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lincR-Ccr2-5′AS and THRIL as potential biomarkers of multiple sclerosis

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Abstract

Background: Multiple sclerosis (MS) is a demyelinating disease affecting the central nervous system (CNS). Long non-coding RNAs (lncRNAs) were believed to play a role in the pathogenesis of neurological disorders including MS. lincR-Ccr2-5′AS is expressed in the T helper2 (Th2) lineage. TNF-α heterogeneous nuclear ribonucleoprotein L (THRIL) causes the induction of TNF-α and regulates innate immune response and inflammation. We investigated the expression of lincR-Ccr2-5′AS and THRIL in MS to clarify their association with MS risk and the clinical features.

Results: LincR-Ccr2-5′AS was significantly downregulated in MS patients (fold change = 0.43±0.29, p = 0.03). The expression level was significantly low in patients with motor weakness and optic neuritis, patients with Expanded Disability Status Scale (EDSS) ≥5.5, and treatment-naïve patients. THRIL was significantly upregulated in MS patients (fold change = 6.18±2, p = 0.02). Its expression was significantly higher in patients with relapsing-remitting multiple sclerosis (RRMS), patients with motor weakness, patients with EDSS ≤5, and patients who received interferon.

Conclusion: Our results showed the downregulation of lincR-Ccr2-5′AS and the upregulation of lncRNA THRIL in MS patients. This differential expression of both lncRNAs may have an important role in MS pathogenesis.

Keywords: Multiple sclerosis, lincR-Ccr2-5′AS, lncRNA, THRIL

Background

Multiple sclerosis (MS) is an autoimmune demyelinating disease. It affects the central nervous system (CNS) and is marked by gliosis and degeneration of axons [1]. It attacks young adults, especially females. It has a variety of symptoms with variable severity [2, 3]. Patients with MS start to present by a relapsing-remitting period in which there are sensory disorders, diplopia, and optic neuritis that become stable over time. However, continuing signs of CNS dysfunction become irreversible [3].

MS pathogenesis involves many genetic and environmental factors [4]. The advance of biomarkers involved in pathogenesis, response to the treatment, and prognosis of the disease could all lead to the optimal clinical management of the disease [5].

Non-coding RNAs (ncRNA) are implicated in human physiology and pathology. Growing evidence suggests a key regulatory role played by ncRNA, especially long non-coding RNA [6]. They are considered an essential regulator of the biological processes, regulating "chromatin remodeling," "gene transcription," and "protein transport" [7]. Recently, lncRNAs are believed to have an essential role in the pathogenesis of many neurological diseases. Few studies investigated the potential role of lncRNAs in MS pathology [8]. LncRNAs are dysregulated in MS patients, especially in peripheral blood mononuclear cells, which propose their enrollment in MS pathogenesis [9].

The exact pathophysiology for the occurrence of MS is still unidentified. It was shown that T helper cells are implicated in MS pathogenesis [10]. The T helper2 (Th2) lineage shows an expression of the lincR-Ccr2-5′ AS, which in return is linked to GATA-binding protein 3 (GATA3), the "master regulator" of Th2 [11]. Diminution of this lncRNA decreases the expression of

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chemokine genes in the Th2 lineage [11]. These chemokines contribute to the enrollment of T lymphocytes and macrophages into the CNS, which is believed to be involved in the pathogenesis of MS [12].

Tumor necrosis factor-alpha (TNF-α) is a cytokine that plays a key role in many autoimmune diseases, such as rheumatoid arthritis (RA) and multiple sclerosis (MS) [13]. It modulates the initiation of the inflammatory process and keeps it all through [13]. Many systemic and local stimuli are involved in the production of TNF-α [14]. THRIL is a lncRNA related to innate immunity. It combines with heterogeneous ribonucleoprotein particle (hnRNPs) which binds to promoter/enhancer regions of the TNF-α gene and causes stimulation of transcription and an elevation in TNF-α gene expression [11]. Levels of TNF-α are significantly elevated in cerebrospinal fluid (CSF) and plasma of MS patients. Besides, they are elevated in CSF of RRMS and secondary progressive MS (SPMS) patients [15].

lincR-Ccr2-5′AS and THRIL have been recently described to be key regulatory molecules sharing a vital role in regulating the immune response. Accordingly, the abnormal expression of lncRNAs has been proposed as an underlying cause of MS pathogenesis.

Given the regulatory role of lincR-Ccr2-5′AS and THRIL in the immune response in MS, we hypothesized that such long non-coding RNA may affect the susceptibility to MS. We aimed to evaluate the relative expression levels of lincR-Ccr2-5′AS and THRIL in MS patients, and to study their potential association with the clinical presentation, disease severity, course, and progression of MS.

Methods

Subjects

This case-control study was conducted on one hundred and thirty-four subjects and was divided into two groups:

- Group I comprised seventy-four patients (83.8% females, 16.2% males, mean age 32.5±8.1years) with MS: relapsing-remitting multiple sclerosis (RRMS) and secondary progressive multiple sclerosis (SPMS) fulfilling the Revised McDonald's criteria for the diagnosis of MS 2010 [16]. They were recruited from Kasr Al-Ainy Multiple Sclerosis Unit (KAMSU)", Cairo University Hospital, and outpatient clinics of the neurology department Fayoum University. We excluded pregnant females,; patients with a history of other autoimmune diseases, cancer, or chronic infectious disease, or patients with recent infection a month before the time of enrollment. Patients on steroid therapy were excluded as well. We recorded full history for all patients and examined them. We assessed the initial symptoms and presentation of MS. MS

manifestations included optic neuritis; sensory symptoms as numbness, itching, or burning pain; or motor symptoms, such as weakness or ataxia. We evaluated the clinical disability using the Expanded Disability Status Scale (EDSS) at the time of patient enrollment. EDSS is the most commonly used scale in MS patients. EDSS, with a scoring system from 0 to 10, reveals the patient's morbidity. While zero-point is a normal neurological examination, 10 points show the MS-related death cases. The patients were classified according to EDSS into 3 groups, EDSS =1.0–3.0, EDSS =3.5–5, and EDSS≥5.5 [17].

- Group II: The control group comprised sixty healthy volunteers, age and sex matched (80% females, 20% males, mean age 30.2±9.2 years), with no family history of MS or any other autoimmune disorders.

Blood sample collection

A vacutainer system was used for collecting blood samples (5ml) from all subjects involved in the study. Samples were collected in tubes with separator gels that lodge between packed cells and the top serum layer, permitted to clot for 15 min, and then centrifuged at 4000×*g* for 10 min. The serum samples were separated from clotted whole blood and stored at –80 °C until use.

RNA extraction

The serum was separated for the quantitative expression of lncRNAs by real-time PCR. MiRNeasy extraction kit (Qiagen, Valencia, CA) was used to extract RNA using QIAzol lysis reagent. NanoDrop 2000 (Thermo scientific, USA) was used to determine the concentration of RNA.

lncRNA expression by quantitative RT-PCR

Sixty nanograms of RNA was used in the reverse transcription (RT) step. The final volume of RT reaction was 20 μl using the RT2 first strand kit (Qiagen, Valencia, CA) as stated by the manufacturer's directions. Complementary DNA (cDNA) was stored at – 80 °C. GAPDH was used as an internal control for lncRNAs [18–20]. Readymade primers and Maxima SYBR Green PCR kit (Thermo, USA) were used for the assessment of lncRNA serum expression. Twenty-five-microliter reaction mixtures were used in RT-PCR. PCR conditions are as follows: 95 °C for 10 min, followed by 45 cycles at 95 °C for 15 s, and 60 °C for 60 s. The gene expression related to the internal control ($2^{-\Delta Ct}$) was calculated. To ensure the specificity of the corresponding RT-PCR reactions, a melt curve analysis was done. The expression levels of the two target genes were calculated using the $2^{-\Delta\Delta Ct}$ equation. The cycle threshold (Ct) value is the number of qPCR cycles required for the fluorescent signal to pass a specified threshold. ΔCt was calculated by

subtracting the Ct values of internal control from those of lincR-Ccr2-5´AS and THRIL. ΔΔCt was calculated by subtracting the ΔCt of the control samples from the ΔCt of the patients' samples. For the control sample, ΔΔCt equals zero, and 20 equals one [21].

Statistical analysis
Data were collected, coded, and entered into Microsoft Access. They were analyzed by the SPSS software version 18 on Microsoft Windows 7. The statistical analysis included a simple descriptive represented by numbers and percentages for qualitative data, standard deviations as a measure of dispersion for quantitative parametric data, inferential statistic test, independent Student's *t*-test, one-way ANOVA test, Chi-square test, and Bivariate Pearson correlation test. The *p* values were calculated based on a Student's *t*-test of the replicate $2^{(-\Delta CT)}$ values for each gene in both the control and patient groups. Logistic analysis was performed to determine the disease predictors. The receiver operating characteristic (ROC) curve was conducted to exemplify

the diagnostic power of both long non-coding RNAs. *P* values less than 0.05 were considered significant.

Results
Demographic and clinical data are summarized in Table 1
Seventy-four patients with MS (RRMS and SPMS types) (83.8% females, 16.2% males, mean age 32.5±8.1 years) were enrolled in this study together with sixty controls. They did not significantly differ in age or sex distribution. Out of 74 patients, 54 patients (73%) had RRMS and 20 patients (27%) had SPMS. 24 patients (32.4%) had optic neuritis, 10 patients (13.5%) had sensory symptoms, while 40 patients (54.1%) had motor symptoms. Four patients (5.4%)had a positive family history. The mean age of the disease onset was 25.9±7.4Y. Mean EDSS was 3.4±1.9; 42 patients (56.8%) had EDSS scores ranging from 1 to 3 and 14 patients (18.9%) had EDSS scores ranging from 3.5 to 5, while 18 patients (24.3%) had EDSS scores ≥ 5.5. Regarding treatment options, 40 patients (54.1%) received interferon and 16 patients (21.6%) received immunosuppressant in the form of

Table 1 Demographic and clinical characteristics of study groups

	MS patients (*n*=74)	Control (*n*=60)	*p*-value
Age [years] mean ±SD	32.5±8.1	30.2±9.2	0.13
Sex [no. (%)]			
Female	62 (83.8%)	48 (80%)	0.59
Male	12 (16.2%)	12 (20%)	
Types of MS [no. (%)]			
RRMS	54 (73%)		
SPMS	20 (27%)		
Initial presentation [no. (%)]			
Optic neuritis	24 (32.4%)		
Sensory symptoms	10 (13.5%)		
Motor symptoms	40 (54.1%)		
Family history of MS [no. (%)]			
Positive	4 (5.4%)	0	
Negative	70 (94.6%)	60 (100%)	
Age of onset (years) [mean ±SD]	25.9±7.4		
EDSS score [mean ±SD]	3.4±1.9		
EDSS = 1–3	42 (56.8%)		
EDSS = 3.5–5	14 (18.9%)		
EDSS ≥ 5.5	18 (24.3%)		
Treatment [no. (%)]			
Treatment naive	18 (24.3%)		
Interferon	40 (54.1%)		
Immunosuppressants			
Azathioprine	12 (16.2%)		
Cyclophosphamide	4 (5.4%)		

MS Multiple sclerosis, *RRMS* Relapsing remittent multiple sclerosis, *SPMS* Secondary progressive multiple sclerosis, *EDSS* Expended disability status scale

azathioprine and cyclophosphamide, while 18 patients (24.3%) were treatment naïve.

Expression levels of lincR-Ccr2-5'AS and THRIL

LincR-Ccr2-5′AS was found significantly downregulated in MS patients compared to controls; the decrease was >2-fold the controls, (fold change, $2^{-\Delta\Delta Ct}$ =0.34, p = 0.03). On the other hand, lncRNA THRIL was found significantly upregulated in MS patients compared to controls with an increase ≥ 6-fold the controls (fold change, $2^{-\Delta\Delta Ct}$ = 6.18, p = 0.02) (Figs. 1 and 2) (Table 2).

The association between lincR-Ccr2-5'AS and THRIL relative expression levels and patients' clinical characters

Table 3 shows the relative expression levels of LincR-Ccr2-5′As and THRIL based on different clinical characters among cases. Regarding lincR-Ccr2-5′AS, there was no significant difference in relative expression levels between RRMS and SPMS patients. The relative expression level was significantly low in patients with motor weakness and optic neuritis compared to those with sensory symptoms (p=0.001). Besides, patients with EDSS ≥5 had significantly lower expression levels than those with EDSS <5.5 (p=0.001). There was a significantly low expression level among treatment-naïve patients and those on immunosuppressants (p=0.001).

Regarding THRIL, patients with RRMS had significantly higher expression levels of THRIL as compared to those with SPMS (p=0.04). Patients who initially had motor symptoms had a significantly higher expression level of THRIL compared to those with sensory and optic neuritis (p=0.001, 0.05 respectively). Patients with EDSS <5 had a significantly higher expression level of THRIL than those with EDSS ≥5.5 (p=0.001). There was a significantly higher expression level among patients who received interferon compared to treatment-naïve patients and those on immunosuppressants (p=0.001).

Correlation between THRIL and lincR-Ccr2-5'AS with other variables among cases

There was no statistically significant correlation between lincR-Ccr2-5′AS and THRIL expression levels. Moreover, there was no statistically significant correlation between the two lncRNAs and the age of patients, or the disease onset age (Table 4).

ROC analysis to determine the diagnostic performance of lincR-Ccr2-5'AS and THRIL

Serum expression levels of lincR-Ccr2-5′AS and THRIL had rational diagnostic performance. For lincR-Ccr2-5′AS, the AUC was 57.1%, cutoff point = 0.49, sensitivity = 71.9%, specificity = 100%, and accuracy= 85.9. For THRIL, the AUC was 0.615, cutoff point = 1.024, sensitivity = 57.1%, specificity = 100%, and accuracy = 78.6%. (Fig. 3) (Table 5).
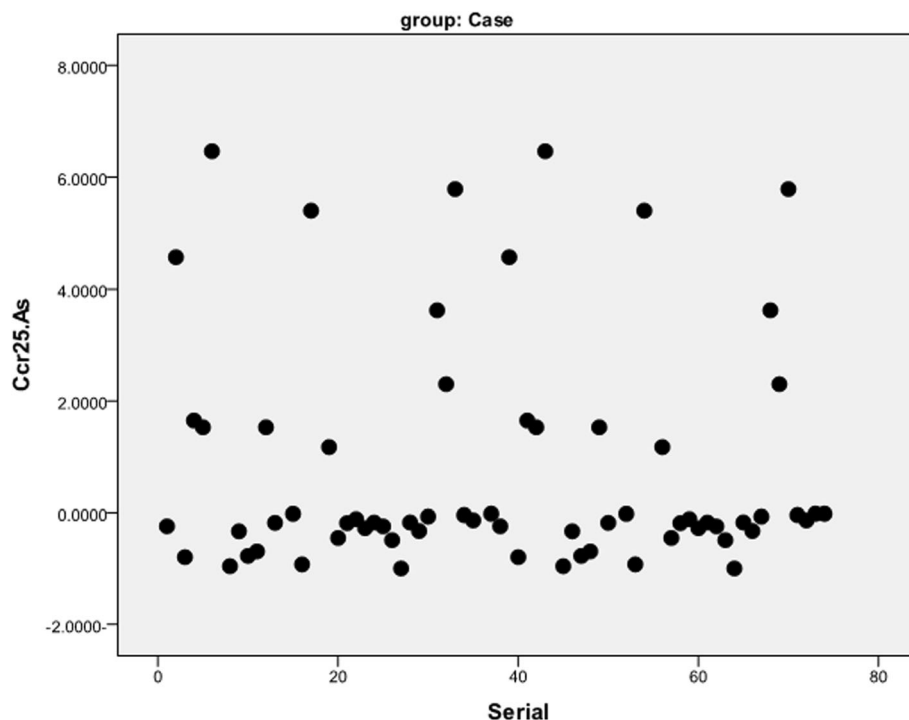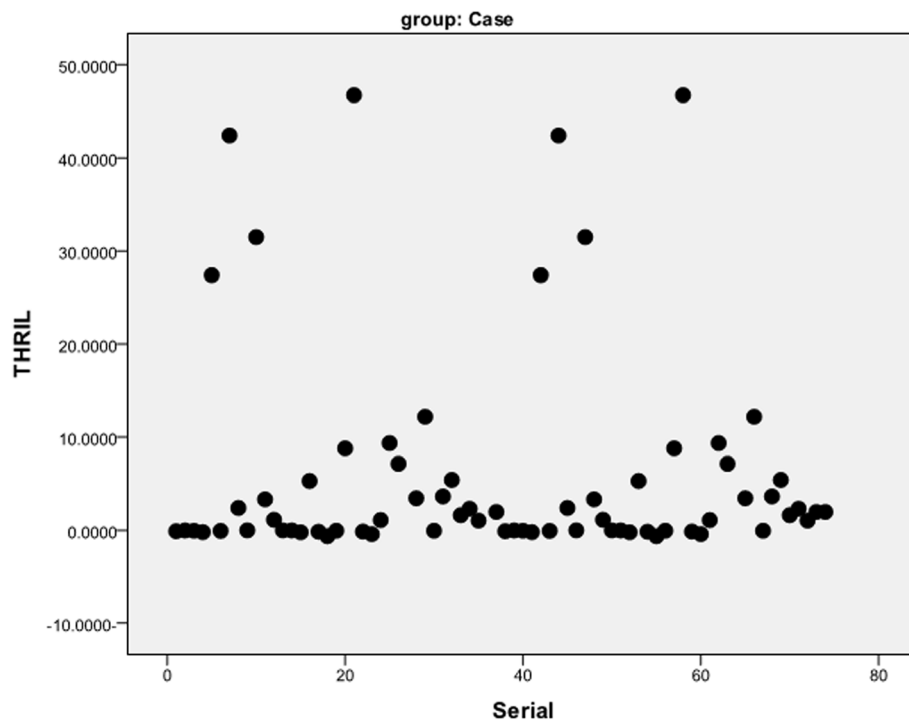

Fig. 1 Dot plot of lincR-Ccr2-5'AS fold change in MS patients

Fig. 2 Dot plot of THRIL fold change in MS patients

Logistical regression analysis

Binary logistical regression analyses were performed to predict the long non-coding RNA associated with an increased risk of multiple sclerosis (Table 6). THRIL was found to be a predictor of MS with a *p*-value <0.05 and OR = 6.45. On the other hand, no significant effect of lincR-Ccr2-5´AS was found.

Discussion

Multiple sclerosis (MS) is a chronic disease with neurodegeneration and inflammation comprising a complex immune mechanism [22]. While there is still insufficient information about its exact underlying genetic factors, many studies showed that transcriptional dysregulation may have a role in MS pathogenesis [23].

Studies show the imperative regulatory role of long non-coding RNA (lncRNA) in the immune system and pathogenesis of different CNS disorders, including MS [24]. These factors are vital regulators of CNS function and would be used in the diagnosis and treatment of CNS disorders [25]. LncRNAs are described in different autoimmune, neurodegenerative, and oncologic diseases. The wide variations in their expression throughout innate immune response and during immune cell differentiation determine their directive role in immunity [26]. Therefore, we aimed to investigate, for the first time, the serum-relative expression levels of lincR-Ccr2-5´AS and lncRNA THRIL in patients with RRMS and SPMS.

Our results showed that lincR-Ccr2-5´AS was significantly downregulated in MS patients compared to the controls. There is still insufficient data about lincR-Ccr2-5´AS in other autoimmune diseases. However, Hu et al. revealed that lincR-Ccr2-5´AS is expressed in Th2 lineage [27]. It is well-known that Th1 and Th2 lymphocytes and their related cytokines are involved in MS pathogenesis [28]. It was found that the ratio of CD4+ CCR3+/CD4+CCR4+ (which represents Th1/Th2 balance) was higher in MS patients [29], which indicates that there is a shift from Th2 to Th1 in the pathogenesis

Table 2 The fold change of LincR-Ccr2-5′As and THRIL gene expression in MS patient group relative to healthy controls

Variables	MS cases (*n*=74) mean fold change($2^{-\Delta\Delta Ct}$) ±SE	*p*-value
LincR-Ccr 2-5' AS	0.43±0.29	0.03[*]
THRIL	6.18±2	0.01[*]

MS Multiple sclerosis
*Significant, *p* values <0.05 are statistically significant

Table 3 Relative expression levels of LincR-Ccr2-5′As and THRIL based on different clinical characters among cases

	LincR-Ccr2-5′As, fold change ($2^{-\Delta\Delta Ct}$), mean± SE	*p*-value	THRIL, fold change ($2^{-\Delta\Delta Ct}$), mean± SE	*p*-value
Types				
RRMS	1.08±0.49	0.2	7.91±2.7	0.04
SPMS	0.057±0.24		1.84±0.83	
Initial presentation				
Optic neuritis	0.622±0.25	0.001	4.91±4.2	0.01[a]
Sensory	4.1±0.91		1.69±0.79	0.05[b]
Motor	0.59±0.12		8.1±2.8	0.001[c]
EDSS score [mean ±SD]				
EDSS=1–3	0.64±0.5	0.01[a]	6.75±3.3	0.02[d]
EDSS=3.5–5	1.13±0.8	0.001[b,c]	9.5±4.5	0.001[e,f]
EDSS ≥ 5.5	0.11±0.2		2.34±0.9	
Treatment				
Treatment-naive	0.17±0.4	0.001[d,e,f]	6.84±5	0.001[g,h]
Interferon	1.45±0.6		7.1±2.8	0.9[i]
Immunosuppressants	0.38±0.1		2.81	

[a]Between patients with EDSS=1–3 and EDSS=3.5–5
[b]Between patients with EDSS=1–3 and EDSS ≥ 5.5
[c]Between patients with EDSS=3.5–5 and EDSS ≥ 5.5
[d]Between patients on interferon and immunosuppressant
[e]Between patients on interferon and treatment naive patients
[f]Between patients on immunosuppressant and treatment naive patients, [g,h]between treatment naive and patients on immunsuppressant,i between treatment naive and patients on interferon

of MS [29]. Besides, GATA3 "the master regulator of Th2" is the transcription factor for lincR-Ccr2-5′AS in Th2 [8]. It was previously found downregulated in MS patients [30]. Previous studies, which support and explain our findings, showed that downregulation of lincR-Ccr2-5′AS decreases the expression of Ccr1, Ccr3, Ccr2, and Ccr5 genes in Th2 lineage [11]. This affects the enrollment of T lymphocytes and macrophages into the CNS which was believed to be involved in the pathogenesis of MS [12].

In our study, a higher relative expression level of lincR-Ccr2-5′AS was reported in patients with sensory manifestations. However, patients with motor manifestations (ataxia) had significantly lower expression levels of lincR-Ccr2-5′AS. To explain this finding, Miller et al. showed that the initial presentation of sensory symptoms is correlated with a decreased risk of conversion of the clinically isolated syndrome (CIS) to MS compared to symptoms that influence movement [31]. A related point to consider is that GATA3 "the master regulator of Th2" is the transcription factor for lincR-

Ccr2-5′AS in Th2 [2]. Besides, the GATA3 expression level was found low in MS patients [30]. Fernando et al. found that the overexpression of GATA3 could reduce the severity of disease and delay the onset of Experimental Autoimmune Encephalomyelitis: an animal model for MS (EAE), and with GATA3 overexpression, a few mice had ataxia [32].

We found that the relative expression level of lincR-Ccr2-5′AS was significantly low in patients with EDSS score ≥5 when compared to those with EDSS<5. To explain these results, Khoury et al. showed that the activation of T-lymphocyte is linked to the disease activity in patients with multiple sclerosis [33].

We found a significant increase in the relative expression level of lincR-Ccr2-5′AS after interferon treatment. This increase could be explained by the "steady-state" of chemokine receptor expression (CCR4) in CD4+T-cell surface during treatment with interferon (IFN), since CCR4 mRNA expression is associated with GATA-3 expression [34]. This confers the effect of interferon with the expression of Th2 chemokine receptors in MS patients toward the normalization [34].

Taking these points together, we can assume the potential role of lincR-Ccr2-5AS' in the genetic susceptibility to MS and its impact on the clinical presentation and the disease course.

Our results showed that lncRNA THRIL was upregulated among MS patients. Besides, it was significantly upregulated in patients with RRMS compared to those

Table 4 Correlation between LincR-Ccr2-5′As and THRIL with other variables among cases

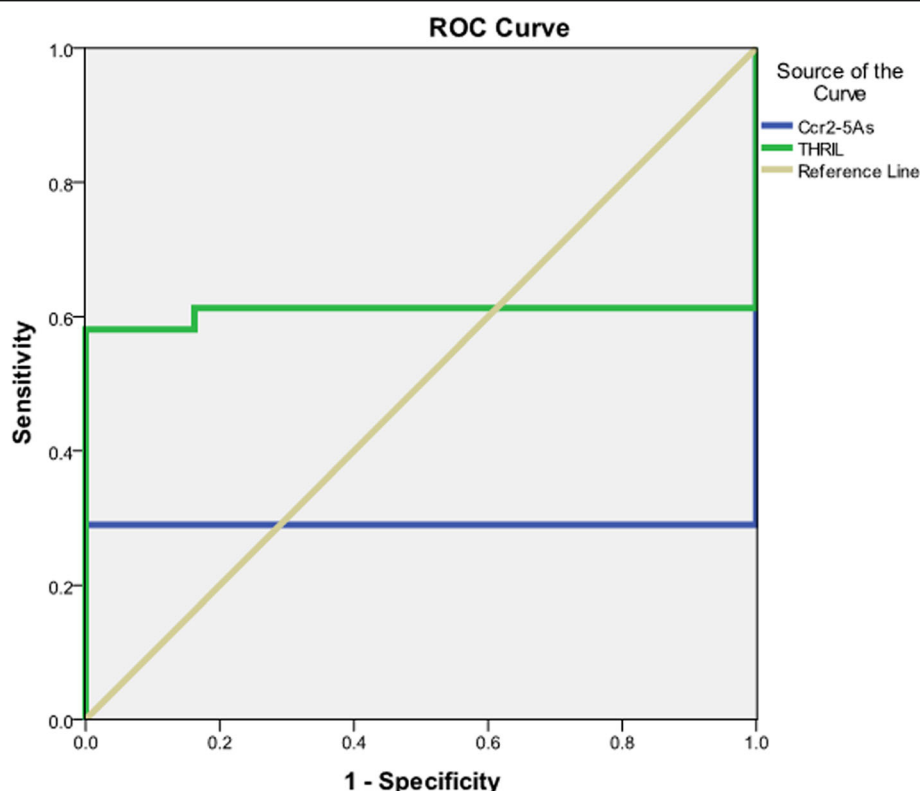| Variables among cases	LincR-Ccr2-5′As		THRIL	
	r	*p*-value	*r*	*p*-value
Age (years)	0.15	0.4	−0.04	0.8
Age of onset (years)	0.05	0.8	− 0.004	0.9
lincR-Ccr2-5′As	--	--	−0.18	0.3

Fig. 3 ROC curve to determine the diagnostic performance of lincR-Ccr2-5′AS and THRIL

with SPMS (p=0.04). In addition, binary logistic regression showed that THRIL could be a predictor of MS with p-value <0.05, OR = 6.45. In agreement with our results, Eftekharian et al. [35] detected a significant THRIL upregulation in MS patients. However, they did not find a significant correlation between THRIL expression levels and EDSS. In contrary to our findings, Li et al. showed reduced THRIL expression levels in the acute phase of Kawasaki disease [36]. This contradiction may be attributed to the altered roles of TNF in the pathogenesis of MS and other autoimmune disorders. Besides, it was reported to induce a pro-inflammatory effect in lipopolysaccharide (LPS)-induced osteoarthritis cell injury model [36].

THRIL was demonstrated to control the expression of the innate immune response genes [26]. THRIL is crucial for TNF-α expression. Obradovic et al. demonstrated the role of TNF-α in MS [15]; they found that MS patients had a higher concentration of TNF-α in

CSF and plasma during relapse and SPMS compared to MS patients during remission. The increased TNF-α expression in SPMS may suppress levels of THRIL by the negative feedback inhibition to regulate TNF-α levels expressed by cells in response to inflammatory stimuli [36]. This negative feedback loop could explain the decreased level of THRIL in our SPMS patients. It is well-known that TNF-α could prompt inflammation, affect the recruitment of the immune cells in tissues, and increase tissue destruction. However, its role in MS needs to be elucidated [37].

Our data showed the potential role of THRIL as a biomarker for genetic susceptibility to MS. Besides, it could have a role as a prognostic biomarker for the prediction of conversion of RRMS to SPMS.

A higher serum level of THRIL was found in patients with EDSS scores ≤5 when compared to those with EDSS≥5.5. We may attribute the variation of its level among different EDSS groups to the conversion of

Table 5 ROC curve analysis to determine the diagnostic performance of LincR-Ccr2-5′As and THRIL

Variable	Sensitivity	Specificity	AUC	Accuracy	Cutoff point
LincR-Ccr2-5′As	71.9%	100%	71.9%	85.9%	0.49
THRIL	57.1%	100%	57.1%	78.6%	1.024

AUC Area under curve

Table 6 Logistic regression analysis to determine the effect of LincR-Ccr2-5′As and THRIL in prediction of MS

Variables	*B*	S.E.	Wald	*P*	Exp (*B*)
LincR-Ccr2-5′As	− 22.604	7234.475	0.00	0.998	0.00
THRIL	1.865	0.806	5.362	**0.021**	6.458
Constant	0.182	0.606	0.091	0.763	1.200

Bold values are significant at $p < 0.05$

patients from inflammation to neurodegeneration in a severe state of the disease, which is accompanied by a drop in several inflammatory biomarkers [38].

In our study, we found a significantly higher expression level of THRIL in patients treated with interferon. This high THRIL level might reflect a continuous subclinical immune activity that interferon could not suppress. Despite the role of TNF in MS pathogenesis, TNF inhibitors worsen the disease condition [39]. On the other hand, TNF antagonisms were shown to cause demyelinating lesions. In general, these findings suggest a diverse TNF role in autoimmunity within CNS [39].

This study has some limitations; there were deficient data for both long non-coding RNAs in different autoimmune diseases. Another limitation is the small number of MS patients. Thus, further large-scale studies should be applied. Previous study demonstrated long non-coding RNAs in peripheral blood mononuclear cells in MS patients [9]. We have tried to use another type of samples in order to estimate long non-coding RNAs; comparative studies could be then performed. Finally, due to the nature of case-control studies, some recall and selection bias could not be avoided.

Conclusion

In conclusion, our study shows that lincR-Ccr2-5′As and THRIL might have a role in genetic susceptibility to MS. Our results demonstrated the downregulation of lincR-Ccr2-5′As and the upregulation of lncRNA THRIL in MS. They may offer good therapeutic targets in the treatment of MS patients. Further studies may be needed to explore the molecular pathways through which lncRNAs influence MS pathogenesis.

Abbreviations

CCR: Chemokine receptor; cDNA: Complementary DNA; CIS: Clinically isolated syndrome; CNS: Central nervous system; CSF: Cerebrospinal fluid; Ct: Cycle threshold; EAE: Experimental autoimmune encephalomyelitis; EDSS: Expanded Disability Status Scale; GATA3: GATA-binding protein 3; GAPDH: Glyceraldehyde 3-phosphate dehydrogenase; hnRNPs: Heterogeneous ribonucleoprotein particle; IFN: Interferon; LncRNAs: Long non-coding RNAs; LPS: Lipopolysaccharide; MS: Multiple sclerosis; ncRNA: Non-coding RNA; RA: Rheumatoid arthritis; RRMS: Relapsing-remitting multiple sclerosis; RT: Reverse transcription; ROC: Receiver operating characteristic; SPMS: Secondary progressive multiple sclerosis; Th2: T Helper2; THRIL: TNF-A and heterogeneous nuclear ribonucleoprotein L; TNFα: Tumor necrosis factor-alpha

Authors' contributions
S.R. and O.G. considered the presented idea and carried out the experiment with support from Asmaa Mohammed. S.R. wrote the manuscript. A.H. together with A. M. collected the samples and interpreted the results. All authors provided critical feedback and supported analysis of data. The authors read and approved the final manuscript.

Availability of data and materials
The authors confirm that data supporting the findings of this study are available within the study.

Declarations

Ethics approval and consent to participate
Written informed consent was provided by all participants. All human studies have been reviewed and approved by the Ethical Committee [committee's reference number, I-211016] at the Faculty of Medicine, Cairo University. It was accomplished by the code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments related to human beings.

Consent for publication
All authors have approved the manuscript for submission.

Competing interests
The authors declare that they have no competing interest.

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