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ORIGINAL ARTICLE

Down-regulation of *ERMN* expression in relapsing remitting multiple sclerosis

Behnaz Salek Esfahani[1] · Jalal Gharesouran[1,2] · Soudeh Ghafouri-Fard[2] · Shahrzad Talebian[1] · Shahram Arsang-Jang[3] · Mir Davood Omrani[4] · Mohammad Taheri[4] · Maryam Rezazadeh[1,5]

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Abstract

Multiple Sclerosis (MS) is a chronic inflammatory disease causing demyelination and neurodegeneration in the central nervous system (CNS). Although the exact etiology of MS is still unclear, both genetic and environmental elements are regarded as causative factors. Environmental factors can induce a cascade of events in immune system leading to neuronal death and nerve demyelination. This paper aims to compare the peripheral transcript levels of *Ermin* (*ERMN*) (a gene with putative role in cytoskeletal rearrangements during myelinogenesis) and *Listerin E3 Ubiquitin Protein Ligase 1* (*LTN1*) (a gene with functions in regulating innate immune system) between relapsing-remitting MS (RR-MS) patients and healthy controls. The results showed a significant decrease in *ERMN* expression ($p = 0.022$); whereas, no significant difference was detected in *LTN1* expression between two groups ($p = 0.935$). The reduction in *ERMN* expression in leukocytes could be the cause of demyelinating process in RR-MS patients. Current findings might also have practical importance in prognosis and targeted therapies.

Keywords Multiple sclerosis (MS) · *ERMN* · *LTN1* · Myelination

Introduction

Multiple sclerosis (MS) is a chronic autoimmune demyelinating and inflammatory disease targeting the central nervous system (CNS). This complex neurodegenerative disease often leads to physical disability with progressive neuroaxonal damage. MS mainly affects young individuals aged between 20- and 40-years. Women are affected about twice as much as men (Huang et al. 2017). According to epidemiological studies, the incidence and prevalence of MS have increased worldwide during the last two decades (Eskandarieh et al. 2016). Genetic predisposition and environmental factors can contribute to the disease penetrance and development. Although the exact etiology of MS is not fully understood, several studies have identified many distinct genetic regions associated with MS (Sawcer et al. 2014). Genetic variation is responsible for almost 30% of the overall disease risk (Dendrou et al. 2015). This immune-mediated disease is triggered by T cells targeting self-antigens in the CNS. Inappropriately activated T cells initiate an inflammatory cascade that cause immune response against myelin sheaths and myelin-forming cells (oligodendrocytes) in genetically susceptible individuals (Baecher-Allan et al. 2018). Axonal injury is also pronounced in white matter in the brain and spinal cord. Plaques are the first lesions in demyelination areas that lead to neurological dysfunction. The heterogeneous manifestation of the condition correlates with the location of these lesions (Friese et al. 2014).

In this paper, we aim to evaluate the expression level of *Ermin* (*ERMN*) and *Listerin E3 Ubiquitin Protein Ligase 1* (*LTN1*) genes that might be involved in the pathogenesis of

✉ Mohammad Taheri
Mohammad_823@yahoo.com

✉ Maryam Rezazadeh
Rezazadehm@tbzmed.ac.ir

[1] Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran

[2] Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[3] Clinical Research Development Center (CRDU), Qom University of Medical Sciences, Qom, Iran

[4] Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran

[5] Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

MS. *ERMN* has been identified as an oligodendrocyte-specific protein. This cytoskeletal molecule is generated at the last stage of myelinogenesis and plays a pivotal role in cytoskeletal rearrangements (Brockschnieder et al. 2006). *LTN1* as a kind of ubiquitin ligase E3, codes for a subunit of the ribosome-associated quality control (RQC) (Bengtson and Joazeiro 2010). Aggregation of nascent polypeptide chains (NC) contributes in neurodegeneration. To prevent toxic protein accumulation, this enzyme targets 60S-nascent chains on ribosomes and initiates efficient nascent chain ubiquitination to signal their degradation in proteasomes (Defenouillère et al. 2016).

Abnormalities in oligodendrocytes and neurodegenerative mechanisms in MS suggest that dysregulation of *ERMN* and *LTN1* expressions might be involved in this disorder. To confirm our hypothesis, we performed quantitative reverse transcription polymerase chain reaction (RT-PCR) to investigate *ERMN* and *LTN1* expression in Relapsing-Remitting MS (RRMS) patients.

Materials and methods

Search strategy

ERMN and *LTN1* genes were selected through an in silico method highlighted in Fig. 1.

Patients and controls

50 RRMS patients and 50 age and gender matched healthy controls was selected for gene expression analysis using quantitative RT-PCR. Blood samples were collected from all study participants. All patients were in the remission phase. Patients were referred to Imam Hussein hospital of Tehran, Tehran, Iran. A neurologist has confirmed the diagnosis of RRMS according to the revised McDonald criteria (Polman et al. 2011). Based on the significant role of HLA- DRB1∗15 in the pathogenesis of MS, in order to have a more pure selection of patients, only HLA-DRB1*15 negative patients were included in the current study. HLA typing had been carried out in a larger cohort of patients previously (Mazdeh et al. 2016). In addition, all of the patients took 20 μg of interferon-beta (IFN-β) three times a week (CinnoVex, Cinagene Company, Iran) and were clinically responders to IFN-β (Sayad et al. 2017; Rahimi et al. 2018). The other exclusion criteria were smoking history and insufficient levels of vitamin D. This study was approved by the local Ethics Committee of Tabriz University of Medical Science. Informed consent was obtained from all participants.

Blood sampling

Five ml of peripheral blood was gathered from all patients and healthy subjects. RNA extraction and cDNA synthesis was performed immediately.

RNA isolation and quantitative RT-PCR

Total RNA was extracted using GeneAll HybridR™ blood RNA extraction kit (cat No. 305–101). After determining RNA concentration and quality by a spectrophotometer, RNA was incubated with DNase to remove genomic DNA contamination. Reverse transcription for mRNA analysis was conducted using the Biosystems High-capacity cDNA Reverse Transcription Kit (PN: 4375575). The specific probes and primers for *ERMN*, *LTN1* and *HPRT1* (the housekeeping gene) were designed using Allele ID 7 software (Premier Biosoft, Palo Alto, USA). The sequences of the probes and primers are listed in Table 1.

Real-time amplifications were performed with the Corbett Rotor Gene 6000 machine (Corbett Life Science) and the

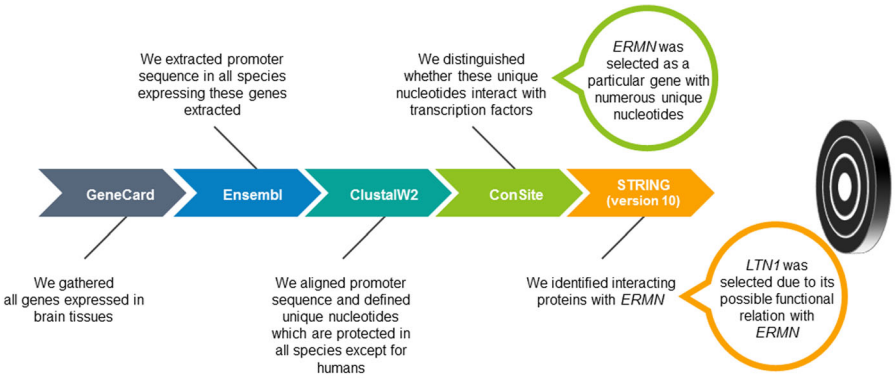

Fig. 1 Search strategy to find genes with putative roles in the pathogenesis of MS

Table 1 Sequences of the primers and probes used in Real Time PCR

Gene name	Primer sequences	Probe sequences
ERMN	F: TGTTGCCTTTATGCTTTCAAACTG R: TCTGCTGCCCACCAATCTTC	AGCCCCTCCCAGTGTCAACCTCAC
LTN1	F: CGCTCAGCTTATTTTGAGTTAGTC R: TGTTGCCTTTATGCTTTCAAACTG	CTGCATTGTGCCAGCGCATTCCAC
HPRT1	F: AGCCTAAGATGAGAGTTC R: CACAGAACTAGAACATTGATA	CATCTGGAGTCCTATTGACATCGC

Biosystems TaqMan®, Universal PCR Master Mix (PN: 4304449). The expression levels of each gene were normalized to the average expression of *HPRT1*.

Statistical analysis

Independent t-test was chosen to compare data collected from patients and control groups. Statistical significance was considered as $p < 0.05$. Pearson correlation coefficient was used to evaluate the correlation level between the variables under study. Statistical analysis was done using SPSS 18 windows statistical package (Chicago, IL, USA).

Results

Subjects

The basic information of the 50 patients (Males = 12, Females = 38) and controls (Males = 13, Females = 37) is summarized in Table 2.

Expression levels of the *ERMN* and *LTN1* genes

Statistical analysis has shown a significant down-regulation in the expression of *ERMN* gene in RR-MS patients compared to healthy controls (P = 0.022). The significance was preserved in female subjects ($P = 0.023$). *LTN1* expression levels were not significantly different between two groups (P = 0.935). Table 3 summarizes the statistical data on *ERMN* and *LTN1* expression levels, respectively.

Correlation between *ERMN* and *LTN1* expression levels and patients' features

There was no correlation between expressions of genes and age of patients. Moreover, expressions of either genes were correlated with disease duration, age at onset, EDSS score or progression index (Fig. 2). However, A significant correlation was observed between the expression levels of *ERMN* and *LTN1* ($P = 0.021$).

Discussion

MS is the leading cause of neurological disability that mainly manifests in young adults, especially women. The majority of the patients initially presents relapsing-remitting form of MS which is characterized by recurrent episodes of neurological deficits and acute exacerbations followed by partial or total recovery (Di Filippo et al. 2018). Moreover, RRMS is considered as an immune-mediated demyelinating disease in which destruction of myelin sheaths and oligodendrocytes along with neurodegeneration are prominent (Xu 2014).

In this study, we applied five distinct bioinformatics databases: GeneCard, Ensembl, ClustalW2, ConSite and STRING (version 10) to select a specific gene whose expression is significantly important in brain tissues. The findings of our precise study determined *ERMN* gene that has numerous unique nucleotides in the promoter site. These unique nucleotides are protected in all species except for humans. Through our survey, we also found LTN1 as an interacting protein with ERMN. Further study on ERMN and LTN1 functions

Table 2 Demographic and clinical characteristics of MS patients and healthy controls

Variables	MS patients	Controls
Female/male [no. (%)]	38(76%)/12(24%)	37(74%)/13(26%)
Age (mean ± SD, years)	39.6 ± 1.28	47.06 ± 2.07
Age range (years)	20–62	25–70
Age of onset (mean ± SD, years)	30.82 ± 1.39	–
Disease duration (mean ± SD, years)	8.76 ± 0.89	–
EDSS score (mean ± SD)	2.38 ± 0.16	–
Progression index	3.58 ± 0.56	–

Table 3 Comparison of *ERMN* and *LTN1* expressions level between MS patients and healthy controls, based on age and sex of the participants ([a] Relative Expression: (LN (Efficiency$^{-(deltaCt)}$)), Reference group = Control, [b] 95% credible intervals)

			ERMN				*LTN1*			
Groups	Controls no.	Patients no.	Relative Expression[a]	SE	*P* value	95% CrI[b]	Relative Expression[a]	SE	P value	95% CrI[b]
Total	50	50	−1.11	0.32	0.022	[−1.75, −0.47]	0.14	0.36	0.935	[−0.57, 0.86]
Males	13	12	0.31	0.47	0.687	[−0.63, 1.21]	0.96	0.98	0.438	[−0.96, 2.86]
Females	37	38	−1.43	0.4	0.023	[−2.21, −0.67]	−0.04	0.39	0.702	[−0.81, 0.73]

underlined the importance of identifying the role of these genes in MS.

In the literature, there are a surprising number of studies on MS pathogenesis; however, the exact mechanism is still unknown and is thought to be a complex interaction between susceptibility genes and environmental factors. In the early stages, activated T lymphocytes secret pro-inflammatory cytokines which trigger the neuroinflammatory cascade of MS. Focal inflammatory lesions are associated with the blood-brain barrier breakdown, demyelination, and axonal damage. Remyelination failure in some areas is assumed to promote degeneration of chronically demyelinated axons and axonal injury. Subsequently, immune-triggered inflammation initiates a cascade of irreversible events that lead to progressive neurodegeneration (Pérez-Cerdá et al. 2016; Lassmann and van Horssen 2011).

Accordingly, our bioinformatics findings along with pathological mechanisms of RR-MS lead us to evaluate the expression levels of *ERMN* and *LTN1* genes which are hypothesized to be involved in the demyelination and neurodegeneration processes of MS. Few studies have been published on *ERMN* and *LTN1* genes.

Ermin is a member of the ezrin-radixin-moesin (ERM) family that has been identified in 2006 as a cytoskeletal protein. This oligodendrocyte-specific protein concentrates in the brain and spinal cord, especially in white-matter-enriched tissues. Ermin is co-localized with β-tubulin in the outer cytoplasmic tongue of the myelin sheath and the paranodal loops of mature myelinated nerves as a marker. Ermin plays a vital role in oligodendrocytes differentiation process and distinctly marks F-actin-rich spikes. More analysis confirmed that Ermin induces cell extensions via its C-terminal region (actin-binding domain) that regulates the dynamic morphological changes of oligodendrocytes during myelination. Ermin reorganizes cell cytoskeleton during the late wrapping and myelin compaction stages of myelination and has a pivotal role in the stability and maintenance of the myelin sheath in adult (Brockschnieder et al. 2006).

A study showed that human Ermin (hErmin) expression is lower in temporal lobe epilepsy (TLE) patients compared to

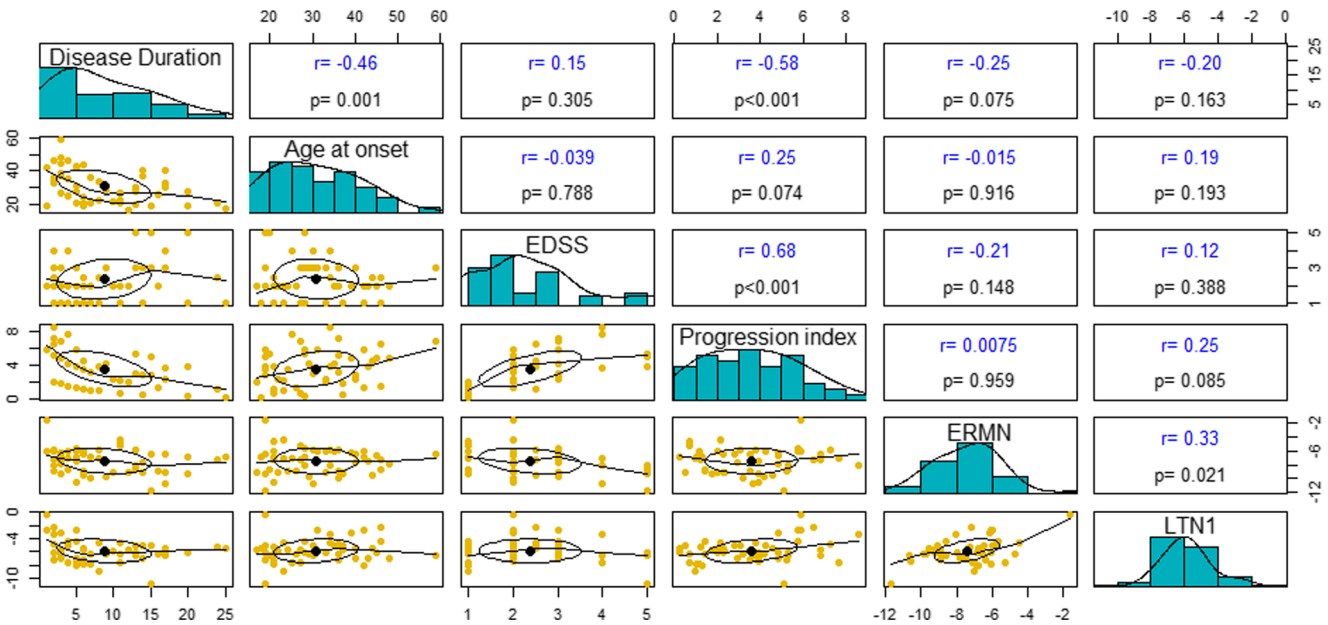

Fig. 2 Correlation between *ERMN* and *LTN1* expression levels and patients' features

healthy controls, suggesting that hErmin may be involved in the epileptic seizures. These findings also provide evidence for the role of oligodendrocytes in epilepsy. They also showed that Ermin is co-localized with two myelin-specific protein markers, CNPase and MBP (Wang et al. 2011).

Using shotgun mass spectrometry analyses, researchers have identified ERMN as an upregulated protein participating in the dysfunction of the dorsolateral prefrontal cortex in schizophrenia (SCZ) (Martins-de-Souza et al. 2009a). Another study using the same approach revealed that ERMN is down-regulated in schizophrenia (SCZ) anterior temporal lobe. ERMN down-regulation could explain why white matter integrity is disrupted in SCZ patients (Martins-de-Souza et al. 2009b).

Besides, investigations revealed that the expression of hErmin is decreased in subependymal giant cell astrocytomas (SEGAs) (Tyburczy et al. 2010). Moreover, other researchers have detected overexpression of the *ERMN* gene in Autism spectrum disorders (ASD) patients as a result of hypomethylation (Homs et al. 2016). Another study conducted in the mouse medial prefrontal cortex (mPFC) brain tissue showed down-regulation of myelin-associated genes, like *ERMN* as an underlying mechanism for myelination reduction in mPFC (Lehmann et al. 2017).

All mentioned findings strongly emphasize on the importance of hErmin and oligodendrocyte in the pathogenesis of CNS disorders.

LTN1 gene codes for listerin E3 Ubiquitin Protein Ligase 1 that takes part in Ribosome quality control (RQC). LTN1 ubiquitylates aberrant and misfolded polypeptide nascent chains (NCs) in 60S ribosomal subunits. Ultimately, LTN1 leads ubiquitinated proteins to proteasomal degradation. Findings show that loss of function in LTN1 leads to mass production of nonstop protein and sensitivity to stress. Consequently, the neurodegenerative phenotype may result from defective protein quality control in Ltn1 mutated mouse (Chu et al. 2009; Choe et al. 2016; Wang et al. 2015).

To the best of our knowledge, no previous studies reported *ERMN* and *LTN1* expression profiling in human MS individuals. The most remarkable result of the present study is that *ERMN* expression in peripheral blood leukocytes was significantly reduced in RR-MS patients in comparison to healthy individuals. According to the underling mechanism of MS, the observed decrease in *ERMN* expression level could be the reason for oligodendrocyte destruction and lack of remyelination in MS patients. Contrary to expectation, we did not find a significant difference in expression levels of *LTN1* between patients and controls. Our finding shows that despite having no correlation between *LTN1* expression and MS onset, *LTN1* expression is significantly associated with *ERMN* expression.

Taken together, these findings highlighted a role for *ERMN* in the pathogenesis of MS. However, these results should be validated in larger sample sizes of patients and controls.

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Compliance with ethical standards

Conflict of interest The authors declare they have no conflict of interest.

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