including T and B cells, and numerous cytokines and chemokines are implicated. LncRNAs modulating the function or differentiation of regulatory T cells (Tregs) may be involved in the pathoetiology of NMO. To assess the involvement of these lncRNAs in this disease, we studied the expression levels of TH2-LCR, MAFTRR, NEST, RMRP, and FLICR in NMO patients and healthy subjects. All of the lncRNAs listed were up-regulated in NMO patients compared with healthy controls. Although the interaction of group and gender factors significantly affected the expression of NEST, RMRP, and TH2-LCR genes, we detected no effect of gender factor on the expression of the examined genes. The highest expression correlation was found between RMRP and TH2-LCR among cases with correlation coefficient 0.73. ROC curve analysis indicated that TH2-LCR, MAFTRR, RMRP, and FLICR had significant prospective diagnostic power (AUC ± SD = 0.99 ± 0.002, 0.97 ± 0.01, 0.91 ± 0.01 and 0.84 ± 0.04, respectively). Best of these genes was TH2-LCR with AUC ± SD = 0.99 ± 0.002, sensitivity = 0.97, specificity = 1, P-value = < 0.0001. RMRP and TH2-LCR had a positive correlation with age and age at onset and a negative correlation with EDSS. Cumulatively, TH2-LCR, MAFTRR, RMRP, and FLICR lncRNAs, particularly TH2-LCR, could be considered as potential contributors to the pathogenesis of NMO disease.

1. Introduction

Neuromyelitis optica spectrum disorder (NMOSD) is an inflammatory autoimmune condition of the central nervous system (CNS) in which the immune system primarily attacks the spinal cord and optic nerves (Jarius et al., 2008). One of the distinguishing characteristics of NMO is detection of aquaporin-4 (AQP4) antibodies in the blood. These antibodies target the AQP4 water channel protein, which is predominantly found in the CNS. This immune response leads to inflammation and damage to the optic nerves and spinal cord, resulting in episodes of optic neuritis and transverse myelitis (Lennon et al., 2004; Lennon et al., 2005). Due to the overlap in symptoms between NMO and multiple sclerosis (MS), misdiagnosis can occur, leading to underreporting and a lower prevalence rate for NMO in the population. However, advancements in diagnostic techniques, including the detection of AQP4 antibodies, have improved the accuracy of distinguishing between NMO and MS, allowing for proper diagnosis and treatment (Mealy et al., 2012). To reduce the number of misdiagnoses, identifying biomarkers that distinguish NMO from MS could be helpful.

The pathogenesis of NMO involves a variety of immune cells and many cytokines and chemokines (Fujihara et al., 2020); however, the exact cause of this disease is still unknown. B and T lymphocytes may be involved in this autoimmune disease since various related cytokines and chemokines have been found in the pathogenic process of NMO disease (Uzawa et al., 2010). Several studies have observed T-cell autoimmune responses against AQP4 water channels and increased levels of Th17 cells and related cytokines in NMO patients, showcasing the crucial role of T-cells in NMO pathogenesis. Additionally, the IgG1 subclass

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of AQP4-targeted antibodies prevalent among most NMO patients relies on T-cell activity, leading to a misdiagnosis of MS and consequently a lower reported prevalence rate for NMO in the population. (Papadopoulos and Verkman, 2012; Uzawa et al., 2014; Wang et al., 2016; Carnero Contentti and Correale, 2021).

Abnormal autoimmune reactions and the development of autoimmune disorders arise from dysfunctions in T-cell populations called CD4+ regulatory T-cells (Tregs). These T-cells play a vital role in regulating autoimmune responses by expressing the transcription factor Foxp3. Dysregulation of genes associated with Treg cells leads to impaired function, contributing to the occurrence of autoimmune disorders (Sakaguchi et al., 2008; Josefowicz et al., 2012).

Long non-coding RNAs (lncRNAs) have been shown to play an essential role in regulating immunological responses and the development of immune cells (Aune and Spurlock, 2016; Ghafouri-Fard et al., 2021). Additionally, the evidence indicates substantial involvement of lncRNAs in MS, rheumatoid arthritis (RA), systematic lupus erythematosus (SLE), and type 1 diabetes mellitus (T1DM) (Wu et al., 2015). LncRNAs play a part in modulating Treg cell differentiation and function (Luo and Wang, 2020). Deregulation of these lncRNAs could therefore contribute to diseases associated with Treg cell dysfunction. FLICR (FOXP3 Regulating Long Intergenic Non-Coding RNA), NEST (IFNG-AS1), MAFTRR (MAF Transcriptional Regulator RNA), TH2-LCR (Th2 Cytokine Locus Control Region), and RMRP (RNA Component of Mitochondrial RNA Processing Endoribonuclease) are five recently identified lncRNAs that are supposed to play a role in the differentiation of Treg cells. We evaluated expression levels of these five Tregs-related lncRNAs in the peripheral blood of NMO patients compared with healthy control subjects.

2. Material and methods

2.1. Research subjects and samples

The ethical committee of Shahid Beheshti University of Medical Sciences has approved the procedures of the study (IR.SBMU.MSP.REC.1401.234). In total, 42 NMO patients (10 males and 32 females) and 50 healthy volunteers with matched ages and sex participated in the study. Cases were diagnosed by clinical evaluations, blood tests, cerebrospinal fluid (CSF) tests, and MRI, and the disease was confirmed by a neurologist. All NMO patients had AQP4 antibody. They had no autoimmune diseases and no malignancies. Control participants also had no history of immune-related or neuropsychiatric disorder. Written informed consent was obtained from all participants, including NMO patients and healthy control subjects. Five-milliliter peripheral blood samples from 42 NMO patients and 50 healthy subjects were collected at Imam Hussein Hospital (Tehran, Iran).

2.2. RNA extraction

Blood samples were used to extract total RNA using the Mammalian Blood RNA Extraction Kit (Viragene, Tehran, Iran) according to the protocol provided in the kit manual. Spectrophotometer and agarose gel electrophoresis were used to evaluate the quality and quantity of the extracted RNAs.

2.3. cDNA synthesis and expression analysis

cDNA was synthesized from the extracted RNA using AddScript cDNA Synthesis Kit (AddBio, Korea). qRT-PCR was used to quantify the expression of TH2LCRR, RMRP, NEST, FLICR, and MAFTRR in all case and control samples. Housekeeping gene B2M was recruited as a reference gene to normalize. Experiments were performed using Ampliqon master mix (Denmark) in StepOnePlus System and, all of them were conducted in duplicate. Details of primers used in the study are demonstrated in Table 1.

2.4. Statistical analysis

GraphPad Prism version 9.0 (GraphPad Software, La Jolla, CA, USA) was used for statistical analysis. We compared the expression levels of five Treg-associated lncRNAs genes, namely MAFTRR, IFNG-AS1 (NEST), FLICR, RMRP, and TH2LCRR of peripheral obtained from NMO patients and healthy controls. The expression levels in each sample were calculated using the Efficiency adjusted Ct of the normalizer gene (B2M) - Efficiency adjusted Ct of the target gene method (comparative –delta Ct method). The normal/gaussian distribution of the values was accessed by the Shapiro-Wilk test. A non-parametric test (Mann-Whitney U test) was used to identify differentially expressed genes between the patients and healthy control groups. Two-way ANOVA and Tukey post hoc tests were used to analyze the effects of main factors (disease and gender) and their interaction on gene expression levels in patients and control subgroups.

Correlations between gene expression levels in both patients and control samples were measured with Spearman's rank correlation coefficient since they were not normally distributed. Also, correlations between gene expression levels, age, disease duration, age at onset, and EDSS scores were measured with Spearman's rank correlation coefficient.

The receiver operating characteristic (ROC) curves were depicted to appraise the diagnostic power of expression levels of differentially expressed genes. Youden's J parameter was measured to find the optimum threshold. P value < 0.05 was considered a significant

3. Results

3.1. General information

Table 2 shows a summary of general data about NMO patients and healthy controls.

3.2. Expression data

As we expected that the expression levels of Treg-associated LncRNAs would differ between cases and controls, we used the real-time PCR method to assess the expression of these genes in the peripheral blood samples of both NMO patients and healthy control groups. Expression levels of all five studied lncRNAs were different among NMO patients and controls (Fig. 1).

Then, we examined the expression levels of these five lncRNAs genes in sex-based subgroups of NMO patients and controls (Fig. 2).

There was a significant effect of group (disease) factor on expression levels of all five Treg-associated lncRNAs genes. However, there was no significant effect of gender factor on expression levels of studied genes.

Table 1
Primer sequences.

Gene	Sequence 5→3	Primer Length (bp)
B2M	F- AGATGAGTATGCCTGCCGTG	20
	R- GCGGCATCTTCAAACCTCCA	20
FLICR	F- GGG CTT TTC CAG AAG GGT CT	20
	R- AGC CCA GGG TTC TAG TCG	18
MAFTRR	F- CTG AAG GGA CAG GAC GGA CAA C	22
	R- GGG GAA AAC CTG GAA AGA GGG A	22
NEST	F- AGC TGA TGG CAA TCT	21
	R- TGA CTT CTC CAG CGT TTT	21
RMRP	F- GTA GAC ATT CCC CGC TTC CCA	21
	R- GAG AAT GAG CCC CGT GTG GTT	21
TH2-LCR	F- GCT CCC CAG GCT TTT GAG ATA	21
	R- TGG TGA TGC TGA AGG GAG AC	20

Table 2
General data of study participants.

Study groups	Parameters	Values	
Patients	Sex (number)	Males	10
		Females	32
	Age (Years, mean ± SD)	Males	39.9 ± 16.52
		Females	37.62 ± 10.12
	Duration (Years, mean ± SD)	Males	2.6 ± 1.34
		Females	2.96 ± 1.37
	age of onset (Years, mean ± SD)	Males	37.3 ± 15.76
		Females	34.65 ± 9.7
	EDSS Start Score	Males	2 ± 2.46
		Females	2.37 ± 1.43
	EDSS Current Score	Males	2 ± 2.21
		Females	2.4 ± 1.58
Controls	Sex (number)	Males	11
		Females	39
	Age (Years, mean ± SD)	Males	37.54 ± 13.32
		Females	40.69 ± 10.91

The interaction of gender and group showed a significant effect on the expression of NEST, RMRP, and TH2LCRR lncRNA genes (table 3).

Expression of all five selected lncRNAs was significantly higher in total NMO patients compared with healthy controls. The highest expression ratio was seen in TH2LCRR and, the expression ratio of NEST was the lowest. Male NMO patients and corresponding controls were compared, and this pattern was also found in them. TH2LCRR, MAFTRR, FLICR, and RMRP had significant overexpression in female patients compared with corresponding controls [expression ratio (95 % CI) = 885 (337–2336), 272.4 (55.7–1332), 22.3 (4.34–114.5) and 10.12 (3.9–26.35), respectively, adjusted P-value = <0.0001]. However, expression of NEST did not differ between female cases and female controls. In female patients compared with male patients, only NEST was significantly under-expressed [expression ratio (95 % CI) = 0.2 (0.04–0.96), adjusted P-value = 0.04]. Although, in male cases versus female controls, all five LncRNAs were significantly overexpressed, in female cases, the expression of three LncRNAs, MAFTRR, RMRP, and TH2LCRR, were significantly higher in comparison to male controls [adjusted P-value = <0.0001]. Finally, no significant difference was observed in the expression of studied lncRNAs between male and female controls (table 4).

In both study groups (patients and controls), we conducted a correlation analysis to assess any potential correlations between the expressions of the studied lncRNAs. We found significant correlations between several pairs of the lncRNAs among patients and healthy controls. A remarkable correlation was revealed between the expression level of NEST and TH2LCRR in both case and control groups (correlation coefficients = 0.39 and 0.32, respectively). Expression of NEST was correlated with expressions of FLICR in patients and RMRP in controls (correlation coefficients = 0.47 and 0.35, respectively). Expressions of FLICR and RMRP were correlated with each other among NMO patients (correlation coefficients = 0.33), but not in the control group. Finally, our data revealed the strongest correlation between RMRP and TH2LCRR among the NMO patients (correlation coefficients = 0.73, P-value = <0.001; Table 5).

We assessed the strength of differentially expressed lncRNAs as a diagnostic marker between NMO patients and controls using ROC curve analysis (Fig. 3). Our data showed TH2LCRR, MAFTRR, RMRP, and FLICR significantly had a potential diagnostic power (AUC ± SD = 0.99 ± 0.002, 0.97 ± 0.01, 0.91 ± 0.01 and 0.84 ± 0.04, respectively). Among these genes, the best diagnostic characteristics were found in TH2LCRR (AUC ± SD = 0.99 ± 0.002, sensitivity = 0.97, specificity = 1, P-value = <0.0001; Table 6).

There was a significant positive correlation between RMRP and TH2LCRR lncRNAs gene expression levels with age and age at onset and a negative correlation with EDSS start (Table 7).

4. Discussion

Regulatory T cells (Tregs), critical regulators of the immune response, play a significant role in maintaining self-tolerance, preventing autoimmune reactions, and controlling inflammation through their inhibitory effects. Therefore, alterations in the number of these cells or disruptions in their inhibitory function could be implicated in the development of autoimmune and inflammatory diseases such as MS, SLE, RA, ankylosing spondylitis (AS), and inflammatory bowel disease (IBD) (Rajendeeran and Tenbrock, 2021). Recent studies have revealed that patients with NMO have decreased expression levels of various genes associated with the regulatory function of Tregs, in comparison to control subjects (Brill et al., 2019; Cai et al., 2023). Foxp3 is one of these genes that produces a key transcription factor in Tregs (Brill et al., 2019). Thus, it is suggested that this group of cells may also be involved in NMO as an inflammatory autoimmune disease. Therefore, it is likely that the genes controlling the development and differentiation of Tregs also contribute to the pathogenesis of NMO disease. LncRNAs that play

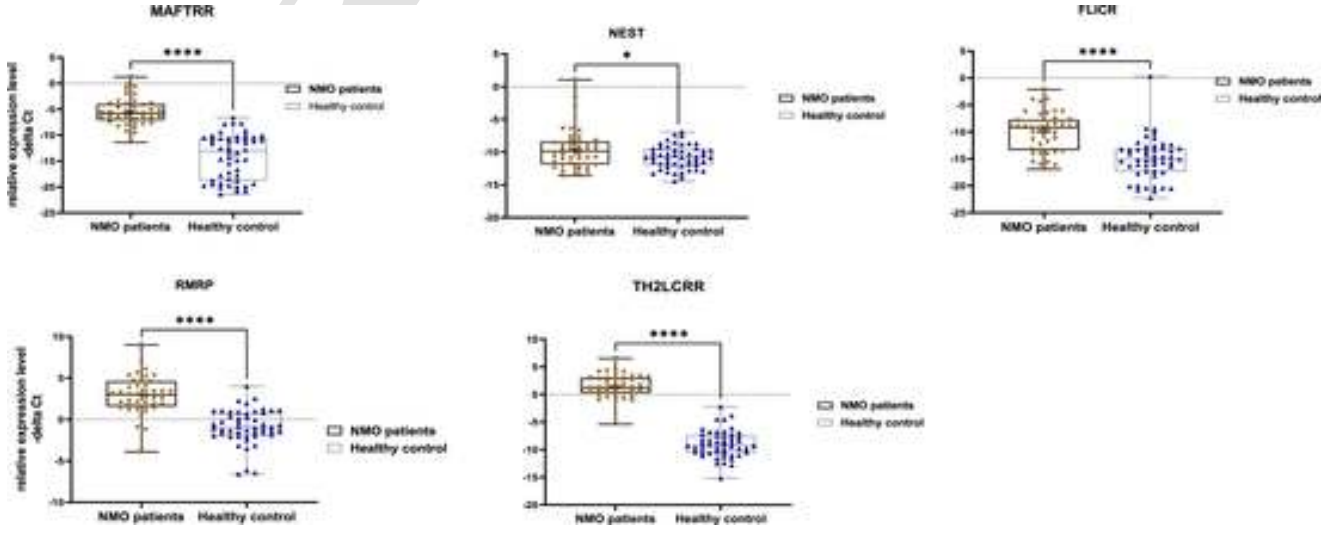

Fig. 1. Relative expression levels of five Treg-associated lncRNAs genes in Neuromyelitis Optica (NMO) patients (total) and healthy controls (total) as described by –delta Ct values (Ct Housekeeping gene- Ct Target gene) (A-E). – delta Ct Data was plotted as box and whisker plots. A non-parametric test (Mann-Whitney U test) was used to identify differentially expressed genes between two groups. (**** P value < 0.0001).

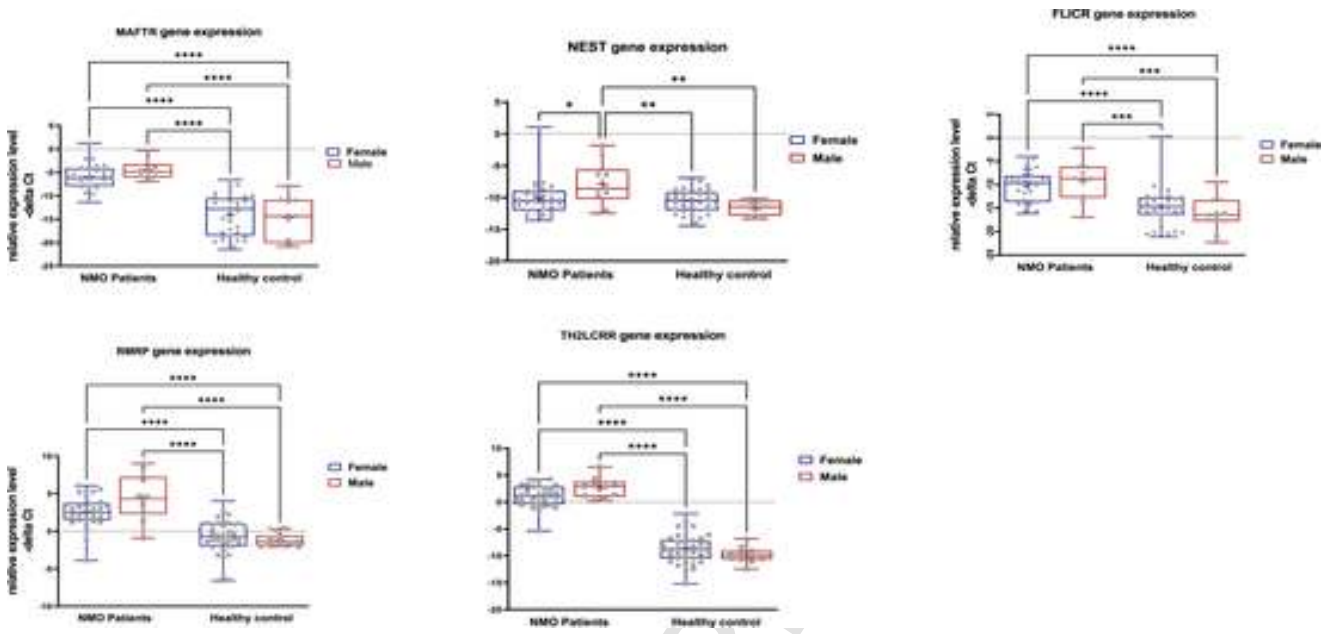

Fig. 2. Relative expression levels of five Treg-associated lncRNAs genes in Neuromyelitis Optica (NMO) patients' subgroups (male and female) versus control subgroups (male and female) as described by –delta Ct values. Two-way ANOVA and Tukey post hoc tests were used to analyze the main effects (disease and gender) and the interaction on gene expression levels in subgroups. (* P value < 0.05, ** P value < 0.001 and **** P value < 0.0001).

Table 3
Graphpad prism output from analysis of effect of Group and Gender (Tests of Between-Subjects Effects) on expression of five Treg-associated lncRNAs genes in NMO cases compared to healthy controls.

Source of Variation	Group effect (Diseases)			Gender effect			interactions		
	SS[1]	F[2]	P value	SS	F	P value	SS	F	P value
MAFTR	1350	100.5	**<0.0001**	2.51	0.18	0.66	17.94	1.33	0.25
NEST	68.92	12.57	**0.0006**	6.68	1.21	0.27	42.95	7.8	**0.006**
FLICR	553	38.75	**<0.0001**	1.54	0.1	0.74	30.12	2.11	0.14
RMRP	322.6	66.42	**<0.0001**	7.94	1.63	0.2	20.52	4.22	**0.04**
TH2LCRR	2014	403	**<0.0001**	0.48	0.09	0.75	30.51	6.11	**0.01**

[1] Sum of Squares.
[2] F of Variance.

Table 4
The results of expression ratio (fold change) of all five Treg-associated lncRNAs genes in peripheral blood of patients with Neuromyelitis Optica (NMO) diseases compared to healthy controls. The expression ratio of each gene (mean and 95 % Confidence interval of mean) is shown. A two-way ANOVA and Tukey post hoc tests were used to analyze the main effects (disease and gender) and their interaction on gene expression levels in subgroups.

		Total patients vs. Controls (42 vs. 50)	Male patients vs. Male Controls (10 vs. 11)	Female patients vs. Female Controls (32 vs.39)	Female patients vs. Male patients (32 vs. 10)	Female control vs. Male Controls (39 vs. 11)
MAFTRR	Expression ratio (95 % CI)	565 (160.9–1991)	1176 (64–16,384)	272.4 (55.7–1332)	0.36 (0.03–4.07)	1.56 (0.16–15.24)
	Adjusted P Value	**<0.0001**	**<0.0001**	**<0.0001**	0.69	0.69
NEST	Expression ratio (95 % CI)	4.16 (1.86–9.31)	12.9 (2.02–82.7)	1.34 (0.49–3.7)	0.2 (0.04–0.96)	1.97 (0.46–8.45)
	Adjusted P Value	**0.0006**	**0.0028**	0.86	**0.04**	0.6
FLICR	Expression ratio (95 % CI)	57.3 (15.7–210)	149 (7.4–2977)	22.3 (4.34–114.5)	0.48 (0.04–5.73)	3.18 (0.3–33.1)
	Adjusted P Value	**<0.0001**	**0.0002**	**<0.0001**	0.86	0.56
RMRP	Expression ratio (95 % CI)	22.16 (10.4–47)	48.2 (8.4–278)	10.12 (3.9–26.35)	0.28 (0.06–1.19)	1.33 (0.34–5.2)
	Adjusted P Value	**<0.0001**	**<0.0001**	**<0.0001**	0.1	0.94
TH2LCRR	Expression ratio (95 % CI)	2304 (1067–4938)	5955 (1009–35,119)	885 (337–2336)	0.34 (0.08–1.48)	2.29 (0.57–9.12)
	Adjusted P Value	**<0.0001**	**<0.0001**	**<0.0001**	0.23	0.39

a regulatory role in differentiating Tregs can be mentioned among these genes. In this study, we assessed the expression levels of five Tregs-associated lncRNAs, namely FLICR, MAFTRR, NEST, RMRP, and TH2-LCR, in the peripheral blood of NMO patients compared to control individuals. We demonstrated that the expression of five lncRNAs was significantly increased in NMO patients compared to healthy controls.

Tregs express the Foxp3 transcription factor, which plays a vital role in maintaining the homeostasis of the immune system and the differentiation of Treg cells. FLICR modulates the expression of this transcription factor in Tregs (Zemmour et al., 2017). This particular lncRNA interacted in conjunction with TGF-B and IL-2 to negatively regulate the expression of Foxp3. Additionally, it is involved in the differentiation of Treg cell subtypes that exhibit low levels of Foxp3 expression (Zemmour et al., 2017). Accordingly, this lncRNA likely contributes to developing autoimmune disorders such as autoimmune diabetes (Zemmour et al., 2017). One of the lncRNAs, MAFTRR, which is specifically expressed in Th1 cells, plays a role in the differentiation process of CD4+ T cells. It achieves this by regulating the expression of the MAF transcription factor. If the expression or activity of this lncRNA is decreased, it will result in a higher tendency for CD4+ T cells to differentiate into Th2 cells (Ranzani et al., 2015). The expression of *MAFTRR* in patients with Hashimoto's Thyroiditis (HT) has shown a significant increase compared to control subjects (Peng et al., 2021). NEST is another lncRNA involved in the regulation of immunological responses; it is triggered by lymphocyte activation (Gomez et al., 2013). This lncRNA regulates the levels of interferon-gamma expression by interacting with WDR5, a component of the H3K3 methyltransferase complex, and mod-

Table 5
Spearman's correlations between RNA expression levels among the NMO patients ($N = 42$) and healthy controls ($N = 50$).

	NEST		FLICR		RMRP		TH2LCRR	
	Patients	Control	Patients	Control	Patients	Control	Patients	Control
MAFTRR	0.3	0.1	−0.07	0.09	0.26	0.17	0.24	0.14
NEST			0.47**	0.14	0.3	0.35*	0.39*	0.32*
FLICR					0.33*	0.07	0.3	0.45
RMRP							0.73**	0.16

* $p < 0.05$.
** $p < 0.001$.

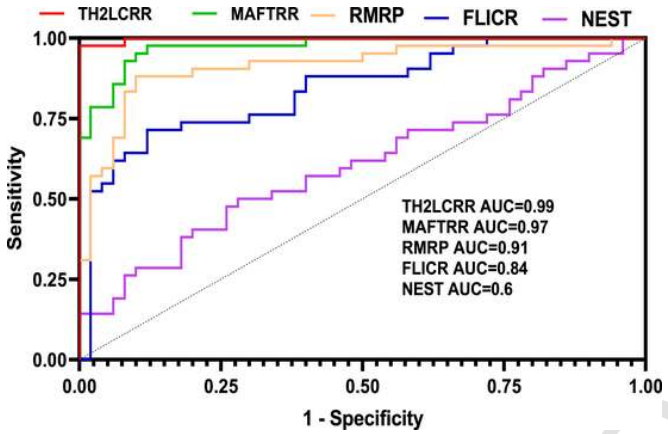

Fig. 3. ROC curves of five Treg-associated lncRNAs genes transcript levels in patients with Neuromyelitis Optica (NMO) disease.

ifying the H3 methylation at the IFN-G gene locus (Gomez et al., 2013). On the other hand, NEST can have an impact on the Th1-stimulated proliferation of Tregs. NEST reduces the expression of CD40A and TFT-bet in CD4+ T cells, leading to a decrease in the proliferation of Tregs (Luo et al., 2017). Therefore, this lncRNA is possibly involved in the pathogenesis of inflammatory diseases. In patients with rheumatoid arthritis, an increase in *NEST* expression has been observed, and a substantial correlation has been found between the high level of this lncRNA and the severity of the disease (Peng et al., 2020). The high level of *NEST* facilitates the pathogenic role of Th1 cell responses in Hashimoto's thyroiditis (HT) patients (Peng et al., 2015). Coronary artery disease (CAD) and IBD are two disorders associated with inflammatory responses in which NEST may be involved (Padua et al., 2016; Xu and Shao, 2018). Another lncRNA is *RMRP*, which has been demonstrated to be overexpressed in T cells of RA patients, and its overexpression have been correlated with the disease duration (Moharamoghli et al., 2019). *RMRP* is implicated in a variety of physiological processes and diseases by affecting several miRNAs. For example, this lncRNA has been identified to be involved in spinal cord injury (SCI) through controlling miR-766–5p/FAM83A axis (Hong et al., 2022). Alternatively, it has been discovered that RMRP facilitates cell growth and prevents cell death by inhibiting miR-34a-5p (Xiao et al., 2019). It is worth noting that miR-34a-5p, by modulating the CXCL10/CXCL11/CXCR3 path, plays a regulatory role in the chemokine signaling pathway (Hart et al., 2020). Therefore, it is possible that *RMRP* is also involved in the regulation of immunological responses through this pathway. In addition, the deregulation of *RMRP* has been identified in patients with various malignancies in several studies (Hussen et al., 2021). Finally, *TH2-LCR* regulates immunological responses through its impact on the production of Th2-related cytokines (Koh et al., 2010). Many cytokines and chemokines have been found at high levels in NMO patients. However, Th2 and Th17-related cytokines are likely to play an important role in inflammatory responses in NMO patients (Uzawa et al., 2014).

The expression level of Treg-related LncRNAs in the autoimmune diseases MS and inflammatory demyelinating polyneuropathies has also been examined in recent studies. Dadyar et al. assessed the expression of FLICR, NEST, MAFTRR, TH2-LCR, and RMRP in MS patients compared with control subjects (Dadyar et al., 2022). In MS patients, the impact of the disease on the expression of RMRP and FLICR genes was observed (Dadyar et al., 2022). Furthermore, Taheri et al. also evaluated expression levels of FLICR, NEST, TH2-LCR, and RMRP in inflammatory demyelinating polyneuropathies patients and healthy controls. Although there was no significant difference in the expression of the studied genes between acute inflammatory demyelinating polyneuropathies (AIDP) and chorionic inflammatory demyelinating polyneuropathies (CIDP) subgroups of patients, RMRP, NEST, and FLICR genes were found to be over-expressed in total patients compared to healthy subjects (Taheri et al., 2022). We observed that all five lncRNAs in NMO patients had a significant increase in expression when compared to control individuals. According to ROC analysis, in inflammatory demyelinating polyneuropathies patients and both AIDP and CIDP subgroups, RMRP and FLICR demonstrated the highest AUC, specificity, and sensitivity so, these two LncRNAs can be considered as potential markers in this disease (Taheri et al., 2022). FLICR has also been known as a possible marker in MS patients (Dadyar et al., 2022). Our finding indicated the potential diagnostic power of the TH2-LCR, MAFTRR, RMRP, and FLICR. TH2-LCR was determined to have the best diagnostic criteria among these genes

Taken together, we revealed that all five Treg-related lncRNAs were up-regulated in NMO patients and we recommended that TH2-LCR, MAFTRR, RMRP, and FLICR, particularly TH2-LCR are involved in the pathogenesis of NMO.

Ethics approval and consent to participate

All procedures performed were in accordance with the ethical standards of the national research committee and with the 1964 Helsinki declaration and its later amendments. Informed consent forms were obtained from all study participants. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Sciences.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Authors' contributions

AH performed the experiment. SE analyzed the data. MT and AH wrote the draft and revised it. AS and SGF designed and supervised the

Table 6
ROC curve analysis for five differentially expressed genes in patients with NMO disease.

TH2LCRR				MAFTRR				RMRP				FLICR				NEST			
AUC ± SD	Sensitivity	Specificity	P Value	AUC ± SD	Sensitivity	Specificity	P Value	AUC ± SD	Sensitivity	Specificity	P Value	AUC ± SD	Sensitivity	Specificity	P Value	AUC ± SD	Sensitivity	Specificity	P V
0.99 ± 0.002	0.97	1	< 0.0001	0.97 ± 0.01	0.97	0.88	< 0.0001	0.91 ± 0.01	0.88	0.9	< 0.0001	0.84 ± 0.04	0.71	0.88	< 0.0001	0.6 ± 0.05	0.5	0.72	0

Table 7
The results of Spearman's rank correlation between expression of five lncRNAs, and clinical data.

	age	sex	Disease duration	age at onset	EDSS start	EDSS current
MAFTRR	.254	.258	.080	.258	−0.020	−0.125
NEST	.210	**.327***	.048	.207	**−0.445****	−0.246
FLICR	.048	.166	.132	.029	**−0.357***	−0.171
RMRP	**.332***	.291	.232	**.327***	−0.115	−0.088
TH2LCRR	**.401****	.291	.044	**.391***	−0.079	−0.033
age		.039	**.415****	**.995****	.058	.038
sex			−0.141	.046	−0.258	−0.158
Disease duration				**.353***	.115	.187
Age at onset					.050	.027
EDSS start						**.408****
EDSS current						

EDSS's Scores was classified into 2 ranges (1–2 and greater than 2).
* significance at the 0.05 level.
** significance at the 0.01 level.

study. AG and MG collected the samples and performed the clinical assessment. All the authors contribute equally and read the submission.

Declaration of Competing Interest

The authors declare they have no conflict of interest.

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Supplementary materials

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