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Association Between Serum MicroRNAs and Magnetic Resonance Imaging Measures of Multiple Sclerosis Severity

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Abstract

IMPORTANCE—MicroRNAs (miRNAs) are promising multiple sclerosis (MS) biomarkers. Establishing the association between miRNAs and magnetic resonance imaging (MRI) measures of disease severity will help define their significance and potential impact.

OBJECTIVE—To correlate circulating miRNAs in the serum of patients with MS to brain and spinal MRI.

DESIGN, SETTING, AND PARTICIPANTS—A cross-sectional study comparing serum miRNA samples with MRI metrics was conducted at a tertiary MS referral center. Two

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Study concept and design: Weiner, Gandhi, Bakshi.

Acquisition, analysis, or interpretation of data: All authors.

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independent cohorts (41 and 79 patients) were retrospectively identified from the Comprehensive Longitudinal Investigation of Multiple Sclerosis at the Brigham and Women's Hospital. Expression of miRNA was determined by locked nucleic acid–based quantitative real-time polymerase chain reaction. Spearman correlation coefficients were used to test the association between miRNA and brain lesions (T2 hyperintense lesion volume [T2LV]), the ratio of T1 hypointense lesion volume [T1LV] to T2LV [T1:T2]), brain atrophy (whole brain and gray matter), and cervical spinal cord lesions (T2LV) and atrophy. The study was conducted from December 2013 to April 2016.

MAIN OUTCOMES AND MEASURES—miRNA expression.

RESULTS—Of the 120 patients included in the study, cohort 1 included 41 participants (7 [17.1%] men), with mean (SD) age of 47.7 (9.5) years; cohort 2 had 79 participants (26 [32.9%] men) with a mean (SD) age of 43.0 (7.5) years. Associations between miRNAs and MRIs were both protective and pathogenic. Regarding miRNA signatures, a topographic specificity differed for the brain vs the spinal cord, and the signature differed between T2LV and atrophy/destructive measures. Four miRNAs showed similar significant protective correlations with T1:T2 in both cohorts, with the highest for hsa.miR.143.3p (cohort 1: Spearman correlation coefficient r_S = −0.452, P = .003; cohort 2: r_S = −0.225, P = .046); the others included hsa.miR.142.5p (cohort 1: r_S = −0.424, P = .006; cohort 2: r_S = −0.226, P = .045), hsa.miR.181c.3p (cohort 1: r_S = −0.383, P = .01; cohort 2: r_S = −0.222, P = .049), and hsa.miR.181c.5p (cohort 1: r_S = −0.433, P = .005; cohort 2: r_S = −0.231, P = .04). In the 2 cohorts, hsa.miR.486.5p (cohort 1: r_S = 0.348, P = .03; cohort 2: r_S = 0.254, P = .02) and hsa.miR.92a.3p (cohort 1: r_S = 0.392, P = .01; cohort 2: r_S = 0.222, P = .049) showed similar significant pathogenic correlations with T1:T2; hsa.miR.375 (cohort 1: r_S = −0.345, P = .03; cohort 2: r_S = −0.257, P = .022) and hsa.miR.629.5p (cohort 1: r_S = −0.350, P = .03; cohort 2: r_S = −0.269, P = .02) showed significant pathogenic correlations with brain atrophy. Although we found several miRNAs associated with MRI outcomes, none of these associations remained significant when correcting for multiple comparisons, suggesting that further validation of our findings is needed.

CONCLUSIONS AND RELEVANCE—Serum miRNAs may serve as MS biomarkers for monitoring disease progression and act as surrogate markers to identify underlying disease processes.

Multiple sclerosis (MS) involves autoimmune-related inflammatory demyelination of the central nervous system white matter and gray matter (GM).[1] The disease may transect axons and cause brain and spinal cord neuronal loss and atrophy.[2] Magnetic resonance imaging (MRI) is a standard tool for the diagnosis and therapeutic monitoring of MS.[3–5] Recent MRI technology allows visualization of tissue atrophy[6,7] and GM involvement[7,8] beyond the traditional focal white matter lesion assessments.[9]

Among lesion measures, T2 hyperintense areas are histologically nonspecific but can estimate the burden of overt demyelinating foci.[9] Persisting T1 hypointense lesions (black holes) specifically indicate irreversible tissue destruction.[10] The total burden of such lesions and the proportion of T2 lesions showing T1 hypointensity[11] provide a better indication of physical disability,[12] quality of life,[13] and unemployment.[14]

Quantification of atrophy with MRI can estimate axonal loss, neuronal loss, and neurodegenerative changes, which complement lesion measurements.[15,16] Brain atrophy, particularly of GM, links to physical disability and cognitive impairment after adjusting for the effect of lesions.[17,18] Spinal cord involvement is associated with disability[19] and provides unique information vs brain MRI.[20,21]

MicroRNAs (miRNAs)are small, noncoding RNAs that regulate gene expression by binding to complementary sequences in untranslated messenger RNA (mRNA) regions, resulting in translational repression or mRNA degradation.[22–24] Dysregulated miRNA expression links to a range of immunologic and other diseases.[25–27] Due to their detectability and stability in plasma, serum, and cerebrospinal fluid[25,27,28] as well as the development of sensitive methods for their detection and quantification,[29] miRNAs are attractive biomarkers.

Previous studies showed changes in miRNA expression in brain tissue, serum and plasma, and immune cells from patients with MS and in experimental autoimmune encephalomyelitis.[30–33] Previous studies have also shown associations between MS disability or disease progression and the expression of miRNAs.[34,35]

Magnetic resonance imaging measures of lesions and atrophy aid in defining the pathobiology reflected by a candidate biomarker.[36] Since miRNAs regulate several cellular pathways, we hypothesized that different miRNA expression patterns would show associations with specific MRI measures of cerebral and spinal cord involvement.

Methods

Participants

As part of the Comprehensive Longitudinal Investigation of Multiple Sclerosis cohort study of clinical, MRI, and blood phenotyping in more than 2000 patients at Brigham and Women's Hospital,[37] we retrospectively identified patients with (1) age 18 to 55 years, (2) MS diagnosis,[38] (3) absence of other major medical disorders, and (4) no more than 30 days between serum and brain MRI acquisition (cohort 1) or both spine and brain MRI acquisition (cohort 2). Patients were evaluated by an MS specialist at the time of MRI for neurologic disability by the Expanded Disability Status Scale (EDSS) score, an ordinal scale ranging from low (0) to high (10) disability.[39] Two independent cohorts (eTable 1 in the Supplement) were tested and analyzed for miRNA-MRI associations. Cohort 1 had 41 participants (7 [17.1%] men), with a mean (SD) age of 47.7 (9.5) years; cohort 2 had 79 participants (26 [32.9%] men), with a mean (SD) age of 43.0 (7.5) years. The study was conducted from December 2013 to April 2016.

The study was approved by the Partners Health Care Human Research Committee. The participants provided written informed consent; there was no financial compensation.

Samples

Serum samples were collected in red-top Vacutainer tubes without additives, centrifuged at 2000 rpm for 10 minutes to separate serum, and stored at −70°C until RNA extraction. Isolation of RNA was then performed (miRcury kit; Exiqon) and converted to

complementary DNA using a synthesis kit (Exiqon). Prepared complementary DNA samples were stored at −20°C. Locked nucleic acid, green-based, real-time quantitative polymerase chain reaction Human Panel I and II (LNA-SYBR; Exiqon) containing 652 miRNAs and 752 miR-NAs were used for profiling the first set and second sets, respectively. Normalization was performed using the mean miRNA expression of 10 miRNAS (hsa.let.7d.3p, hsa.miR.103a.3p, hsa.miR.106a.5p, hsa.miR.126.3p, hsa.miR.15b.5p, hsa.miR.19a.3, hsa.miR.20a.5p, hsa.miR.30b.5p, hsa.miR.425.5p, and hsa.miR.92a.3p), with the best stability index determined by Norm Finder software. Normalized cycle quantification (Cq) was calculated as mean Cq – assay Cq.

MRI Acquisition

Patients were scanned on one of two 1.5T MRI units (Signa; GE Healthcare). For brain imaging, the sequences covered the whole head; were free of intersection gaps; and included (1) axial, T2-weighted, conventional spin-echo dual-echo (repetition time (TR)/echo time 1 (TE1)/TE2, 3000/30/80 milliseconds; voxel size, 0.9375 × 0.9375 × 3 mm); (2) axial T1-weighted spinecho (TR/TE, 467–733/20 milliseconds; voxel size, 0.9375 × 0.9375 × 3 mm); (3) (cohort 2 only) sagittal, 3-dimensional, magnetization-prepared rapid gradient-echo (TR/TE, 8.6/3.8 milliseconds; voxel size, 0.94 × 0.94 × 1.2 mm); and (4) axial T1-weighted spin-echo imaging, which was repeated 5 to 7 minutes after a 0.1-mmol/kg intravenous infusion of gadolinium. For the cervical spinal cord imaging (cohort 2), patients were scanned on the same MRI unit, including axial T2-weighted fast spin-echo covering the whole cervical spinal cord, with a voxel size of 0.35 × 0.35 × 3mm. The mean (SD) TR was 3561 (431) milliseconds, and the TE was 103 (1.3) milliseconds. Thus, cohort 2 had a 3-dimensional, high-resolution brain acquisition and a spinal cord image set in addition to conventional brain imaging that was obtained in both cohorts.

MRI Analysis

Lesion Measures—Whole-brain T1 hypointense (T1LV) and T2 hyperintense (T2LV) lesion volume (both cohorts) and whole cervical (C1 to C7) spinal cord T2LV (cohort 2) were expert traced using an edge-finding tool in Jim, version 7 software (Xinapse Systems Ltd, http://www.xinapse.com/). Brain T2 lesions required hyperintensity on both the early- and late-echo T2 images. Brain T1 hypointense lesions (black holes) were defined as containing some degree of visible hyperintensity on the T2 images but were free of gadolinium enhancement (to reduce the detection of benign and transient lesions).[40] As an index of the destructive potential of the lesions, we calculated the intrapatient ratio of T1LV to T2LV (T1:T2) based on its unique and valuable role in previous studies.[11,41,42]

Brain Atrophy—For cohort 2, given the availability of a high-resolution image set, we used a fully automated algorithm to derive normalized whole-brain parenchymal (BPV) and GM(GMV) volumes(SIENAX, version 5.0; Analysis Group,http://fsl.fmrib.ox.ac.uk).[14,43] Given the ineffective contouring of the deep central and posterior foss a GM from the SIENAX output, we performed manual masking of the GMV maps to remove those regions to calculate the cortical GMV (cGMV). For cohort 1 (Figure 1), we relied on the dual-echo images to estimate brain atrophy using a fully automated pipeline (SPM, version 12; Wellcome Trust Centre for Neuroimaging, www.fil.ion.ucl.ac.uk/spm/). This derived brain

parenchymal fraction (BPF) (a validated estimation of whole-brain atrophy[6]) normalized as the ratio of brain parenchymal tissue (GM plus white matter) volume divided by the intracranial volume.[44] We also calculated the global cerebral GM fraction (GMF) in an analogous manner.

Spinal Cord Atrophy—In cohort 2, axial T2-weighted images of the cervical cord (C1-C5) were applied to a validated active surface tool[45] using the Jim, version 7 software package. Manual adjustments were made as necessary to capture the spinal cord contour. Spinal cord volume from C1 to C5 was normalized by dividing by the number of axial sections[46] to generate the upper cervical spinal cord area (UCCA). Our group has previously shown that cord volumes derived from T2-weighted images are similar to those obtained from T1-weighted images.[47]

Reliability and Blinding—Analysis of MR images was performed by operators (F.K., R.C., S. Tauhid, S. Tummala, S.L.D., and G.K.) blinded to clinical and biomarker information. Five metrics required operator input (brain T2LV, brain T1LV, cGMV, UCCA, and spinal cord T2LV). Regarding brain T2LV, brain T1LV, and UCCA, our procedures and their high reliability have been well established in previously published studies.[47,48] In the present data set, intrarater reliability was high as assessed from 5 randomly chosen cases. For spinal cord T2LV, the mean coefficient of variation was 3.53%; for cGMV, the mean coefficient of variation was 0.44%.

Statistical Analysis

Spearman correlation coefficients (r_s) estimated the association between miRNA expression (normalized Cq) and MRI and disability (EDSS score). Spearman correlations were used so that participants with miRNA levels below detection limits (missing or undetected values) could contribute to the analysis. Undetected expression values were assigned a value lower than the smallest observed value from all participants. *P* Values were adjusted for multiple comparisons using the false discovery rate. The miRNA-MRI associations were observed as either pathogenic, shown by high miRNA expression associated with high MRI severity, or protective, shown by association with lower MRI-indicated severity. In the interpretation of the correlation coefficients, MRI-indicated severity was reflected in 2 possible directions depending on the metric: (1) for lesion severity (T2LV and T1:T2), a higher number represented increased disease severity, and (2) for atrophy measures (BPF, GMF, BPV, cGMV, and UCCA), a higher number represented lower disease severity (less atrophy). Demographic characteristics were compared using 2-sample, 2-tailed *t* tests for continuous variables and χ^2 tests for dichotomous variables. Data analysis was performed from April 2016 to June 2016. Statistical analysis was completed in R, version 3.0.2 (http://www.r-project.org) and Stata/IC, version 14 (http://www.stata.com).

Results

Correlation Between MRI and Disability

The MRI measures of destructive pathology (T1:T2 ratio and atrophy), but not brain and spinal cord T2LV, showed significant associations with EDSS (eTable 2 in the Supplement).

The strongest correlation was for atrophy of the brain (GMF: $r_S = -0.507$; $P < .001$; BPV: $r_S = -0.470$; $P < .001$) and spinal cord (UCCA: $r_S = -0.389$; $P < .001$).

Correlation Between miRNAs and MRI

The 10 miRNAs showing the strongest MRI association in each cohort are provided in Tables 1 and 2 (eTable 3 and eTable 4 in the Supplement present all correlations). In cohort 1, the miRNAs that demonstrated strong correlations were similar for BPF and GMF. However, the miRNAs associated with lesions (T2LV and T1:T2 ratio) were mostly different from those associated with atrophy, suggesting different pathologic processes underlying focal lesions (inflammatory demyelination) vs neurodegeneration (axonal and neuronal loss). Similar to those in cohort 1, miRNAs in cohort 2 that showed a strong correlation with whole-brain atrophy also indicated a strong correlation with cerebral GM atrophy. However, a different set of miRNAs correlated with spinal cord vs brain atrophy. In addition, only a small overlap was found between miRNAs associated with brain vs spinal cord T2LV. Thus, there was a topographic specificity in brain- vs spinal cord–related miRNAs. Although we found several miRNAs associated with MRI outcomes, none of these remained significant after false discovery rate correction.

Correlations Common Between miRNAs and MRI in Both Cohorts

Four miRNAs showed similar significant protective correlations with T1:T2 in both cohorts, with the highest for hsa.miR.143.3p (cohort 1: Spearman correlation coefficient $r_S = -0.452$, $P = .003$; cohort 2: $r_S = -0.225$, $P = .046$); the others included hsa.miR.142.5p (cohort 1: $r_S = -0.424$, $P = .006$; cohort 2: $r_S = -0.226$, $P = .045$), hsa.miR.181c.3p (cohort 1: $r_S = -0.383$, $P = .01$; cohort 2: $r_S = -0.222$, $P = .049$), and hsa.miR.181c.5p (cohort 1: $r_S = -0.433$, $P = .005$; cohort 2: $r_S = -0.231$, $P = .04$). In the 2 cohorts, hsa.miR.486.5p (cohort 1: $r_S = 0.348$, $P = .03$; cohort 2: $r_S = 0.254$, $P = .02$) and hsa.miR.92a.3p (cohort 1: $r_S = 0.392$, $P = .01$; cohort 2: $r_S = 0.222$, $P = .049$) showed similar significant pathogenic correlations with T1:T2; hsa.miR.375 (cohort 1: $r_S = -0.345$, $P = .03$; cohort 2: $r_S = -0.257$, $P = .022$) and hsa.miR.629.5p (cohort 1: $r_S = -0.350$, $P = .03$; cohort 2: $r_S = -0.269$, $P = .02$) showed significant pathogenic correlations with brain atrophy. Thus, a common set of miRNAs was as sociated with measures of destructive pathology/neurodegeneration in both cohorts. The miRNAs that were significantly correlated in the same direction with MRI are summarized in Table 3 and Figure 2. Four miRNAs (hsa.miR.142.5p, hsa.miR.143.3p, hsa.miR.181c.3p, and hsa.miR.181c.5p) were significantly correlated (protective) with T1:T2 ratio in both cohorts, with hsa.miR.143.3p correlating the highest (cohort 1: $r_S = -0.452$, $P = .003$; cohort 2: $r_S = -0.225$, $P = .046$). Two miRNAs (hsa.miR.486.5p and hsa.miR.92a.3p) significantly correlated (pathogenic) with T1:T2 ratio in both cohorts. Two miRNAs (hsa.miR.375 and hsa.miR.629.5p) significantly correlated (pathogenic) with brain atrophy in both cohorts. Thus, a common set of miRNAs was associated with measures of destructive pathology/ neurodegeneration in both cohorts.

Correlations Between miRNAs and Disability

Several miRNAs showed significant associations with EDSS score in both cohorts (Tables 1 and 2). Seven of these associations remained significant after false discovery rate correction (Table 1).

Discussion

We evaluated the association between serum miRNA and MRI measures of disease severity in MS, including lesions and atrophy, in the brain and spinal cord. Several key findings emerged: both protective and pathogenic associations were found between miRNAs and MRI; a topographic specificity, different between the brain and spinal cord, was identified; a pathobiologic specificity was found with T2 lesions showing a different miRNA signature vs atrophy and destructive measures; and T2 lesions demonstrated the weakest link to both miRNA and disability.

MicroRNAs have been proposed as biomarkers relevant to diagnosis, stage of disease, and response to treatment in MS.[33–35,53] Magnetic resonance imaging techniques have long contributed to the diagnosis and monitoring of the disease.[3] Imaging advances have allowed measurement of heterogeneous pathologic processes, allowing clustering of patients based on these differences.[15,56] To further understand the correlation between pathogenic and protective miRNAs and the relevance of their association with MRI in the present study, we performed an Ingenuity Pathway Analysis (https://targetexplorer.ingenuity.com/) to identify the most relevant experimentally observed gene targets of the miRNAs.

Among the identified miRNAs, reduced expression of hsa.miR.143.3p correlated with increased T1:T2 ratio as a protective association. Previous studies indicate that hsa.miR.143.3p is inversely associated with brain tissue damage, with decreased levels in the serum in persons with Alzheimer disease, minimal cognitive impairment, and vascular dementia vs the levels in healthy individuals.[50] Deregulated expression of hsa.miR.143 is present in synaptoneurosomes isolated from prion-infected mice at both asymptomatic and symptomatic stages of the disease.[57] The expression of hsa.miR.143 in neuronal stem cells is increased following the administration of insulinlike growth factor-1[58] (known to support the proliferation and enhanced survival of neuronal stem cells).[59] An analysis of the downstream cascade of hsa.miR.143 has exposed many potential targets, such as platelet-derived growth factor receptor-α, which play a significant role in the differentiation of oligodendrocytes, as trocytes, and neuroprogenitors.[60] Another target, mitogen-activated protein kinase, regulates differentiation of neuronal stem cells.[61] Taken together, the above results suggest the potential role of hsa.miR.143.3p in neuroprotection and repair, consistent with the present findings.

Similarly, hsa.miR.142.5p, which also showed a protective correlation with T1:T2 ratio in our study, is known to be downregulated in cerebrospinal fluid and plasma from patients with Alzheimer disease.[62] As predicted by pathway analysis, hsa.miR.142.5p targets nuclear factor erythroid-like 2 and plays a key role in regulating genes involved in responses to free radical production upon injury and inflammation.[63]

The pathogenic miRNA, hsa.miR.92a.3p, showed a pathogenic relationship with T1:T2 ratio in our study. This miRNA belongs to the miR17–92 cluster, which is overexpressed in B cells[55] and plasma[34] in patients with MS vs healthy individuals serving as controls. In mice, miR17–92 favors proinflammatory Th17 polarization.[64] Furthermore, CD4 T-cell–specific

ablation of the miR17–92 cluster mitigates experimental autoimmune encephalomyelitis by inhibiting the effector function of Th17 cells.

Another pathogenic miRNA in our study with regard to T1:T2 ratio (hsa.miR.486.5p) primarily targets phosphatase and tensin homologue (PTEN) and forkhead Box O1 (FOXO1).[65,66] Both PTEN and FOXO1 are involved in the PI3K/Akt signaling pathway. T-cell receptor and cytokine signaling activate this pathway, which leads to Akt phosphorylation and inactivation of FOXO1, which is a factor critical for T-cell homeostasis, homing, and Treg induction. PTEN is a suppressor of the phosphatidylinositol 3-kinase/protein kinase B (P13K/Akt) pathway[67,68]; therefore, enhanced expression of hsa.miR.486.5p may lead to downregulation of PTEN and FOXO1, followed by activation of the P13K/Akt pathway and T-cell dysfunction. The other pathogenic miRNA with regard to T1:T2 ratio in our study, hsa.miR.92a.3p, also targets PTEN[69,70] and other proteins, including IKAROS family zinc finger 1 (IKZF1),[71] which is a transcription factor that has been shown to regulate hematopoietic cell development. Studies indicate that IKZF1 represses the expression of Tbet transcription factor, a regulator of Th1 cells. In addition, IKZF1 represses the B-cell production of IgG2c, an IgG whose levels are upregulated in autoimmune disorders.[72,73] Therefore, one can speculate that hsa.miR.92a.3p plays a pathogenic role by repressing gene targets known to regulate immune cell activity and prevent inflammation.

miRNA hsa.miR.181c.5p showed a protective correlation with T1:T2 ratio in our study. This miRNA targets TRA, CD69, and AICDA—all known to mediate proinflammatory pathogenic effects by modulating T-cell activation or autoantibody production by B cells.[74–79] Therefore, a reduction in protective miRNA levels may enhance inflammatory responses by increased expression of its targets. Another target of hsa.miR.181c.5p includes matrix metallopeptidase 14 (MMP14),[80] which is implicated in cell migration and infiltration. Loss of MMP14 in mice was shown to impair monocyte migration, transendothelial invasion, cytokine release, and infiltration of T cells into sites of inflammation.[81] These findings suggest that the presence of MMP14 due to low levels of this protective miRNA may correlate with disease progression by allowing entry of immune cells into the central nervous system.

An important observation in this study was the topographic specificity of the relationships that we detected between miRNAs and MRI. Different sets of miRNAs were associated with spinal cord MRI measures of disease severity (lesions or atrophy) vs brain measures. This divergence is in line with previous laboratory and clinical studies showing that specific genetic susceptibility is associated with a predilection for spinal cord lesions[82] and that different effector T-cell subsets induce predominantly spinal cord (Th1) or brain (Th17) parenchymal infiltration and inflammation.[83] In further support of this concept, a study found no correlation between brain and spinal cord MRI involvement in MS.[21] Other studies have shown the complementary information obtained by combining brain and spinal cord MRI to characterize disease severity.[20,84]

We observed a limited overlap between miRNAs associated with cerebral lesions (T2LV) vs those associated with tissue destruction (ie, atrophy [BPF, BPV, GMF, and cGMV] or severe

lesions [T1:T2]). Such findings are consistent with the weak association that has been detected between T2LV and brain atrophy.[15,56] These observations suggest a decoupling between focal inflammatory demyelination in white matter and diffuse cerebral neurodegeneration.[15] A range of factors other than T2 lesions may be more important determinants of tissue loss, such as remyelination and repair capacity,[85] meningeal inflammation,[86] microglial proliferation,[87] and toxins or oxidative stress.[88]

Regarding lesion measures, T2LV showed the weakest association with either disability or miRNA expression. This observation is consistent with the notion that T2 lesions are nonspecific for the nature and severity of tissue injury, but the development of lesional T1 hypointensity[9] has a higher clinical relevance. This would explain why a T1:T2 lesion index shows more utility than T2LV in tracking therapeutic effects and providing clinical relevance in MS.[11,89]

Strengths and Limitations

To our knowledge, this is the first study that includes 2 large, independent patient cohorts; a highly specific, locked nucleic acid–based, quantitative polymerase chain reaction platform for miRNA expression[29]; and quantitative MRI analysis that includes the brain and spinal cord. One of the limitations is the lack of traditional discovery and validation phases, that is, the patients in cohort 2 were different from those in cohort 1 in their treatment history and the miRNA panel. However, this difference might indicate better generalizability of our findings given their consistency despite these variations. Among the miRNAs showing a significant correlation with disability, several correlations remained significant after correcting for multiple comparisons. In contrast, none of the correlations with MRI remained significant following this correction. These limitations temper our conclusions and suggest the need for further validation of our findings in future studies. Some of the miRNAs that significantly correlated with disease severity measures in the present study have also been related to MS or experimental autoimmune encephalomyelitis (Table 3). However, these correlations do not necessarily represent causation, although the evidence described above suggests such a relationship. Circulating miRNAs could be a byproduct of routine microvesicle secretion and cell death or be actively secreted to contribute to intracellular communication.[90]

Conclusions

Further studies in larger MS cohorts, other neurodegenerative diseases, and healthy individuals serving as controls may indicate whether the miRNAs identified in this study are exclusive to MS or generalized. A study with longitudinal design, with serum samples drawn at disease onset, would allow the evaluation of the ability of miRNA expression to determine subsequent disease evolution.

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Key Points

Question

Are serum microRNAs associated with brain and spinal cord magnetic resonance imaging involvement in multiple sclerosis?

Findings

In this cross-sectional cohort study of 120 patients with multiple sclerosis, microRNA magnetic resonance imaging associations were both pathogenic and protective. A topographic specificity differed for the brain vs the spinal cord, and the microRNA signature differed between magnetic resonance imaging T2 lesions and atrophy measures.

Meaning

Serum microRNAs may serve as multiple sclerosis biomarkers for monitoring disease progression and act as surrogate markers to identify distinct underlying disease processes, such as inflammation vs tissue destruction.

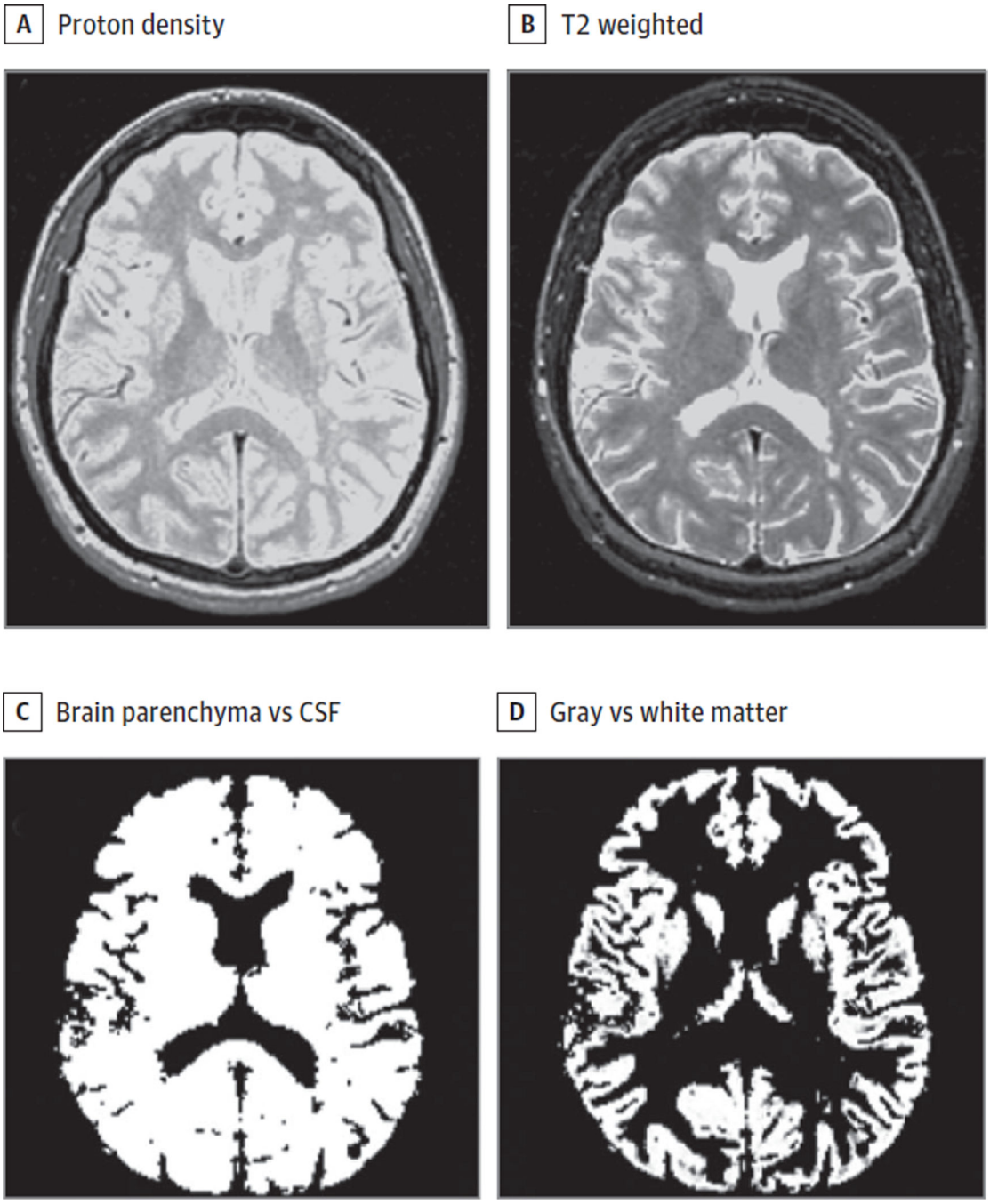

Figure 1. Magnetic Resonance Imaging (MRI) Segmentation Procedure to Derive Brain Volume (Atrophy) Measures in Cohort 1

Native axial dual-echo proton density (A) and T2-weighted (B) images were applied to a fully automated statistical parametric mapping pipeline (version 12), resulting in an intracranial volume segmented into brain vs cerebrospinal fluid (CSF) compartments used to derive brain parenchymal fraction (C) and gray vs white matter maps (D) used to derive global cerebral gray matter fraction. A patient with relapsing-remitting multiple sclerosis from cohort 1 is shown.

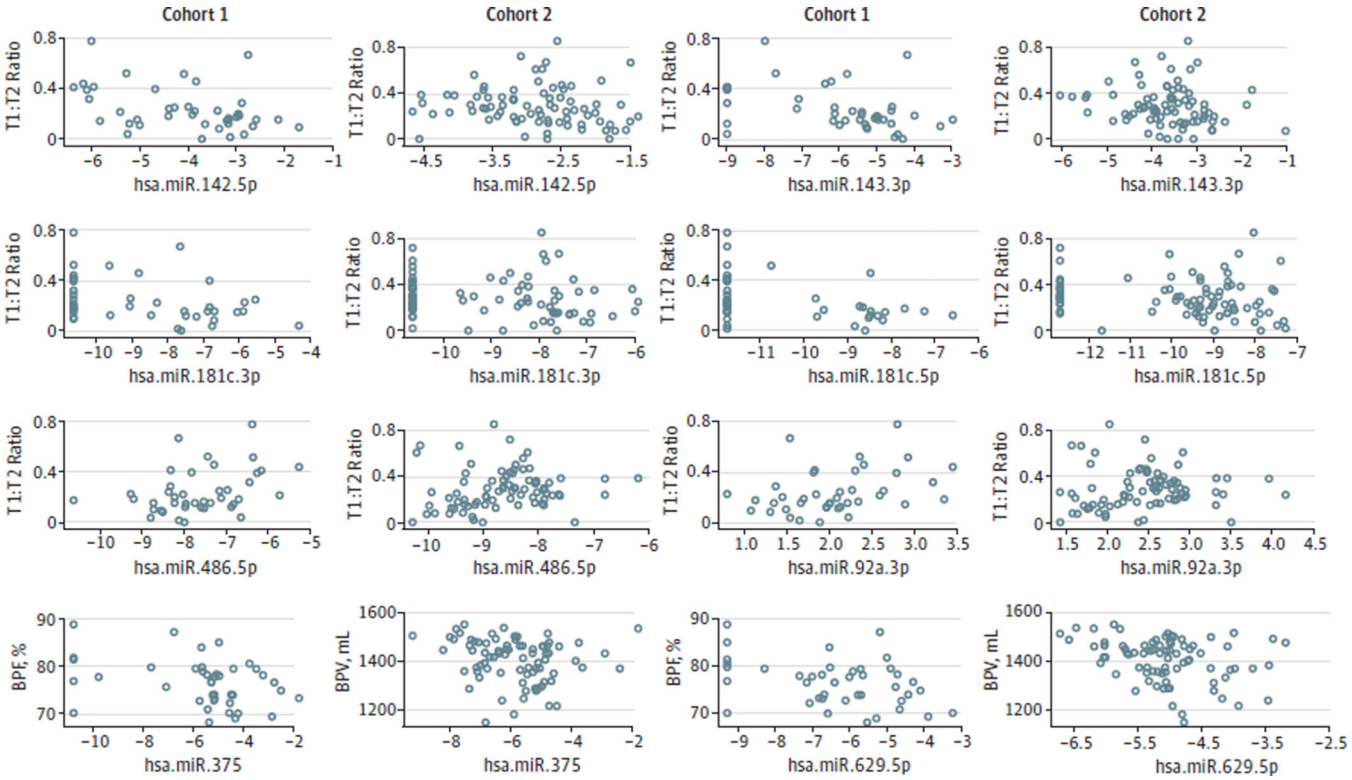

Figure 2. MicroRNA (miRNA) Correlation With Magnetic Resonance Imaging (MRI) Measures of Disease Severity

miRNAs showed significant correlations with MRI measures of disease severity in both cohort 1 and cohort 2. BPF indicates whole-brain parenchymal fraction; BPV, whole-brain parenchymal volume; and T1:T2, ratio of T1 hypointense to T2 hyperintense lesion volume.

Table 1

Cohort 1: Highest Correlations Between miRNAs and Clinical/MRI Data

miRNA	Spearman Correlation Coefficient[a]	Protective/ Pathogenic miRNA	*P* Value	
			Uncorrected	Corrected[b]
Brain T2LV				
hsa.miR.628.3p	0.506	Pathogenic	.001	.32
hsa.miR.195.5p	0.464	Pathogenic	.002	.48
hsa.miR.92b.3p	0.412	Pathogenic	.007	.80
hsa.miR.145.5p	0.407	Pathogenic	.009	.80
hsa.miR.32.3p	−0.378	Protective	.02	.80
hsa.miR.296.5p	−0.369	Protective	.02	.80
hsa.miR.511	0.367	Pathogenic	.02	.80
hsa.miR.501.5p	−0.361	Protective	.02	.80
hsa.miR.627	0.359	Pathogenic	.02	.80
hsa.miR.139.3p	0.356	Pathogenic	.02	.80
Brain T1:T2 Ratio				
hsa.miR.331.3p	−0.490	Protective	.001	.27
hsa.miR.338.3p	−0.476	Protective	.002	.27
hsa.miR.433	−0.471	Protective	.002	.27
hsa.miR.143.3p	−0.452	Protective	.003	.32
hsa.miR.181c.5p	−0.433	Protective	.005	.34
hsa.miR.183.5p	0.432	Pathogenic	.005	.34
hsa.miR.142.5p	−0.424	Protective	.006	.36
hsa.miR.365a.3p	−0.416	Protective	.007	.36
hsa.miR.92a.3p	0.392	Pathogenic	.01	.50
hsa.miR.337.3p	−0.390	Protective	.01	.50
BPF				
hsa.miR.101.3p	0.493	Protective	.001	.52
hsa.miR.324.3p	0.411	Protective	.008	.63
hsa.miR.19a.3p	0.389	Protective	.01	.63
hsa.miR.21.5p	0.388	Protective	.01	.63
hsa.miR.548l	−0.384	Pathogenic	.01	.63
hsa.miR.211.5p	−0.378	Pathogenic	.02	.63
hsa.miR.590.5p	0.377	Protective	.02	.63
hsa.miR.99b.3p	−0.374	Pathogenic	.02	.63
hsa.miR.32.5p	0.374	Protective	.02	.63
hsa.miR.660.5p	0.371	Protective	.02	.63
Whole-brain GMF				
hsa.miR.101.3p	0.547	Protective	<.001	.11

miRNA	Spearman Correlation Coefficient[a]	Protective/ Pathogenic miRNA	*P* Value	
			Uncorrected	Corrected[b]
hsa.miR.324.3p	0.494	Protective	.001	.20
hsa.miR.379.3p	−0.480	Pathogenic	.001	.20
hsa.miR.340.3p	−0.469	Pathogenic	.002	.20
hsa.let.7a.5p	−0.466	Pathogenic	.002	.20
hsa.miR.19a.3p	0.430	Protective	.005	.32
hsa.miR.20a.5p	0.423	Protective	.006	.32
hsa.let.7e.5p	−0.422	Pathogenic	.006	.32
hsa.miR.15b.3p	0.420	Protective	.007	.32
hsa.miR.106b.5p	0.410	Protective	.008	.35
EDSS				
hsa.miR.19a.3p	−0.627	Protective	<.001	.005
hsa.miR.101.3p	−0.608	Protective	<.001	.005
hsa.miR.30e.5p	−0.569	Protective	<.001	.01
hsa.miR.19b.3p	−0.562	Protective	<.001	.01
hsa.miR.29c.3p	−0.553	Protective	<.001	.02
hsa.miR.32.5p	−0.520	Protective	<.001	.04
hsa.miR.195.5p	0.503	Pathogenic	.001	.049
hsa.miR.142.5p	−0.450	Protective	.003	.14
hsa.miR.27a.3p	−0.443	Protective	.004	.14
hsa.miR.136.3p	−0.440	Protective	.004	.14

Abbreviations: BPF, brain parenchymal fraction; EDSS, Expanded Disability Status Scale; GMF, gray matter fraction; miRNA, microRNA; MRI, magnetic resonance imaging; T1:T2, ratio of T1 hypointense to T2 hyperintense lesion volume; T2LV, T2 hyperintense lesion volume.

[a]Estimated Spearman correlation coefficients are shown: the sign of the Spearman correlation indicates the direction of association between the miRNA expression level and the MRI measure of disease activity; its value reflects the strength of the association. In the interpretation of the meaning of the correlation coefficients, MRI-indicated severity was reflected in 2 possible directions: (1) for lesion severity (T2LV, T1:T2 ratio), a higher number represented increased disease severity, and (2) for atrophy measures (BPF, GMF), a higher number represented lower disease severity (less atrophy). The miRNA-MRI associations were observed in either direction: (1) a pathogenic relationship shown by a higher miRNA expression was associated with greater severity of MRI-indicated involvement, and (2) a protective relationship shown by a higher miRNA expression was associated with lower severity of MRI-indicated involvement.

[b]Corrected for multiple comparisons using false discovery rate.

Table 2

Cohort 2: Highest Correlations Between miRNAs and Clinical/MRI Data

miRNA	Spearman Correlation Coefficient[a]	Protective/Pathogenic miRNA	*P* Value	
			Uncorrected	Corrected[b]
Brain T2LV				
hsa.miR.210.3p	0.359	Pathogenic	.001	.50
hsa.miR.362.5p	−0.333	Protective	.003	.50
hsa.miR.92a.1.5p	0.327	Pathogenic	.003	.50
hsa.miR.1914.3p	0.324	Pathogenic	.004	.50
hsa.miR.330.5p	−0.305	Protective	.006	.66
hsa.miR.577	0.296	Pathogenic	.008	.66
hsa.miR.572	−0.292	Protective	.009	.66
hsa.miR.154.3p	0.281	Pathogenic	.01	.66
hsa.miR.30b.5p	−0.279	Protective	.01	.66
hsa.miR.671.5p	−0.274	Protective	.02	.66
Brain T1:T2 Ratio				
hsa.miR.548a.3p	−0.365	Protective	<.001	.18
hsa.miR.515.5p	−0.348	Protective	.002	.18
hsa.miR.27b.3p	−0.348	Protective	.002	.18
hsa.miR.223.3p	−0.345	Protective	.002	.18
hsa.miR.574.3p	−0.344	Protective	.002	.18
hsa.miR.23b.3p	−0.342	Protective	.002	.18
hsa.miR.23a.3p	−0.337	Protective	.002	.18
hsa.miR.197.3p	−0.332	Protective	.003	.18
hsa.miR.25.5p	0.323	Pathogenic	.003	.18
hsa.miR.1271.5p	−0.317	Protective	.005	.25
BPV				
hsa.miR.484	−0.392	Pathogenic	<.001	.19
hsa.miR.627.5p	0.335	Protective	.003	.41
hsa.miR.671.5p	0.330	Protective	.003	.41
hsa.miR.320b	−0.323	Pathogenic	.004	.41
hsa.miR.362.5p	0.322	Protective	.004	.41
hsa.miR.30a.3p	−0.317	Pathogenic	.004	.40
hsa.miR.548d.5p	0.300	Protective	.007	.48
hsa.miR.486.5p	−0.288	Pathogenic	.01	.48
hsa.miR.132.5p	0.287	Protective	.01	.48
hsa.miR.135a.5p	0.281	Protective	.01	.48
cGMV				
hsa.miR.484	−0.386	Pathogenic	<.001	.24

miRNA	Spearman Correlation Coefficient[a]	Protective/Pathogenic miRNA	*P* Value	
			Uncorrected	Corrected[b]
hsa.miR.610	−0.329	Pathogenic	.003	.58
hsa.miR.627.5p	0.312	Protective	.005	.58
hsa.miR.340.5p	0.306	Protective	.006	.58
hsa.miR.188.5p	−0.302	Pathogenic	.007	.58
hsa.miR.7.5p	−0.301	Pathogenic	.007	.58
hsa.miR.934	−0.285	Pathogenic	.01	.58
hsa.miR.556.5p	−0.279	Pathogenic	.01	.58
hsa.miR.615.3p	0.277	Protective	.01	.58
hsa.miR.25.3p	−0.277	Pathogenic	.01	.58
Spinal T2LV				
hsa.miR.132.5p	−0.349	Protective	.002	.88
hsa.miR.548j.5p	0.325	Pathogenic	.003	.88
hsa.miR.937.3p	0.305	Pathogenic	.006	.88
hsa.miR.342.5p	0.285	Pathogenic	.01	.88
hsa.miR.433.3p	0.273	Pathogenic	.02	.88
hsa.miR.150.5p	0.263	Pathogenic	.02	.88
hsa.miR.155.5p	0.263	Pathogenic	.02	.88
hsa.miR.10a.5p	0.261	Pathogenic	.02	.88
hsa.miR.202.5p	−0.254	Protective	.02	.88
hsa.miR.943	0.244	Pathogenic	.03	.88
Normalized UCCA				
hsa.miR.130b.3p	−0.350	Pathogenic	.002	.56
hsa.miR.135a.5p	0.337	Protective	.002	.56
hsa.miR.148b.5p	0.329	Protective	.003	.56
hsa.miR.374a.5p	0.272	Protective	.02	.86
hsa.miR.101.3p	0.261	Protective	.02	.86
hsa.miR.1538	−0.249	Pathogenic	.03	.86
hsa.miR.1468.5p	−0.247	Pathogenic	.03	.86
hsa.miR.1247.5p	0.245	Protective	.03	.86
hsa.miR.190a.5p	0.245	Protective	.03	.86
hsa.miR.30a.3p	−0.242	Pathogenic	.03	.86
EDSS				
hsa.miR.199a.5p	−0.380	Protective	<.001	.23
hsa.miR.25.5p	0.350	Pathogenic	.002	.23
hsa.miR.551b.3p	−0.350	Protective	.002	.23
hsa.miR.496	−0.349	Protective	.002	.23
hsa.miR.301a.3p	−0.323	Protective	.004	.37
hsa.miR.181c.3p	−0.320	Protective	.004	.37

miRNA	Spearman Correlation Coefficient[a]	Protective/Pathogenic miRNA	*P* Value	
			Uncorrected	Corrected[b]
hsa.miR.301b	−0.308	Protective	.006	.37
hsa.miR.136.3p	−0.305	Protective	.006	.37
hsa.miR.15a.5p	0.298	Pathogenic	.008	.37
hsa.let.7b.5p	0.294	Pathogenic	.009	.37

Abbreviations: BPV, whole-brain parenchymal volume; cGMV, cortical gray matter volume; EDSS, Expanded Disability Status Scale; miRNA, microRNAs; MRI, magnetic resonance imaging; T1:T2, ratio of T1 hypointense to T2 hyperintense lesion volume; T2LV, T2 hyperintense lesion volume; UCCA, upper cervical spinal cord area.

[a]Estimated Spearman correlation coefficients are shown: the sign of the Spearman correlation indicates the direction of association between the miRNA expression level and the MRI measure of disease activity; its value reflects the strength of the association. In the interpretation of the meaning of the correlation coefficients, MRI-indicated severity was reflected in 2 possible directions: (1) for lesion severity (T2LV, T1:T2 ratio), a higher number represented increased disease severity, and (2) for atrophy measures (BPV, cGMV, and UCCA), ahigher number represented lower disease severity (less atrophy). The miRNA-MRI associations were observed in either direction: (1) a pathogenic relationship shown by a higher miRNA expression was associated with greater severity of MRI-indicated involvement, and (2) a protective relationship shown by a higher miRNA expression was associated with lower severity of MRI-indicated involvement.

[b]Corrected for multiple comparisons using false discovery rate.

Table 3

miRNAs Showing Significant Correlations With MRI Measures in Both Cohort1 and Cohort2

miRNA	Description	Relevant Pathways Involved[a]	Cell Types/Tissues Expressing	Relevant Findings in Previous Publications
hsa.miR.142.5p	Negative correlation with T1:T2 ratio (protective)	Axonal guidance signaling, IL-6 signaling, B-cell activating factor signaling	PBMCs, neurons	Upregulated in both PBMCs and brain white matter lesions from patients with MS and mouse model; normalizes with BMT and GA treatment; in patient's serum, negative correlation with EDSS[49]
hsa.miR.143.3p	Negative correlation with T1:T2 ratio (protective)	NF-κB signaling pathway, T-cell receptor signaling pathway, leukocyte transendothelial migration	CD4 T cells, macrophages, dendritic cells, neurons	Markedly decreased in serum of patients with AD vs age-matched controls[50]
hsa.miR.181c.3p	Negative correlation with T1:T2 ratio (protective)	Axonal guidance signaling, TGF-β signaling	B cells, retinal cells, and in brain	Upregulated in MS CSF vs other neurologic diseases, upregulated in RR MS vs SP MS[28]
hsa.miR.181c.5p	Negative correlation with T1:T2 ratio (protective)	TGF-β signaling pathway, neurotrophin signaling pathway, T-cell receptor signaling pathway	Brain	Released from the brain following ischemia and associated with neurogenesis[51]
hsa.miR.375	Negative correlation with BPF (pathogenic)	No known targets	Brain, spinal cord	CSF levels lower in patients with AD vs age- and sex-matched HCs[52]
hsa.miR.486.5p	Positive correlation with T1:T2 ratio (pathogenic)	Integrin signaling, leukocyte extravasation signaling	Muscle	Correlates with EDSS; upregulated in patients with MS vs HCs, patients with progressive MS, those with other neurologic diseases, and those with other autoimmune diseases[53]
hsa.miR.629.5p	Negative correlation with BPF (pathogenic)	IL-10 signaling, axonal guidance signaling	Whole blood	Lower expression found in blood samples from patients with MS treated with natalizumab and developed PML vs patients who did not develop PML[54]
hsa.miR.92a.3p	Positive correlation with T1:T2 ratio (pathologic)	B-cell receptor signaling, chemokine signaling, neuregulin signaling	Brain, B cells	Its cluster, miR17-92, is overexpressed in B cells and plasma of patients with MS vs controls[34,55]

Abbreviations: AD, Alzheimer disease; BMT, bone marrow transplantation; BPF, brain parenchymal fraction; CSF, cerebrospinal fluid; EDSS, Expanded Disability Status Scale; GA, glatiramer acetate; HC, healthy controls; IL, interleukin; miRNA, microRNA; MRI, magnetic resonance imaging; MS, multiple sclerosis; NF-κB, nuclear factor κB; PBMCs, peripheral blood mononuclear cells; PML, progressive multifocal leukoencephalopathy; RR, relapsing-remitting; SP, secondary progressive; T1:T2, ratio of T1 hypointense to T2 hyperintense lesion volume; TGF-β, transforming growth factor β.

[a]Ingenuity pathway analysis.