Comprehensive evaluation of serum microRNAs as biomarkers in multiple sclerosis

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ABSTRACT

Objective: To identify circulating microRNAs (miRNAs) linked to disease stage and disability in multiple sclerosis (MS).

Methods: Sera from 296 participants including patients with MS, other neurologic diseases (Alzheimer disease and amyotrophic lateral sclerosis), and inflammatory diseases (rheumatoid arthritis and asthma) and healthy controls (HCs) were tested. miRNA profiles were determined using LNA (locked nucleic acid)-based quantitative PCR. Patients with MS were categorized according to disease stage and disability. In the discovery phase, 652 miRNAs were measured in sera from 26 patients with MS and 20 HCs. Following this, significant miRNAs ($p < 0.05$) from the discovery set were validated using quantitative PCR in 58 patients with MS, 30 HCs, and in 74 samples from other disease controls (Alzheimer disease, amyotrophic lateral sclerosis, asthma, and rheumatoid arthritis).

Results: We validated 7 miRNAs that differentiate patients with MS from HCs ($p < 0.05$ in both the discovery and validation phase); miR-320a upregulation was the most significantly changing serum miRNA in patients with MS. We also identified 2 miRNAs linked to disease progression, with miR-27a-3p being the most significant. Ten miRNAs correlated with the Expanded Disability Status Scale of which miR.199a.5p had the strongest correlation with disability. Of the 15 unique miRNAs we identified in the different group comparisons, 12 have previously been reported to be associated with MS but not in serum.

Conclusions: Our findings identify circulating serum miRNAs as potential biomarkers to diagnose and monitor disease status in MS.

Classification of evidence: This study provides Class III evidence that circulating serum miRNAs can be used as biomarker for MS. *Neurol Neuroimmunol Neuroinflamm* 2016;3:e267; doi: 10.1212/NXI.0000000000000267

GLOSSARY

AD = Alzheimer disease; **ALS** = amyotrophic lateral sclerosis; **AUC** = area under the receiver operating characteristic curve; **CLIMB** = Comprehensive Longitudinal Investigation of Multiple Sclerosis at Brigham and Women's Hospital; **EDSS** = Expanded Disability Status Scale; **FDR** = false discovery rate; **HC** = healthy control; **KEGG** = Kyoto Encyclopedia of Genes and Genomes; **LNA** = locked nucleic acid; **miRNA** = microRNA; **MS** = multiple sclerosis; **PPMS** = primary progressive multiple sclerosis; **RA** = rheumatoid arthritis; **RRMS** = relapsing-remitting multiple sclerosis; **SPMS** = secondary progressive multiple sclerosis.

Multiple sclerosis (MS) is considered an autoimmune demyelinating disease of the CNS. Currently, MRI is the most frequently used biomarker to diagnose and monitor MS. It is imperative to identify blood biomarkers in MS that can aid in disease diagnosis, identify disease stage, and provide a link to disability accumulation.

microRNAs (miRNAs) are small noncoding RNAs that regulate the expression of genes at the posttranscriptional level by binding to complementary sequences in the 3′ or 5′ UTR (untranslated

region) of the target messenger RNA.[1–4] Previous studies[5–17] have investigated miRNA expression in the CNS, in immune cell populations in MS, and in experimental autoimmune encephalomyelitis (reviewed in references 18–22).

In addition to cells, miRNAs have been detected in several body fluids[23] where they are highly stable as they are resistant to circulating ribonucleases.[24] Circulating miRNAs are stable following extended storage, freeze–thawing, and extreme pH.[24,25] Their stability, along with the development of sensitive methods for their detection and quantification,[20] makes circulating miRNAs as ideal candidates for biomarkers. Investigators have identified changes in circulating miRNAs in a number of disease processes (reviewed in references 26–28) and we previously reported changes in circulating plasma miRNAs in patients with MS.[29] In the present study, we investigated serum miRNAs in MS as part of the NIH common fund UH2 initiative. We found that several serum miRNAs are differentially expressed in MS, associate with disease stage, and correlate with disability.

METHODS The aim of this study was to identify circulating miRNAs that are linked to disease, disease stage, and disability in MS. We performed a comparison of MS to healthy controls (HCs), MS compared to other diseases, relapsing-remitting compared to progressive patients, and correlation with the Expanded Disability Status Scale (EDSS). This study provides Class III evidence that circulating serum miRNAs accurately identify patients with MS.

Participants. Samples from patients with MS were obtained from the CLIMB Study (Comprehensive Longitudinal Investigation of Multiple Sclerosis at Brigham and Women's Hospital). CLIMB is an ongoing longitudinal cohort study that follows more than 2,000 patients with clinical examinations, MRI, and blood sampling done on a yearly basis. Patients are diagnosed with MS as defined by the 2010 revisions to the McDonald criteria.[30]

Patients with secondary progressive MS (SPMS) were defined as patients with MS who had a relapsing onset, evidence of disease progression defined as an increase of at least 1.5 points on the EDSS for patients with baseline EDSS score of 0, an increase of at least 1 point on the EDSS for patients with initial EDSS score between 1 and 5, or an increase of 0.5 point for patients with an initial EDSS score of ≥5.5. EDSS increase was subsequently maintained or increased for at least 180 days. Patients with primary progressive MS (PPMS) were classified as such according to 2010 revisions to the McDonald criteria definitions.[30]

Patients with MS selected for this study had not received treatment with steroids in the past month; glatiramer acetate, interferon beta, fingolimod, dimethyl fumarate, or teriflunomide in the past 3 months; or other disease-modifying therapies in the past 6 months including cyclophosphamide, rituximab, daclizumab, methotrexate, and natalizumab. HCs were obtained from the Brigham PhenoGenetic Cohort study and from healthy participants enrolled in the CLIMB Study. For disease controls, amyotrophic lateral sclerosis (ALS) samples were obtained from James Berry (Massachusetts General Hospital), Alzheimer disease (AD) samples from David Bennett (Rush, Chicago), asthma samples from Scott Weiss (Channing Laboratory, Boston),[31] and rheumatoid arthritis (RA) samples from Brigham and Women's Hospital biorepository.

Standard protocol approvals and consent forms. A signed informed consent was received from all of the participants in the various studies. Secondary use approval was obtained from the institutional review board for other disease samples (IRB 2013P002181/BWH).

Figure 1   Flowchart
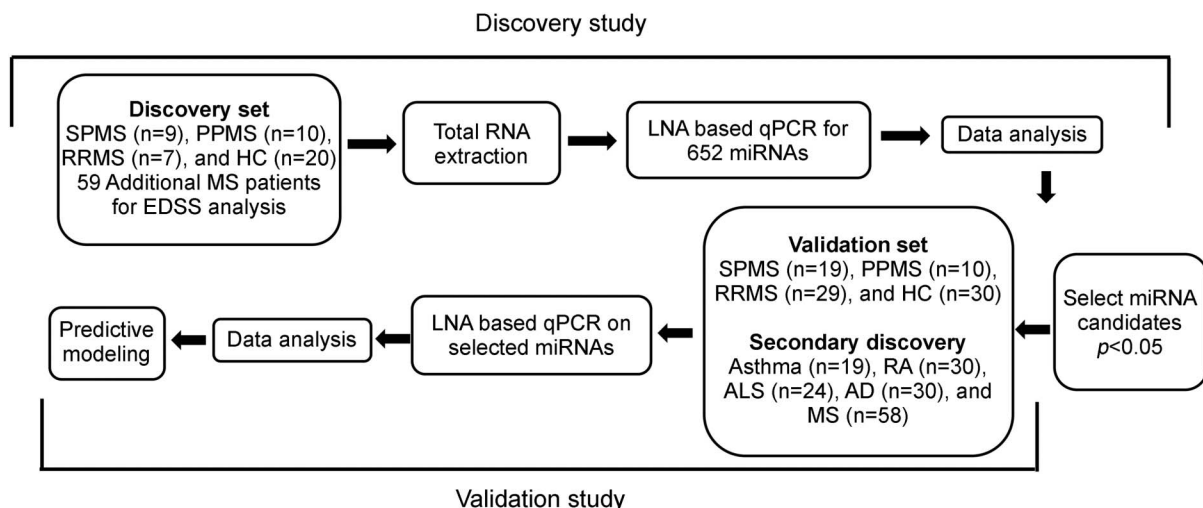
Flowchart showing the study design. AD = Alzheimer disease; ALS = amyotrophic lateral sclerosis; EDSS = Expanded Disability Status Scale; HC = healthy control; LNA = locked nucleic acid; miRNA = microRNA; MS = multiple sclerosis; PPMS = primary progressive multiple sclerosis; qPCR = quantitative PCR; RA = rheumatoid arthritis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

Study design. The study involved 2 phases: a discovery and a validation phase (figure 1). In the discovery phase, 652 miRNAs were measured in participants from 4 groups: relapsing-remitting multiple sclerosis (RRMS) (n = 7), SPMS (n = 9), PPMS (n = 10), and HC (n = 20). The demographic characteristics of these groups are shown in table 1. For diagnostic biomarkers, we compared all patients with MS to HCs, patients with relapsing MS to inflammatory disease controls (asthma and RA), and patients with progressive MS to neurologic diseases (ALS, AD). For disease stage biomarkers, the patients with progressive MS (SPMS and PPMS) were compared to the patients with RRMS. In addition, patients with SPMS were directly compared to patients with RRMS. For EDSS disability correlations, all patients with MS contributed to the analysis; in addition, 59 untreated patients with RRMS contributed to EDSS disability correlations. The miRNAs were rank ordered for each of the biomarker categories (diagnostic, disease stage, and disability).

Based on the rank order from the discovery phase, a subset of the miRNAs was measured in the larger validation set of participants from the 4 groups: RRMS (n = 29), SPMS (n = 19), PPMS (n = 10), and HC (n = 30). The demographic characteristics of the 4 groups in the validation set are shown in table 1. The diagnostic biomarker miRNAs identified in the discovery phase were tested on the other disease controls: asthma (n = 19), RA (n = 30), ALS (n = 24), and AD (n = 30). The demographic characteristics of the other disease controls are also shown in table 1.

Samples and methods. Blood samples were collected in glass red-top serum vacutainer tubes without additives (BD, Franklin Lakes, NJ); serum tubes were kept at room temperature for 30 to 60 minutes. Each sample was centrifuged at 2,000 rpm for 10 minutes to separate serum and then stored at −70°C until RNA extraction. Serum was frozen within 2 hours of the blood draw. RNA was isolated using the miRcury kit (Exiqon, Woburn, MA) and converted to complementary DNA using a synthesis kit from Exiqon following the manufacturer's instructions. Prepared complementary DNAs were stored at −20°C until use. Locked nucleic acid (LNA) SYBR green–based real-time PCR Human Panel I and II (Exiqon) containing 652 miRNAs were used for profiling in the discovery phase. Normalization was performed using the mean expression of the miRNAs with the best stability index using NormFinder. We used 10 normalizing miRNAs in the discovery phase (let.7d.3p, miR.103a.3p, miR.106a.5p, miR.126.3p, miR.15b.5p, miR.19a.3, miR.20a.5p, miR.30b.5p, miR.425.5p, and miR.92a.3p) and 4 normalizing miRNAs in the validation phase (miR-15b-5p, miR-19a-3p, miR-126-3p, and miR-425-5p). Of note, all normalizers used in the validation phase were also used in the discovery phase. The formula used to calculate the normalized Cq values is: Normalized Cq = average Cq − assay Cq.

To identify pathways that might be regulated by the miRNAs that were differentially expressed between the groups compared, we used the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.[32]

Table 1	Demographics of patients and HCs				
	Discovery				
	Multiple sclerosis				
	RRMS	**SPMS**	**PPMS**	**EDSS cohort**	**HC**
No. of participants	7	9	10	59	20
Mean age (SD), y	50 (6.37)	50 (6.43)	57 (7.46)	40 (9.46)	33 (9.2)
Females, %	71	67	50	80	70
Mean EDSS score (SD)	0.5 (0.8)	5.8 (1.42)	5.1 (2.31)	1.6 (1.3)	—
Mean disease duration (SD), y	8.7 (1.49)	17.0 (7.72)	16.6 (5.16)	8.8 (6.38)	—
	Validation				
	RRMS	**SPMS**		**PPMS**	**HC**
No. of participants	29	19		10	30
Mean age (SD), y	36 (7.6)	46 (6.9)		47 (5.5)	43 (12)
Females, %	66	68		50	70
Mean EDSS score (SD)	1.1 (0.93)	6.1 (1.2)		5.3 (2.1)	—
Mean disease duration (SD), y	3.4 (3.03)	15.2 (4.8)		10.87 (5.93)	—
	Other diseases				
	Asthma	**AD**		**ALS**	**RA**
No. of participants	19	30		24	30
Mean age (SD), y	23 (1.33)	74 (3.53)		56 (9.4)	56 (10.3)
Females, %	42	70		33	80

Abbreviations: AD = Alzheimer disease; ALS = amyotrophic lateral sclerosis; EDSS = Expanded Disability Status Scale; HC = healthy control; PPMS = primary progressive multiple sclerosis; RA = rheumatoid arthritis; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

Statistical analysis. For diagnostic biomarkers, patients with MS and HCs in the discovery set were compared for each miRNA using a Wilcoxon rank sum test. A Wilcoxon rank sum test was used so that participants with miRNA levels below the limit of detection (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. All miRNAs were rank ordered based on the p value. To select miRNAs for the validation phase, we chose up to 40 miRNAs based on the p value with the requirement that the miRNA was expressed by a least 50% of the participants in at least one of the groups and the p value was less than 0.05. If more than 40 miRNAs were significantly differentially expressed between the groups compared, only the top-ranked 40 were selected. In the validation phase, each miRNA identified based on the discovery phase was compared between the patients with MS and HCs using the same approach as in the discovery phase. The receiver operating characteristic curve is a graphical approach for investigating the sensitivity and specificity at all possible cutoff values for a predictor, and the area under the receiver operating characteristic curve (AUC) provides an estimate of the miRNA's ability to discriminate the groups compared.[33] The associated p value and AUCs were calculated for each miRNA. To account for the multiple miRNAs investigated in the validation phase, p values from the validation phase analyses were also adjusted for multiple comparisons using the false discovery rate (FDR).[34] We also compared the miRNA expression level between the groups using a proportional odds model to adjust for age and sex. The proportional odds model is a generalization of the Wilcoxon rank sum test that allows adjustment for other variables.[35] In addition to the separate analysis of each miRNA, we combined all miRNAs that showed a significant association with a validation group after correction using the FDR to fit a multivariate logistic regression model.

For the disease stage biomarkers, the same set of analyses as for the diagnostic biomarkers was performed except that patients with RRMS were compared to patients with SPMS/PPMS. Furthermore, given the particular interest in comparison of RRMS and SPMS, these 2 groups were also compared. For the disability biomarkers, the same approach was followed except that Spearman correlation coefficient was used to estimate the association between each miRNA and the EDSS score. An miRNA was defined as significantly differentially expressed in the validation phase if it was expressed by a least 50% of the participants in at least one of the groups, the Wilcoxon test p value/Spearman correlation p value was less than 0.05, and the same direction of expression (up- or downregulated) was observed in both phases.

Statistical analysis was completed using the statistical packages R (www.r-project.org) and Stata/IC version 14 (www.stata.com).

RESULTS Diagnostic biomarkers. In the discovery phase, we identified 167 miRNAs that were differentially expressed between patients with MS and HCs (table e-1 at Neurology.org/nn). After filtering using the selection criteria, 40 miRNAs were chosen for further validation. In the validation phase, we found 7 miRNAs of the 40 selected from the discovery phase that were significantly differentially expressed in MS compared to HCs. Among these 7 miRNAs, 6 were identified as significantly different after correcting for multiple comparisons using FDR (table 2). The results remain unchanged on adjustment for age and sex. From this analysis, we found that miR.320a provided the best AUC (0.707) discriminating patients with MS from HCs (table 2, figure 2, A and D). When all 6 miRNAs that remained significant after correcting for multiple comparisons were included in a multivariable logistic regression model, the AUC for the combined model was 0.795 (figure 2A).

To further validate the specificity of this miRNA signature for MS, the 40 miRNAs identified from the discovery set were compared between patients with MS and patients with other neurologic diseases (ALS and AD) as well as patients with other inflammatory diseases (asthma and RA) (table e-2). Of the 7 miRNAs validated as differentially expressed between MS and HCs, 4 miRNAs were also differentially expressed when comparing MS to other neurologic diseases (miR.320a, miR.486.5p, miR.320b, and miR.25.3p). In addition, 2 common miRNAs (miR.486.5p and miR.25.3p) were significantly

Table 2   Validated serum miRNA differentially expressed in MS vs HCs

miRNA	Validation								Discovery, Wilcoxon p value
	No. expressed		Mean expression		Wilcoxon p value	Adjusted p value[a]	FDR value	AUC	
	MS	HC	MS	HC					
hsa.miR.320a	58	30	0.65	0.20	<0.01	<0.01	0.03	0.71	<0.01
hsa.miR.486.5p	58	30	1.53	0.89	<0.01	<0.01	0.03	0.69	<0.01
hsa.miR.320b	58	30	−1.26	−1.68	<0.01	0.01	0.03	0.69	<0.01
hsa.miR.25.3p	58	30	0.99	0.57	<0.01	<0.01	0.03	0.69	<0.01
hsa.let.7c.5p	58	30	−4.06	−3.69	0.01	<0.01	0.03	0.68	<0.01
hsa.miR.140.3p	58	30	−0.33	−0.62	0.01	<0.01	0.05	0.67	<0.01
hsa.miR.365a.3p	58	30	−4.58	−4.20	0.05	0.05	0.24	0.63	<0.01

Abbreviations: AUC = area under the receiver operating characteristic curve; FDR = false discovery rate; HC = healthy control; miRNA = microRNA; MS = multiple sclerosis.
[a] Adjusted p value from proportional odds model adjusting for age and sex.
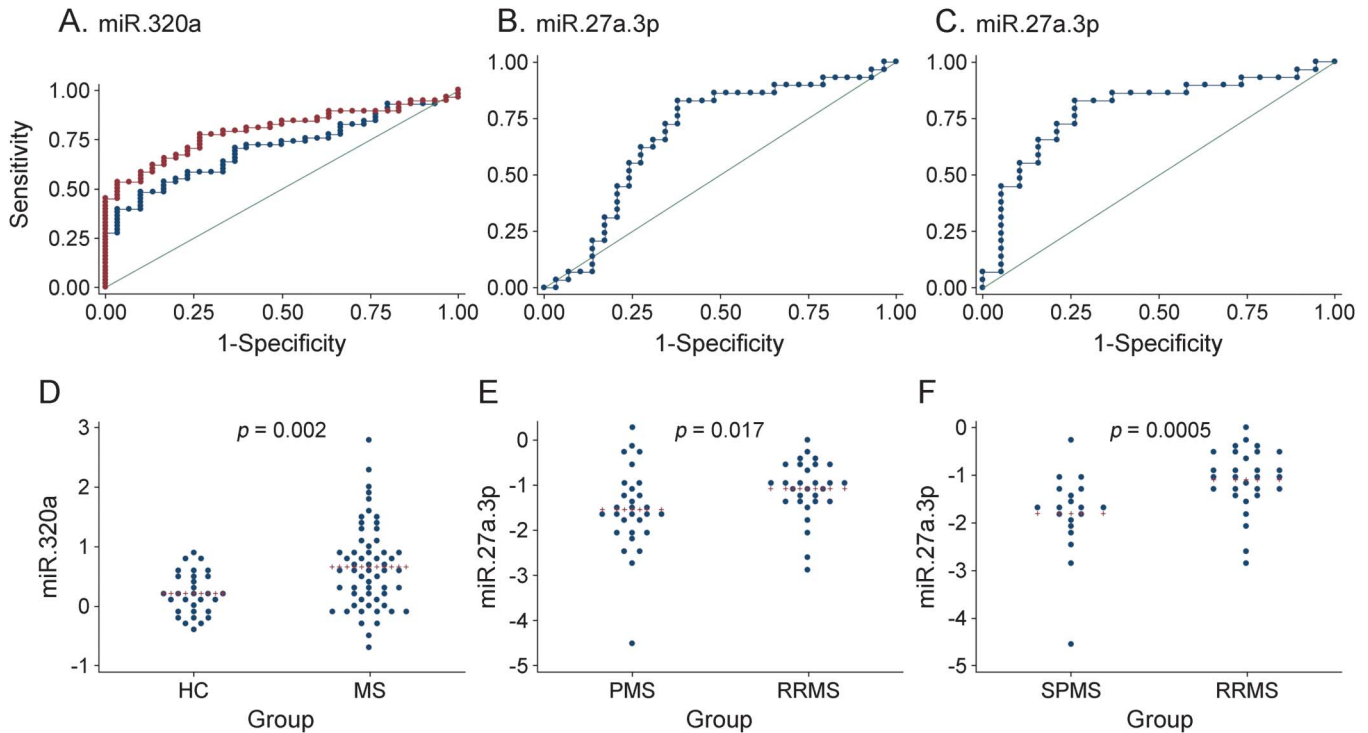
A) ROC curve for healthy controls vs. MS patients: Blue: ROC curve for best single miRNA, miR.320a AUC = 0.707. Red: ROC curve for combination of six miRNAs (hsa.miR.320a + hsa.miR.486.5p + hsa.miR.320b + hsa.miR.25.3p + hsa.let.7c.5p + hsa.miR.140.3p), AUC = 0.795. (B) ROC curve for patients with RRMS vs patients with PMS. Blue: ROC curve for hsa.miR.27a.3p. AUC = 0.68. (C) ROC curve for patients with RRMS vs patients with SPMS. Blue: ROC curve for hsa.miR.27a.3p. AUC = 0.78. (D) Scatterplot for the expression of miR.320a in HCs vs patients with MS. (E) Scatterplot for the expression of miR.27a.3p in patients with PMS vs RRMS. (F) Scatterplot for the expression of miR.27a.3p in patients with SPMS vs RRMS. AUC = area under the receiver operating characteristic curve; HC = healthy control; miRNA = microRNA; MS = multiple sclerosis; PMS = progressive multiple sclerosis; ROC = receiver operating characteristic; RRMS = relapsing-remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

different in both MS vs HC and MS vs other inflammatory disease comparisons. After adjusting for age and sex, the results remain unchanged.

To further assess whether the miRNAs are specific for the neurodegenerative or inflammatory aspects of the disease, we restricted our comparisons so that relapsing patients were compared to patients with other inflammatory diseases and progressive patients were compared to patients with other neurologic diseases. Of the 40 miRNAs chosen in the discovery phase for the comparison of HCs and MS, 15 showed a statistically significant difference comparing RRMS and other inflammatory diseases, and all of these remained significant after correcting for multiple comparisons (table e-3). For the comparison of progressive MS and other neurologic diseases, 16 showed a significant difference, and 9 remained significant after correcting for multiple group comparison (table e-4).

Disease category biomarkers. To identify disease category biomarkers, relapsing patients were compared to progressive patients (SPMS and PPMS) in both the discovery and validation phases. In the discovery phase, we identified 21 miRNAs that were differentially expressed between the groups (table e-5). miR.27a.3p was the only miRNA validated that differentiated progressive patients from relapsing patients; the AUC for miR.27a.3p was 0.68 (figure 2, B and E). The results were similar after adjustment for age and sex.

Given the potential difference between SPMS and PPMS, we also compared RRMS to SPMS only. In the discovery phase, we identified 27 miRNAs that were differentially expressed between groups (table e-6). In the validation phase, miR.27a.3p and miR.376b.3p were significantly differentially expressed in RRMS compared to SPMS in the same direction in the discovery and validation phases. miR.27a.3p provided the best AUC (0.78), and only this miRNA remained significant after correcting for multiple comparisons (figure 2, C and F). The results were similar after adjustment for age and sex.

In the comparison of RRMS to PPMS, none of the miRNAs selected from the discovery phase was validated in the validation phase.

Disability biomarkers. We investigated the association between miRNAs and disability as measured by the EDSS. In the discovery phase, using all untreated patients with MS (plus 59 additional patients with RRMS), 103 miRNAs were significantly associated

with EDSS score (table e-7), and 40 of these miRNAs were selected for further validation. In the validation phase, 10 miRNAs were significantly associated with EDSS score (table 3). Nine miRNAs remained significantly correlated to EDSS after adjusting for age (table 3). Two (miR.199a.5p and miR.142.5p) of these associations remained statistically significant after correcting for multiple comparisons using FDR. The best correlation with the EDSS was observed with miR.199a.5p (figure e-1), and the estimated Spearman correlation coefficient in the validation set was −0.435 with a corresponding *p* value of 0.0006.

DISCUSSION We found that circulating serum miRNAs are differentially expressed in MS vs HCs and in RRMS vs SPMS. Furthermore, we identified specific miRNAs that are linked to disability accumulation. The miRNAs were identified in a discovery set and were then validated in a larger independent cohort. We found that miRNAs that are associated with progression are also associated with disability. The strength of our study lies in the 2 independent cohort design, the comparison to other diseases, and the sample of untreated patients to minimize potential treatment effect that confounds most biomarker studies. We used the LNA-based quantitative PCR platform from Exiqon, which was shown to have the highest specificity in a recent study comparing 12 different miRNA expression platforms for serum.[36]

KEGG Pathway Analysis showed that some significant, differentially expressed miRNAs target important immune functions and the maintenance of neuronal homeostasis. For example, miR.27a.3p, the strongest miRNA that distinguishes RRMS from SPMS and RRMS from PMS (upregulated in the relapsing form as compared to the progressive form) shows a strong link to both the neurotrophin signaling pathway and the T cell receptor signaling pathway. Other studies have shown that miR.27a.3p targets multiple proteins of intracellular signaling networks that regulate the activity of nuclear factor κB and mitogen activated protein kinases (MAPKs).[37] As a consequence, miR-27a inhibits differentiation of Th1 and Th17 cells and promotes the accumulation of Tr1 and Treg cells.[37] It has also been shown that miR.27.3p is upregulated in MS active brain lesions and that the level of miR.27a.3p in CSF is reduced in patients with dementia due to AD.[38]

Of all the miRNAs, miR.486.5p was identified in the largest number of comparisons. It correlates with EDSS; it is upregulated in MS compared to HC, to other neurologic diseases, as well as compared to other inflammatory diseases. This particular miRNA was found to be associated with transforming growth factor-β signaling pathways and is a known tumor suppressive miRNA.[39]

We found that miR.320a is upregulated in MS when compared to HCs and other neurologic diseases. miR.320a has been previously described to be highly expressed in B cells of patients with MS and was suggested to contribute to increased blood–brain barrier permeability due to regulation of matrix metallopeptidase-9.[40] Pathway analysis links this miRNA to the cell-to-cell adhesion pathways and also thus it may be linked to blood–brain barrier permeability.

In the context of other studies that have evaluated the role of serum miRNAs as biomarkers in MS, ours is the most comprehensive with the largest sample size that uses 2 independent cohort designs. We have previously reported circulating plasma miRNAs as potential biomarkers in MS. Although miR.140.3p and the let.7c.5p were found to be dysregulated and validated in both studies, it is known that there is limited overlap between serum and plasma miRNAs, which may relate in part to miRNAs released during coagulation.[41]

Table 3	Validated serum miRNA correlated with disability in patients with multiple sclerosis							
	Validation					**Discovery**		
miRNA	**No. expressed**	**Spearman estimate**	***p* Value**	**FDR**	**Age-adjusted *p* value**	**No. expressed**	**Spearman estimate**	***p* Value**
hsa.miR.199a.5p	58	−0.44	<0.01	0.03	<0.01	72	−0.33	<0.01
hsa.miR.142.5p	58	−0.41	<0.01	0.03	<0.01	85	−0.39	<0.01
hsa.miR.877.5p	41	0.34	<0.01	0.11	<0.01	75	0.34	<0.01
hsa.miR.25.3p	58	0.33	0.01	0.11	0.01	85	0.33	<0.01
hsa.miR.486.5p	58	0.32	0.02	0.13	0.01	85	0.37	<0.01
hsa.miR.337.3p	46	−0.30	0.02	0.14	<0.01	61	−0.34	<0.01
hsa.miR.199a.3p	58	−0.29	0.03	0.17	0.07	72	−0.35	<0.01
hsa.miR.320b	58	0.28	0.03	0.17	0.02	85	0.28	0.01
hsa.miR.142.3p	58	−0.27	0.04	0.17	<0.01	85	−0.35	<0.01
hsa.miR.376b.3p	48	−0.27	0.04	0.17	0.03	61	−0.36	<0.01

Abbreviations: FDR = false discovery rate; miRNA = microRNA.

In addition to the analysis of the separate miRNAs, the logistic regression model including all validated miRNAs showed an increase in the AUC, which indicates a combination of miRNAs might improve predictive accuracy relative to single miRNAs. Although a model including validated miRNAs is one potential model, it is important to note that alternative model building strategies including more than the validated miRNAs as candidates could lead to improved predictive accuracy. These approaches including the LASSO (least absolute shrinkage and selection operator) are the focus of current research in our group.[42]

One limitation of our study is that participant samples were collected from a single MS center. Further external validation of our results will require investigating samples from patients at other centers. We are currently performing such multicenter studies, which may also increase the power of our results. A second limitation of our study is the relatively small number of participants who contributed to each group comparison. Future work will require larger sample sizes to ensure that we have sufficient power to detect miRNAs with smaller effect sizes. In addition, because of the overlap of a single miRNA expression among the groups compared as shown in figure 2, it may be that it will require more than a single miRNA to serve as a diagnostic marker for MS or MS subgroups.

Although miRNAs have been studied in cells and the CNS of patients with MS, ours is the first comprehensive investigation of serum miRNAs. Circulating miRNAs offer a sophisticated immunologic assessment of disease that can be easily measured in serum. It is known that MS is a heterogeneous disease and a major unmet need is a biomarker that can serve as the basis for the care of the individual patient with MS over time. Currently, physicians must treat patients in an empirical manner with little basis for choosing one drug over another and no clear rationale for changing or combining therapy. Although MRI provides guidance in this regard, it cannot be used frequently and provides no information about the biology of the disease. Although additional investigation is required, our study suggested that circulating miRNAs could serve as biomarkers to diagnose MS, monitor disease status, and provide a link to degree of disability accumulated.

AUTHOR CONTRIBUTIONS

Keren Regev, MD: drafting the manuscript for content, study concept and design, analysis and interpretation of data, and acquisition of data. Anu Paul, PhD: analysis or interpretation of data, acquisition of data, and revising the manuscript for content. Brian Healy, PhD: drafting the manuscript for content, study concept, statistical analysis, and interpretation of data. Felipe von Glenn, MD, PhD: acquisition of data and revising the manuscript for content. Camilo Diaz-Cruz, MD: acquisition of data and revising the manuscript for content. Taha Gholipour, MD: acquisition of data. Maria Antonietta Mazzola, MD: revising the manuscript for content. Radhika Raheja, PhD: revising the manuscript for content. Parham Nejad: acquisition of data. Bonnie I. Glanz, PhD: design and patient selection. Pia Kivisakk, MD, PhD: design, patient selection, and collection. Tanuja Chitnis, MD: design, patient selection, and collection. Howard L. Weiner, MD: revising the manuscript for content, study concept and design, study supervision and coordination, and obtaining funding. Roopali Gandhi, PhD: drafting and revising the manuscript for content, study concept and design, analysis and interpretation of data, study supervision and coordination, and obtaining funding.

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