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Differences in the Differential Expression of MicroRNAs Between Patients with Familial Multiple Sclerosis and Those with Sporadic Multiple Sclerosis

Ailesel Multipl Skleroz ve Sporadik Multipl Skleroz Hastaları Arasında MikroRNA'ların Diferansiyel Ekspresyon Farklılıkları

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Abstract

Objective: Multiple sclerosis (MS) is a heterogeneous disease with clinical and immunological features. Most MS cases occur sporadically, but a considerable proportion of patients have a family history of MS. The etiology and pathophysiology of MS remain unclear. Recent epidemiological and gene expression studies have indicated that dysregulation of microRNAs (miRNAs) may play a role in MS pathogenesis. This study aimed to evaluate the differential expression of miRNAs in sporadic MS (sMS) and familial MS (FMS) patients.

Materials and Methods: This cross-section, single-center study was conducted in 20 FMS and 10 sMS patients and 8 healthy controls. The patients were in the remission. In total, 2,549 miRNA genes were screened in the blood mononuclear cells from the whole blood samples of MS patients depending on miRBase 21. Differential expression of miRNAs in MS patients was identified compared with the control group, and miRNAs with a fold change ≥2 were validated using reverse transcription-polymerase chain reaction. Differentially expressed miRNAs were then compared between FMS and sMS patients.

Results: Initial findings showed that miR-5100 and hsa-miR-16-2-3p were increased and miR-432-3p was decreased in FMS compared with sMS, whereas miR-548-aa, hsa-miR-142-3p, and miR-451-b were increased in both sMS and FMS, but miR-548-v was increased only in sMS. Some miRNAs showed the same expression patterns in both groups.

Conclusion: Differential expression of certain miRNAs may be a useful biomarker in the diagnosis of MS. This study showed that miRNAs may discriminate between FMS and sMS cases and MS subtypes, as indicated in earlier studies.

Keywords: Multiple sclerosis, familial MS, sporadic MS, miRNA expression

Öz

Amaç: Multipl skleroz (MS), klinik ve immünolojik özellikler açısından heterojen bir hastalıktır. MS olgularının çoğu sporadik meydana gelir, ancak hastaların önemli bir kısmında ailede MS öyküsü vardır. MS hastalığının etiyolojisi ve patofizyolojisi hala net değildir. Son epidemiyolojik ve gen ekspresyonu çalışmaları, mikroRNA'ların (miRNA'lar) disregülasyonunun MS'in patogenezinde rol oynayabileceğini göstermektedir. Bu çalışmanın amacı, hem sporadik MS (sMS) hem de ailesel MS (FMS) hastalarında miRNA'ların diferansiyel ekspresyonlarını değerlendirmektir.

Gereç ve Yöntemler: Bu kesitsel tek merkezli çalışma 20 FMS ve 10 sMS hastası ve 8 sağlıklı kontrolle gerçekleştirilmiştir. Hastaların remisyon döneminde olma şartı aranmıştır. Toplamda 2.549 miRNA geni, MS hastalarının tam kan örneklerinden izole edilen

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mononükleer kan hücrelerinde miRbase 21'e dayalı olarak taranmıştır. MS hastalarında miRNA'lerin diferansiyel ekspresyonları kontrol grubuyla karşılaştırılarak belirlenmiş ve ≥2 kat değişim olan miRNA'ların RT-PCR ile validasyonu yapılmıştır. Daha sonra, diferansiyel eksprese olan miRNA'lar FMS ve sMS hastaları arasında karşılaştırılmıştır.

Bulgular: İlk bulgular, sMS'ye kıyasla FMS'de miR-5100 ve hsa-miR-16-2-3p ekspresyonlarının arttığını ve miR-432-3p ekspresyonun azaldığını gösterirken, miR-548-aa, hsa-miR-142-3p ve miR-451-b'nin ekspresyonunun hem sMS hem de FMS'de arttığını, ancak miR-548-v'nin ekspresyonunun yalnızca sMS'de arttığını göstermiştir. Bazı miRNA'lar her iki grupta da aynı ekspresyon paternini göstermiştir.

Sonuç: Belli miRNA'ların diferansiyel ekspresyonları MS teşhisinde yararlı bir biyobelirteç olabilir. Bu çalışma, miRNA'ların FMS ve sMS olguları arasında olduğu kadar daha önceki çalışmalarda belirtildiği gibi MS alt tipleri arasında da ayırt edici olabileceğini göstermiştir.

Anahtar Kelimeler: Multipl skleroz, ailevi MS, sporadik MS, miRNA ekspresyonu

Introduction

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS) with a life-time risk of 1/400 and affecting primarily young females (1).

MS has four subtypes first defined in 1996 by the US National Multiple Sclerosis Society Advisory Committee on Clinical Trials in MS and was then revised in 2013 (2): relapsing remitting MS (RRMS), primary progressive MS (PPMS), secondary progressive MS (SPMS), and progressive relapsing MS. The majority of MS cases presents with RRMS and most of RRMS cases occur sporadically. However, a considerable proportion of cases have family history. In a meta-analysis, the prevalence of familial MS (FMS) was reported as 12.6% with monozygotic twins having the highest risk as 27%. The incidence of developing MS for the first-degree relatives ranges from 2% to 5% (3).

Although MS was discovered more than a century ago, the etiology and pathophysiology of the disease remain unclear. The most significant genetic association was found with human leukocyte antigen (HLA) region. The incidence of MS is higher in patients with HLA-DR2 (DR1501) serotype (4). Nevertheless, recent epidemiological and gene expression studies indicated that dysregulation of microRNAs (miRNAs) may also have a role in MS pathogenesis (5).

miRNAs are small, single-stranded, non-coding RNA molecules consisting approximately of 22 nucleotides (range, 19-25 nucleotides) that mediate mRNA translation repression or mRNA degradation. They control the expression of more than 2/3 of protein-coding genes in mammals and play a role in various biological process in the immune system and in neuroinflammation. They function as immune regulators by repressing target genes at the posttranscriptional level, which is essential for immune homeostasis and preventing autoimmune diseases (6).

Aberrant miRNA expression is responsible for the pathogenesis of various diseases such as neurodegeneration, autoimmunity, and cancer (7). MS is also associated with aberrant miRNA expressions. Up-regulated or down-regulated miRNAs have been reported in MS patients compared to healthy controls (8-11).

Identifying the miRNA expressions specific to MS subtypes help clinicians in establishing diagnosis and treatment planning. It is important to identify whether FMS differs from sporadic MS (sMS) regarding demographic, clinical, genetic, and radiological characteristics; accordingly, FMS cases can be identified and other family members prone to MS are diagnosed early. This study aimed to evaluate differential miRNA expressions in the peripheral blood mononuclear cells (PBMC) of FMS patients comparing with sMS patients.

Materials and Methods

This cross-sectional, single-center study included 32 MS patients, who visited Neurology Department of Dokuz Eylül University Medical Faculty between December 2014 and November 2016. The control group included eight healthy individuals from the hospital staff. The patients were required to be in remission period and analyzed in two groups: patients with FMS (n=20) and patients with sMS (n=12). Patients who had comorbidities such as demyelinating diseases other than MS and cancer were excluded. The study was approved by the Non-interventional Clinical Research Ethics Committee of Dokuz Eylül University (approval no: 2014/34-21, date: 06.11.2014) and Scientific Research and Publishing Ethics Committee of Dokuz Eylül University (decision no: 1, date: 08.11.2022) and conducted in accordance with the Helsinki Declaration as revised in 2013. Informed consents of the participants were obtained.

The participants were investigated in detail regarding demographic (age, sex), clinical (MS sub-type, disease duration), laboratory [oligoclonal band (OCB), cerebrospinal-fluid (CSF)-serum total protein, albumin, immunoglobulin G (IgG) index], and radiological characteristics [cranial and spinal magnetic resonance imaging (MRI)]. Lesions on MRI were evaluated separately as cortical-subcortical, corpus callosum, periventricular, cerebellar, mesencephalon, pons, and bulbus on axial, coronal and sagittal sections, T2 (spin-spin/transverse relaxation time)/Flair, T1 (spin-lattice/longitudinal relaxation time) and Gd (gadolinium) series.

RNAs were isolated from PBMC in blood samples of MS patients taken into tubes containing EDTA for routine

blood analysis. RNA concentrations and their purity were determined by spectrophotometric method. Thereafter, the mRNA samples were stored at -80 ºC until the time of analysis.

Differential miRNA expressions (up-regulated and down-regulated) were first identified by microarray analysis. For this purpose, totally 2,549 miRNA genes in the MiRBase 21 (http://www.mirbase.org/) were screened and then miRNAs with a fold change ≥2 were validated using real-time polymerase chain reaction (PCR) after reverse transcription of miRNAs to complementary DNA (cDNA).

The cDNAs were first diluted by 80x (5 ul cDNA +395 ul water) and housekeeping gene and cDNA were checked using spike-in primers. SNORD48 and U6 were used as housekeeping genes. The mixture containing Snord48 and U6 primers were used to evaluate the expression of miRNAs in RT-PCR. The expression of miRNAs were then normalized to the SNORD48 and U6 housekeeping genes. Regarding CT values for SNORD48 and spike-in outcomes of the cDNAs, those with a CT value between 15 and 29 (CT15-29) were analyzed for miRNAs using miRNA LNA™ primer sets (EXIQON). Relative quantitation of the outcomes was performed and calculated using Δ/Δ CT approach (12).

Statistical Analysis

Data were analyzed using the IBM SPSS Statistics for Windows, Version 22.0. (IBM Corp., Armonk, NY, USA). Kolmogorov-Smirnov test was used to test the parametric test assumptions. The Mann-Whitney U test and chi-square test were used to determine the differences of miRNA expression between FMS and sMS patients. A p-value <0.05 was considered statistically significant.

Results

Of 32 MS patients included, 20 had FMS (mean age 37.6±14.2 years, 16 females) and 12 had sMS (mean age, 35.5±9.3 years, 6 females). Patients' demographic and clinical characteristics are demonstrated in Table 1. The FMS and sMS groups were comparable for age (p>0.05). The mean disease duration was 95.8±106.1 months and 134.9±113.1 months in the patients with sMS and FMS, respectively, with no significant difference between the groups (p>0.05). All patients in the FMS group were first-degree relatives from 10 families (three couples of mother-daughter, three couples of sister-sister, three couples of sister-brother, and a couple of father-daughter). Patients in both groups were in remission period. RRMS was the most common type of MS in both groups (100% in sMS and 80% in FMS group). There was no significant difference between the groups regarding subtypes of MS (p>0.05). The mean expanded disability status scale (EDSS) score was higher in FMS cases than in sMS cases (2.9±0.4 vs. 1.6±0.2), but did not reach the level of statistical significance (p>0.05). The OCB was positive in 88.9% and 72.7% of the FMS and sMS groups, respectively (p>0.05).

Table 1. Demographic and clinical characteristics of the patients with sMS and FMS

	Patients with		p-value
Demographic and clinical features	**sMS**	**FMS**	
Age, year, mean ± SD	35.5±9.3	37.6±14.2	>0.05
Disease duration, month, mean ± SD	95.8±106.1	134.9±113.1	>0.05
Clinical phenotype, n (%)			>0.05
CIS	0 (0)	1 (5)	
RRMS	12 (100)	16 (80)	
SPMS	0 (0)	3 (15)	
EDSS score, mean ± SD	1.6±0.2	2.9±0.4	>0.05
Laboratory findings			
OCB, n (%)	8 (72.7)	16 (88.9)	>0.05
IgG index	1.1±0.6	0.6±0.3	>0.05
MRI findings, n (%)			
Pons T2 lesion	4 (40)	3 (16.7)	**0.023**
Cervical T2 lesion	8 (80)	9 (52.9)	**0.017**
Upper thoracic T2 lesion	4 (40)	2 (12.5)	**0.038**
Lower thoracic T2 lesion	6 (60)	5 (31.3)	**0.019**

sMS: Sporadic multiple sclerosis, FMS: Familial multiple sclerosis, SD: Standard deviation, CIS: Clinically isolated syndrome, RRMS: Relapsing remitting multiple sclerosis, SPMS: Secondary progressive multiple sclerosis, EDSS: Expanded Disability Status Scale, OCB: Oligoclonal band, IgG: Immunoglobulin G, MRI: Magnetic resonance imaging, T2: Spin-spin/transverse relaxation time

With regard to the MRI findings, sMS cases had significantly more lesions in the pons (p=0.023), cervical spinal cord (p=0.017), and thoracic spinal cord (p=0.038 for upper thoracic and p=0.019 for lower thoracic).

Up-regulated miRNAs

Analysis of miRNA expression revealed that 12 miRNAs were up-regulated only in the FMS cases and 65 miRNAs were up-regulated only in the sMS cases (Table 2). Genetic analysis revealed that miR-5100 was significantly up-regulated in the FMS cases comparing with the sMS cases. While hsa-miR-16-2-3p was up-regulated only in FMS cases, miR-548-U was up-regulated only in sMS cases. There were 53 miRNAs up-regulated in both FMS and sMS groups (Table 3). Gene analysis revealed up-regulated miR-451-b, miR-142-3p and miR-548aa in both groups.

Table 2. Up-regulated miRNAs only in the patients with FMS and only in the patients with sMS

FMS		sMS					
miRNA	Fold increment	miRNA	Fold increment	miRNA	Fold increment	miRNA	Fold increment
hsa-miR-5100	2.18, 3.2	hsa-miR-6500-5p	2.96	hsa-let-7f-1-3p	2.34, 2.5, 2.42	hsa-miR-629-3p	2.13
hsa-miR-4633-5p	2.04	hsa-miR-466	8.49, 8.53, 8.07, 6.37	hsa-miR-4290	3.22, 3.59	hsa-let-7b-3p	2, 2.01
hsa-miR-125a-3p	2.08	hsa-miR-3190-5p	2.65	hsa-miR-574-5p	3.24, 2.49	hsa-miR-3656	2.19, 2.18
hsa-miR-486-3p	2.4	hsa-miR-4713-5p	2.82	hsa-miR-933	2.43, 2.58	hsa-miR-381-3p	2.33
hsa-miR-30e-5p	2.09	hsa-miR-5704	2.55, 2.36	hsa-miR-300	4.06	hsa-miR-485-3p	2,76, 2.11
hsa-miR-125 a-3p	2.14, 2.08	hsa-miR-654-3p	2.39	hsa-miR-2278	3.79	Has-miR-4646-3p	2.19
hsa-miR-4286	2.55	hsa-miR-4725-5p	2.61, 2.65	hsa-miR-6072	3.29	hsa-miR-3160-5p	2.19
hsa-miR-335-3p	2.01	hsa-miR-3935	3.52, 2.66	hsa-miR-328-3p	2.09	hsa-miR-640	2.5
hsa-miR-139-3p	3.25	hsa-miR-551a	2.36	hsa-miR-1273c	2.39	hsa-miR-483-3p	2.13
hsa-miR-3907	2.33	hsa-miR-764	2.26	hsa-miR-5194	2.21, 2.49	hsa-miR-299-5p	2.46, 2.51
hsa-miR-6126	2.27	hsa-miR-6127	2.07, 2,06	hsa-miR-3940-5p	2.48	hsa-miR-595	2.63
hsa-miR-16-2-3p	4.86	hsa-miR-3191-5p	2.14	hsa-miR-3665	2.17	hsa-miR-3646	2.65, 2.69
		hsa-miR-449c-3p	2.24, 3.48, 2.98	hsa-miR-3150b-5p	2.52	hsa-miR-3940-3p	2.49, 2.78
		hsa-miR-1275	2.43	hsa-miR-766-3p	2.1	hsa-miR-605-5p	2.46, 2.05
		hsa-miR-4281	2.01	hsa-miR-4493	2.6, 2.28	hsa-miR-320a	2.3
		hsa-miR-5195-3p	-	hsa-miR-5787	2.6, 2.88	hsa-miR-3162-3p	2.62, 2.2
		hsa-miR-135a-3p	5.06	hsa-miR-328-3p	2.09	hsa-miR-574-3p	2.31, 2.59
		hsa-miR-1273c	2.39	hsa-miR-4728-3p	2.29	hsa-miR-6508-5p	2.56
		hsa-miR-4254	3.77, 3.92	hsa-miR-5001-5p	2.25	hsa-miR-4312	2.34
		hsa-miR-1255b-2-3p	3.22, 2.35	hsa-miR-3927-5p	2.14	hsa-miR-609	2.02
		hsa-miR-149-5p	2.62, 2.72	hsa-miR-4649-3p	2.01, 2.37	hsa-miR-584v	4.59
		hsa-miR-647	4.13, 4.17, 4.26, 3.04	hsa-miR-4695-3p	2.1, 2.31	-	

miRNA: MicroRNA, FMS: Familial multiple sclerosis, sMS: Sporadic multiple sclerosis

Down-regulated miRNAs

There were 10 miRNAs down-regulated only in FMS patients and 19 miRNAs down-regulated only in sMS patients (Table 4). MiRNA analysis revealed that 14 miRNAs were down-regulated both in FMS and sMS cases (Table 5). Gene analysis demonstrated that miR-432-3p was down-regulated in FMS vs. sMS cases.

Discussion

In the present study, genetic analysis revealed that miR-5100 was significantly up-regulated but miR-432-3p was significantly down-regulated in FMS cases comparing with sMS cases, suggesting that these two miRNAs can be used as biomarkers in discriminating between FMS and sMS. Additionally, hsa-miR-16-2-3p was up-regulated only in FMS, whereas miR 548-U was up-regulated only in sMS. However, miR-451-b, miR-548aa and hsa-miR-142-3p were up-regulated in both groups.

MS is a chronic neurodegenerative disease of the CNS and develops due to the combination of genetic and environmental factors. Although not being a hereditary disorder, it can be seen among family members with FMS accounting for

Table 3. Up-regulated miRNAs both in the patients with FMS and sMS

miRNA	Fold increment		miRNA	Fold increment	
	FMS	sMS		FMS	sMS
hsa-miR-3688-3p	10.42	2.37; 9.96	hsa-miR-33b-3p	2.51	2.87, 4.25
hsa-miR-371b-5p	9.41, 8.11	11.25, 12.7	hsa-miR-449b-3p	2.45, 2.2	5.38, 4.92
miRNABrightCorner30	4.62	5.62	hsa-miR-6085	2.34; 2.92	2.95, 2.56
hsa-miR-135b-5p	4.57, 3.44	5.27; 7.47	hsa-miR-1238-5p	2.03	4.14, 3.29
hsa-miR-491-3p	6.65, 6.24, 6.24; 6.97	12.12, 11.13, 13.07, 10.86	hsa-miR-197-3p	2.09, 2.14	4.14, 4.16
hsa-miR-4428	4.08	3.94	hsa-miR-4455	2.2; 2.42	4.77, 4.92
hsa-miR-3129-3p	3.36	3.34, 2.82	hsa-miR-491-5p	3.54	8.64
hsa-miR-142-3p	7.17, 3.42	3	hsa-miR-494-3p	2.32	2.69
hsa-miR-4694-5p	4.27, 3.75	7.48, 6.26	hsa-miR-665	3.65	3.68
hsa-miR-423-5p	3.02	2.67	hsa-miR-4310	2.14	3.81
hsa-miR-4668-5p	5.57	4.58	hsa-miR-30d-5p	2.29, 2.19	2.12
hsa-let-7d-3p	3.7, 2.11	4.51, 2.78	hsa-miR-2116-3p	2.07	3.3, 2.35
hsa-miR-1260b	2.6	3.56, 3.59	hsa-miR-1296-5p	2.25, 2.11	4.47, 4.49
hsa-miR-4787-5p	3.05	2.31, 3.7	hsa-miR-4275	2.26	2.78
hsa-miR-1260a	3.15	3.41, 3.63	hsa-miR-1181	3.88	3.75
hsa-miR-6125	2.58, 2.55	3.61, 3.64	hsa-miR-4485-3p	3.73, 2.27	3.98
hsa-miR-4515	2.07, 2.27	2.37, 2.13	hsa-miR-1915-3p	2.48, 2.59	3.64, 3.46
hsa-miR-4446-5p	3.76, 4.05	8.48, 9.31	hsa-miR-211-3p	2.36, 2.38	2.22
hsa-miR-937-3p	2.64, 2.21, 2.04	4.18, 4.55, 4.44, 4.29	hsa-miR-3149	5.8	15.71
hsa-miR-6514-3p	3.92, 2.21, 2.2, 2.04, 2.64	4.44, 4.29, 4.18, 4.55	hsa-miR-487b-3p	2.86	6.04
hsa-miR-32-3p	4.71, 4.71, 3.37, 2.69	12.56, 10.12, 7.17, 8.68	hsa-miR-885-5p	2.74, 2.25	4.12, 5.17
hsa-miR-4465	3.74, 4.15	3.57, 3.29	hsa-miR-6504-3p	4.12	9.94
hsa-miR-2861	2.02, 2.16	2.87, 2.85	hsa-miR-3681-3p	2.86, 3.76	7.49, 9.99
hsa-miR-6499-3p	2.52, 2.57	5.31, 5.46	hsa-miR-548aa	3.17	5.84
hsa-miR-5011-5p	5.1	17.87	hsa-miR-136-5p	3.6	6.04
hsa-miR-4685-5p	3.53, 4.02	4.28, 4.93	hsa-miR-6514-3p	3.49, 3.92	9.61, 10.61
hsa-miR-5010-3p	2.45, 2.56	5.84, 5.87		-	-

miRNA: MicroRNA, FMS: Familial multiple sclerosis, sMS: Sporadic multiple sclerosis

12.5% of the MS cases (3). A biomarker that distinguishes MS from other demyelinating diseases is critically valuable. Biomarkers may shed light to the diagnosis of MS subtypes, prediction of disease period and course, treatment choice and success, as well as considering new therapies, and prediction of prognosis. Identification of FMS cases is also important to determine family members who are prone to developing MS. Detection of biomarkers associated with the natural history of MS including inflammation, demyelination, oxidative stress and axonal injury is of great importance. Gene expression profiling is a beneficial tool to provide information about molecular pathways involved in MS pathogenesis.

Table 4. Down-regulated miRNAs only in the patients with FMS and only in the patients with sMS

FMS		sMS	
miRNA	Fold decrement	miRNA	Fold decrement
hsa-miR-877-3p	-2.04	hsa-miR-133b	-2.77
hsa-miR-675-3p	-2.77	hsa-miR-23a-3p	-4.76
hsa-miR-320d	-2.32	hsa-let-7a-5p	-9.09
hsa-miR-432-3p	-2.5	hsa-miR-107	-6.66, -0.09
hsa-miR-320e	2.22	hsa-miR-5096	-2.08
hsa-miR-1180-3p	-2.32	hsa-miR-17-5p	-7.14
hsa-miR-4707-5p	-2.32	hsa-miR-185-5p	-4.76
hsa-miR-642b-3p	-2.08	hsa-miR-4646-5p	-2.04
hsa-miR-1236-5p	-2.43	hsa-miR-4639-3p	-2.43
hsa-miR-1224-5p	-2, -2.04	dmr_316	-3.33
		hsa-miR-521	-2.38
		hsa-miR-96-5p	-8.33
		hsa-miR-4650-5p	-2.38
		hsa-miR-211-5p	-2.38
		hsa-miR-342-3p	-8.33
		hsa-miR-566	-2.08
		hsa-miR-193b-3p	-3.03
		dmr_3	-4.34, -7.69
		hsa-miR-4307	-3.57

miRNA: MicroRNA, FMS: Familial multiple sclerosis; sMS: Sporadic multiple sclerosis

Table 5. Down-regulated miRNAs both in the patients with FMS and sMS

miRNA	Fold decrement		miRNA	Fold decrement	
	FMS	sMS		FMS	sMS
has-miR-623	-4.16, -2.56, -3.70	-4, -3.12, -2.70	has-miR-5196-5p	-4.76	-5.55
has-miR-181d-5p	-4, -2.77	-5, -3.44	has-miR-550a-3-5p	-3.57	-4
has-miR-4710	-2.5, -3.03	-2.38, -2.63	has-miR-4306	-2.63, -2.38	-3.03, -2.7
has-miR-331-3p	-3.7	-6.25	has-miR-660-5p	-5.88	-8.33
has-miR-4271	-2.08	-2.04	has-miR-4700-3p	-2.38	-2.85
has-miR-4632-5p	-3.125	-2.77	has-miR-3676-5p_v19.0	-2.85	-2.32
has-miR-4728-5p	-2.22	-3.03	has-miR-1343-3p	-4	-4.76

miRNA: MicroRNA, FMS: Familial multiple sclerosis; sMS: Sporadic multiple sclerosis

Studies have demonstrated that dysregulation of miRNAs play a role in MS pathogenesis (5). Circulating miRNAs are good candidates to be diagnostic biomarkers for MS as they stay stable for a long time and are non-invasive, cheap and time-saving for disease monitoring (13). Moreover, they can be used to assess disease severity, to monitor progression, and to assess therapeutic responses. Besides, unique miRNA signatures may allow discrimination between MS subtypes.

Most studies have focused on miRNA expression in MS patients compared with healthy controls. Otaegui et al. (8) demonstrated that miRNA expression is significantly different between RRMS patients and healthy controls. In a recent review, miR-18b, miR-493, miR-599, miR-145, miRNA-26a, miR-149-5pand miR-708-5p were up-regulated in PBMC of MS patients (9). Using PCR, Nuzziello et al. (10) found up-regulated miR-652-3p, miR-125a-5p, miR-185-5p, miR-320a, miR-942-5p, and miR-25-3p in MS patients vs. healthy controls. Another study have revealed decreased expression of miR-10 (p=0.0002), miR-21 (p=0.0014) and miR-124 (p=0.0091) in RRMS patients vs. healthy controls and concluded that miR-10, miR-124 and miR-21 are promising diagnostic tools for MS (11).

Recently, numerous studies have also been performed to distinguish MS subtypes. A study investigating miRNA expression in CSF of patients with different types of MS found dysregulated miR-20a-5p and miR-320b expression in serum samples of PPMS patients comparing with RRMS and other neurological diseases. Additionally, it was found that miR-26a-5p and miR-485-3p were down-regulated in PPMS vs. RRMS, whereas miR-142-5p was up-regulated in RRMS patients vs. patients with other neurological diseases. They also demonstrated that let-7b-5p and miR-143-3p were down-regulated in CSF samples of patients with PPMS vs. patients with other neurological diseases (14). In our study, we also found that miR-142-3p was up-regulated in both FMS and sMS cases. These findings suggest that FMS and/or sMS may be associated with different dysregulated miRNAs than in other types of MS.

Discrimination between FMS and sMS cases is also important because identification of miRNAs specific to FMS may provide early diagnosis of family members likely to develop MS via screening for signature miRNAs for familial cases before the onset of symptoms or, probably, occurrence of neurodegeneration.

In our study, of 173 dysregulated miRNAs identified, 12 were up-regulated in only FMS cases. Although not significant, miR-16-2-3p was up-regulated in only FMS cases, as was previously demonstrated by Keller et al. (15). The genetic analysis revealed that miR-5100 was increased only in FMS cases. Since miR-5100 was associated with various types of cancer (16,17), dysregulation of miR-5100 in MS needs to be investigated in further studies with larger patient populations.

Similar to the findings of the study by Gandhi et al. (7), in which increased miR-30e expression was shown in RRMS cases, we identified up-regulated miR-30e only in FMS cases, which was not significant in genetic analysis. Likewise, up-regulation of miR-125a only in FMS cases was not significant in the genetic analysis. Consistent with the results of our study, studies have reported increased miR-125a expression in MS patients (18).

It was determined that some miRNAs have been up-regulated only in sMS cases. Up-regulation of none of these miR-RNAs, except for miR-548-U, was found significant in the genetic analysis. Other miRNAs up-regulated only in sMS cases in our study included miR-584 and miR-1275, which were found to be up-regulated in the blood cells of RRMS patients in earlier studies (19,20). Additionally, Gandhi et al. (7) reported up-regulated miR-449b, let-7f, miR-574-3p, let-7b, let-7d and miR-135a in the plasma of RRMS patients, which is consistent with the findings of our study; however, they did not compare these miRNAs between FMS and sMS cases. Although the present study failed to find a significant difference, earlier studies have suggested that miR-135a and miR-574-3p can be considered potential biomarkers in distinguishing RRMS cases from both controls and SPMS cases; moreover, a correlation was demonstrated between these miRNAs and EDSS, disease duration and frequency of relapses (6), which were not analyzed in the present study.

Dysregulation of let-7d found in the present study was also confirmed by Piket et al. (13) in RRMS vs. SPMS cases. In our study, miR-629 and miR-328 were also up-regulated in sMS patients. Although up-regulation of miR-629 was confirmed in Chinese population (18), miR-328 was found to be down-regulated in the PBMC of MS patients as compared to healthy controls (21).

In the present study, we identified up-regulated let-7b-3p in sMS cases. However, a study found significant reduction in -5p form (let-7b-5p) in the CSF of patients with progressive MS vs RRMS. In the non-progressive phase, they demonstrated that let-7b-5p was inversely associated with inflammation; whereas, it was negatively correlated with clinical disability in progressive MS and the authors concluded that let-7b-5p can be used as a biomarker for disease course (22). Nevertheless, two other studies failed to demonstrate a difference in the expression of let-7b between MS patients and healthy controls (23).

In addition to these miRNAs up-regulated in either FMS or sMS cases, we also found that some miRNAs were up-regulated in both FMS and sMS cases (Table 3). Studies have shown that miR-142-3p was up-regulated in the PBMCs of MS patients (19-21). In the present study, we also identified significantly up-regulated miR-142-3p in both FMS and sMS cases. Likewise, miR-211-3p was up-regulated in both groups in the present study; however, different from the present study, Cox et al. (24) demonstrated down-regulation of -5p form of miR-142 (miR-142-5p) in all MS subtypes. Given that they also used microarray analysis,

this difference may be attributed to small sample size in the present study or to the ethnic differences between the cohorts, which warrants further analysis.

Keller et al. (19) detected up-regulated mir-491-5p in the PBMCs of MS patients. miR-32 and miR-197 were among the miRNAs up-regulated in both FMS and sMS cases in the present study. While up-regulation of miR-32 in MS was supported by Yang et al. (18), they detected significantly down-regulated miR-197 in the PBMCs of MS cases vs. healthy controls. We also identified significant up-regulation of miR-548aa in both groups, warranting further investigation about the role of miR548 in MS.

There were 14 miRNAs down-regulated in all RRMS cases (familial and sporadic) in the present study. Likewise, Cox et al. (24) found that miR-623 was down-regulated in all MS types. In the same study, miR-17 was also down-regulated in all MS cases, whereas we determined up-regulation of miR-17 in only sMS cases suggesting that this can be used as a marker to distinguish sporadic cases from familial cases. While we determined that miR-660 was down-regulated in both groups, a study reported up-regulation of miR-660 in the plasma of patients with RRMS (23). Although miR-211-3p was up-regulated in both groups in the present study, -5p form (miR-211-5p) was down-regulated in sMS cases. Likewise, down-regulated miR-211-5p in all subtypes of MS as compared to healthy controls was also reported (24).

Ebrahimkhani et al. (25) investigated exosomal miRNAs and reported differently expressed nine miRNAs including miR-342-3p and miR-432-5p in RRMS and in progressive MS; in the present study, however, -3p form (miR-342-3p) was down-regulated in sMS cases. Likewise, -3p form of miR-432 (miR-432-3p) was also significantly down-regulated in FMS cases, making it a candidate biomarker to discriminate between FMS and sMS cases.

We identified decreased levels of miR-96 in the sMS cases, which was shown to be involved in the remission phase of MS (8). Different from the present study, another study reported increased plasma levels of miR-96 in RRMS cases (7). In the present study, miR-1180 was also down-regulated in the FMS patients, whereas it was found to be increased in the PBMC of Chinese MS patients (18), which can be attributed to the ethnic differences. Moreover, we identified down-regulated miR-23a in sMS cases. Gandhi et al. (7) reported that miR-23a detected in body fluids can discriminate between MS subtypes and might be associated with certain parameters including EDSS score and disease duration. In the present study, however, we did not analyze the correlation between miRNAs and EDSS score and disease duration, which can be considered a limitation of the study.

Although it was reported in a study that miR-155 is the one of the most consistently dysregulated miRNA in MS (20), we failed to identify any in the present patient population. Differences between studies regarding differentially expressed miRNAs might be resulted from the differences in sample sizes and cohorts, and body fluids and methods used to detect miRNAs.

Our study has some limitations including small sample size and absence of correlation analysis between significantly different miRNA expressions and disease parameters such as EDSS score and disease duration. Nevertheless, this study is one of the limited studies investigating the differences between FMS and sMS regarding miRNA expression and contributes to the discrimination of FMS from sMS. Showing that miR-5100, miR-548aa and miR-548-U may be associated with MS pathogenesis and that miR-5100 and miR-432-3p are potential biomarkers to differentiate FMS from sMS makes the present study valuable.

Conclusion

In this study, both different and similar expressions of miRNAs were observed in familial and sporadic cases, some of which were supported by earlier studies. We showed that miR-5100 and hsa-miR-16-2-3p expressions were increased but miR-432-3p expression was decreased in FMS, whereas miR-548-v was increased in sMS. These data suggest that expression of certain miRNAs may be a useful biomarker for MS diagnosis and may discriminate between not only MS subtypes, as was indicated in earlier studies, but also between FMS and sMS cases.

The clinical relevance and significance of these miRNA genes identified in this study should be investigated in different clinical samples in further studies with larger patient cohorts.

Ethics

Ethics Committee Approval: The study was approved by the Non-interventional Clinical Research Ethics Committee of Dokuz Eylül University (approval no: 2014/34-21) and Scientific Research and Publishing Ethics Committee of Dokuz Eylül University (decision no: 1, date: 8.11.2022) and conducted in accordance with the Helsinki Declaration as revised in 2013.

Informed Consent: Informed consents of the participants were obtained.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Surgical and Medical Practices: H.G., H.A.U., T.P., Z.A., D.K., P.Ö., E.İ., Concept: H.G., H.A.U., T.P., Z.A., D.K., Design: H.G., H.A.U., T.P., Z.A., D.K., Data Collection or Processing: H.G., H.A.U., T.P., Z.A., D.K., Analysis or Interpretation: H.G., H.A.U., T.P., Z.A., D.K., Literature Search: H.G., H.A.U., T.P., Z.A., D.K., Writing: H.G., H.A.U., T.P., Z.A., D.K.

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References

1. Compston A, Coles A. Multiple sclerosis. Lancet 2002; 359: 1221-31.
2. Lublin FD, Reingold SC, Cohen JA, Cutter GR, Sørensen PS, Thompson AJ, et al. Defining the clinical course of multiple sclerosis: the 2013 revisions. Neurology 2014; 83: 278-86.
3. Harirchian MH, Fatehi F, Sarraf P, Honarvar NM, Bitarafan S. Worldwide prevalence of familial multiple sclerosis: A systematic review and meta-analysis. Mult Scler Relat Disord 2018; 20: 43-7.
4. Muñoz-Culla M, Irizar H, Otaegui D. The genetics of multiple sclerosis: review of current and emerging candidates. Appl Clin Genet 2013; 6: 63-73.
5. Mansoor SR, Ghasemi-Kasman M, Yavarpour-Bali H. The role of microRNAs in multiple sclerosis. Int Rev Immunol 2022; 41: 57-71.
6. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 2004; 116: 281-97.
7. Gandhi R, Healy B, Gholipour T, Egorova S, Musallam A, Hussain MS, et al. Circulating microRNAs as biomarkers for disease staging in multiple sclerosis. Ann Neurol 2013; 73: 729-40.
8. Otaegui D, Baranzini SE, Armañanzas R, Calvo B, Muñoz-Culla M, Khankhanian P, et al. Differential micro RNA expression in PBMC from multiple sclerosis patients. PLoS One 2009; 4: e6309.
9. Gao Y, Han D, Feng J. MicroRNA in multiple sclerosis. Clin Chim Acta 2021; 516: 92-9.
10. Nuzziello N, Vilardo L, Pelucchi P, Consiglio A, Liuni S, Trojano M, et al. Investigating the Role of MicroRNA and Transcription Factor Co-regulatory Networks in Multiple Sclerosis Pathogenesis. Int J Mol Sci 2018; 19: 3652.
11. Abolghasemi M, Ashrafi SA, Asadi M, Shanehbandi D, Etehad SS, Poursaei E, et al. Altered miR-10, miR-24a, miR-124, and miR-21 expression in peripheral blood mononuclear cells of patients with multiple sclerosis. Preprint. Available from: https://assets.researchsquare.com/files/rs-1490169/v1/ea525601-112a-4281-982e-c97463c22b37.pdf?c=1648750902.
12. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative C(T) method. Nat Protoc 2008; 3: 1101-8.
13. Piket E, Zheleznyakova GY, Kular L, Jagodic M. Small non-coding RNAs as important players, biomarkers and therapeutic targets in multiple sclerosis: A comprehensive overview. J Autoimmun 2019; 101: 17-25.
14. Muñoz-San Martín M, Gómez I, Quiroga-Varela A, Gonzalez-Del Río M, Robles Cedeño R, Álvarez G, et al. miRNA Signature in CSF From Patients With Primary Progressive Multiple Sclerosis. Neurol Neuroimmunol Neuroinflamm 2022; 10: e200069.
15. Keller A, Leidinger P, Steinmeyer F, Stähler C, Franke A, Hemmrich-Stanisak G, et al. Comprehensive analysis of microRNA profiles in multiple sclerosis including next-generation sequencing. Mult Scler 2014; 20: 295-303.
16. Wei Z, Lyu B, Hou D, Liu X. Mir-5100 Mediates Proliferation, Migration and Invasion of Oral Squamous Cell Carcinoma Cells Via Targeting SCAI. J Invest Surg 2021; 34: 834-41.
17. Zhang HM, Li H, Wang GX, Wang J, Xiang Y, Huang Y, et al. MKL1/miR-5100/CAAP1 loop regulates autophagy and apoptosis in gastric cancer cells. Neoplasia 2020; 22: 220-30.
18. Yang D, Wang WZ, Zhang XM, Yue H, Li B, Lin L, et al. MicroRNA expression aberration in Chinese patients with relapsing remitting multiple sclerosis. J Mol Neurosci 2014; 52: 131-7.
19. Keller A, Leidinger P, Lange J, Borries A, Schroers H, Scheffler M, et al. Multiple sclerosis: microRNA expression profiles accurately differentiate patients with relapsing-remitting disease from healthy controls. PLoS One 2009; 4: e7440.
20. McCoy CE. miR-155 Dysregulation and Therapeutic Intervention in Multiple Sclerosis. Adv Exp Med Biol 2017; 1024: 111-31.
21. Ma X, Zhou J, Zhong Y, Jiang L, Mu P, Li Y, et al. Expression, regulation and function of microRNAs in multiple sclerosis. Int J Med Sci 2014; 11: 810-8.
22. Mandolesi G, Rizzo FR, Balletta S, Stampanoni Bassi M, Gilio L, Guadalupi L, et al. The microRNA let-7b-5p Is Negatively Associated with Inflammation and Disease Severity in Multiple Sclerosis. Cells 2021; 10: 330.
23. Søndergaard HB, Hesse D, Krakauer M, Sørensen PS, Sellebjerg F. Differential microRNA expression in blood in multiple sclerosis. Mult Scler 2013; 19: 1849-57.
24. Cox MB, Cairns MJ, Gandhi KS, Carroll AP, Moscovis S, Stewart GJ, et al. MicroRNAs miR-17 and miR-20a inhibit T cell activation genes and are under-expressed in MS whole blood. PLoS One 2010; 5: e12132.
25. Ebrahimkhani S, Vafaee F, Young PE, Hur SSJ, Hawke S, Devenney E, et al. Exosomal microRNA signatures in multiple sclerosis reflect disease status. Sci Rep 2017; 7: 14293.