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**Review Article**

# The Role of Epigenetics in Multiple Sclerosis

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## Abstract

Multiple sclerosis (MS), an autoimmune chronic inflammatory, demyelinating disease, has affected over 2.5 million people in the world, who are mostly in young adulthood ages. As the burden of this disease would highly influence the socioeconomic status of the societies, as well as the patient's quality of life, any progress in better understanding the pathophysiology of this disease would be valuable. MS is caused by a series of cell-mediated immune mechanisms involving $CD4^+$ T-cell reactivation against CNS. Also, as the involvement of both innate and acquired immunities, different risk factors have been proposed for MS. Environmental factors such as smoking, Epstein-Barr virus infection, sun exposure and vitamin D, body mass index, gut microbiota, and melatonin disturbance may affect gene expression patterns through epigenetic changes, and therefore, play roles in disease occurrence. These epigenetic changes could be categorized as alterations in DNA methylation, histone modifications and non-coding RNAs. Moreover, the reversibility of these epigenetic changes could be potentially considered as therapeutic targets. Therefore, several experimental and preclinical studies have investigated medications for reversing the pathologic epigenetic changes in MS. Accordingly, the current review was conducted to gather the current findings on the role of epigenetics in the pathophysiology and also treatment of MS.

**Keywords:** DNA Methylation; Epigenetics; MicroRNA; Multiple Sclerosis

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## Introduction

Multiple sclerosis (MS) is an autoimmune, chronic inflammatory, demyelinating disease that only affects the central nervous system (CNS)(1). Approximately 2.5 million people worldwide are affected by MS, which is noticeably more common in northern and southern latitudes. The disease onset is mostly in young adulthood, with a double-fold prevalence among females compared to males (2,3). It has been estimated that most patients with MS experience relapse-remission pattern (RRMS) about 85% (4), which half of them progress to secondary progressive MS (PMS) in 10 years (5).

It is currently believed that as an autoimmune disease, MS is caused by a series of cell-mediated immune mechanisms involving $CD4^+$ T-cell reactivation against CNS. Both innate and adaptive immunities are actively involved in inflammation, demyelination, and neurodegenerative processes of MS disease. $CD4^+$ T-cells stimulate immune cells such as B-cells, $CD8^+$ cells, mast cells, granulocytes, and monocytes. The Antigen Presenting Cells (APCs) reactivate $CD4^+$ T-cells to produce cytokines and chemokines, which aggravate the inflammation by inducing myelin phagocytosis by affecting microglia and astrocyte activation (6,7).

Although specific causes of MS are not clearly understood yet, they are believed to have a multifactorial origin that includes a combination of several genetic and environmental factors. Recent studies have suggested different intrinsic and extrinsic factors for the etiology of MS, which have also been approved by The Americas Committee for Treatment and Research in Multiple Sclerosis (ACTRIMS) Forum (8).

The roots of genetic evidence come from the number of disease occurrences in families, especially first-degree relatives of MS patients who are at greater risk for developing the disease compared to the general population (9). Till 2019, Up to 230 genetic variants have been firmly associated with an increased risk of developing MS. These genes are mostly involved in the regulation of immune response, which is common among other autoimmune diseases as well. Although all of these findings may suggest an etiological root for MS, we are still far from fully decoding MS genetic complexities (10,11).

Accordingly, genome-wide association studies and meta-analyses have identified genes that may cause susceptible individuals to develop the disease. Many of these genes play a role in the immune system, with a prominent role for major histocompatibility complex (MHC) class II molecules, and in particular, the *HLA-DRB1* alleles (3,12).

## Genetics to Epigenetic

As there is a low concordance in disease occurrence among monozygotic twins, it could be concluded that genetics is not the only etiologic factor for disease. Meanwhile, different studies on monozygotic co-twins indicated no significant evidence for genetic or epigenetic differences that could explain this discordance (13). Studies on monozygotic twins are the most powerful evidence for the role of epigenetics in MS. The most common proposed mechanism is DNA methylation. Prior studies have shown evidence of different representing types of DNA methylation in $CD4^+$ T-cells in monozygotic twins with and without diagnostic signs of the disease (14). A large genome-wide association study (GWAS) designated *ATXN1* as the most plausible gene associated with multiple sclerosis (MS) risk within a disease locus mapping at 6p22.3 (15). Also, there was no evidence of genetic association with clinical course, severity of disease, or month of birth. Although in the previous studies, neither gender difference nor genetic association (e.g., *DRB1*15:01*) has been found, some animal investigations have indicated different methylation and expression levels of X genes (*FOXP3*) in the different inheritance of X chromosome from either of the parents, which may possibly explain the cause of different concordance in gender. However, analysis with respect to age at onset replicated the previously suggested association with the *DRB1*15:01* (3,16).

As a multifactorial disease, different etiologic factors, other than genetic ones, have been proposed to play a role in the pathophysiology of MS. One of these risk factors could be epigenetic changes, including DNA methylation patterns, histone modifications, and non-coding RNAs, which may be caused by environmental factors such as smoking, Epstein-Barr virus (EBV) infection, sun exposure and vitamin D (vit. D), body

mass index (BMI), gut microbiota, melatonin disturbances, and other factors (17-20) .

Considering a dynamic process, the epigenetic changes occur during differentiation and in response to environmental factors (21). Also, epigenetics is one of the most promising heritable mechanisms influencing gene expression with no alteration in DNA sequences, which is associated with the incidence of MS (22). To find MS genetic loci that are linked to a worsening of disability over time and to create and test ensemble genetic learning models that can identify people with MS (PwMS) who are at risk of getting worse in the future. Associated with a risk of disability getting worse, the majority are near or tagged to 13 genomic regions rich in pathways for the biosynthesis of steroids and peptide hormones (23).

## DNA Methylation

One of the most important epigenetic modifications is DNA methylation, which is associated with transcriptional repression through the addition of methyl groups to cytosine by DNA methyl transferases (24). DNA methylation occurs in unique regions of the genome, known as CpG islands, that contain more than 50% cytosine and guanine nucleotides. Importantly, this process could be responsible for most of the aberrant gene expressions involved in several neurological diseases (25,26).

The first association of DNA methylation at *HLA-DRB1* was observed in detecting methylation signal at chromosome 6p21, in which a peak signal at *HLA-DRB1* and 74 CpGs were associated with MS. Importantly, and besides the significant effect of *DRB1* methylation, 55 non-*HLA* CpGs also exhibited differential methylation, notably in some genes which were in association with MS disease (27).The most recent studies have also indicated an association of differentially methylated regions (DMRs) with MS. The alterations in the methylation status of DMRs include hypomethylation at *HLA-DRB1* and hypermethylation at *HLA-DRB5* in the relapsing-remitting (RRMS) MS. In addition, hypermethylation of several other *MHC* loci and also two non-*MHC* DMRs were identified in association with disease, which were mainly located at chromosomes 1 and 8 (28).

79 differentially methylated CpGs were associated with MS. Different genome-wide studies showed significant differences in DNA methylation profiling between $CD8^+$ T-cells, $CD4^+$ T-cells, and whole blood DNA in MS patients. The methylation profile of $CD8^+$ T-cells was distinctive from $CD4^+$ T-cells (29). Despite the strong evidence in hypermethylation of $CD8^+$ T-cells, there was no association with $CD4^+$ T-cells, whole blood DNA, or MS risk gene *HLA-DRB1* locus in the $CD8^+$ T-cells (29,30) .

When compared among two different subtypes of MS, the DNA methylation CpG sites and methylation alterations were more commonly found in primary progressive MS (PPMS) patients than in RRMS. Interestingly, while methylation alteration in PPMS mainly included hypermethylation, hypomethylation was detected in RRMS patients. In these studies, 60% of DMSs detected on CpG-islands and CpG-shores (31).

Commonly methylated in RRMS patients, the CpG sites in the *L1PA2* subfamily could be significant in the hypermethylation of repetitive elements *LINE-1* in these patients. Recent studies demonstrated that the methylation level of the CpG sites within the Alu, LINE-1, and SAT-α repetitive elements was elevated in RRMS patients. Moreover, expanded disability status scale (EDSS) values were associated with differential methylation in Alu and LINE-1 elements as well (32,33).

Some other studies indicated significant increases in mRNA levels of DNA methyl-transferase enzymes (*DNMTs*). In studies on MS patients' demyelinated hippocampus, mRNA levels of DNA de-methylation enzymes and the total hydroxy-methylated levels were downregulated. Also, some differentially methylated positions (DMPs) were highly detected in those disease-affected hippocampus (34). All of these results reinforce the role of epigenetics in the pathophysiology of MS.

Significantly altering the gene expression levels, the proximal promoter of interleukin-2 receptor-α has been found remarkably hypomethylated in MS patients with subsequent higher levels of gene expression in T-cells (35). As proven in the latest progress, methylation patterns of *RUNX3*, *CDKN2A*, *SOCS1*, and *NEUROG1* genes were interestingly different between controls and MS patients but not between patients in relapse and remission phases (36) .

Prior studies found that RRMS patients express

significantly lower levels of *TET2* and *DNMT1* (37). In another study, the demethylating enzyme TET3 showed a lower expression level in secondary progressive MS (SPMS) patients than in the control group. TET3 enzyme is determined to be significantly related to the top three genes, including *APLP2* (amyloid beta (A4) precursor-like protein 2), *SLC25A11* (solute carrier family 25, member 11), and *ATP6AP2* (ATPase, H+ transporting, lysosomal accessory protein 2)(38). The *APLP2* gene plays a role in brain development, mainly by controlling neural stem cells.

The decreased levels of SLC25A11 in the process of brain ischemia could be evidence of its role in the failure of energy metabolism (39). Finally, the expression of ATP6AP2 is detected in the late stages of adult neurogenesis in the hippocampus of animals (149). Besides, the clinical SPMS could possibly happen due to an altered balance in the expression of DNA methylating and demethylating enzymes. These changes in DNA methylation are in a balance of demethylation responding to environmental stimuli, which is involved in MS patients (38).

Comparison of the PAD2 enzyme expression in the white matter of MS patients with healthy controls showed significant upregulation in the enzyme level. The study also proved that the over-expression was associated with the *PAD2* promoter demethylation, which is located in a CpG island (40).

Although in different studies, hyper/hypomethylated repetitive elements were observed to be associated with MS, there is no evidence of a relationship between methylation status and disease activity, phase of MS, days since relapse, year of onset, multisystem disorder, spinal cord relapse or the presence of oligo-clonal bands in CSF (33). Recent studies confirmed that the methylation and gene expression stages of m6A-associated genes in RRMS samples had been substantially better than those in revolutionary MS (PMS) cerebrospinal fluid samples evaluation (4). Dynamic methylation of m6A-RNAs is probably a new diagnostic biomarker to early distinguish PMS from RRMS and may provide a better prognosis for the disease (41,42).

Recently, preclinical access showed increased ataxin-1 levels with enhanced ATXN1 mRNA (43). Different hypomethylated sites within the *ATXN1* genomic sequence of B cells have been reported in the clinical pathogenesis of this disease. These changes may be mediated by mRNA-upregulated TET1 according to RNA analysis (44). New studies on EAE mice have shown an increase in the Treg function of the *HDAC7 R166H* variant compared with conventional CD4 T cells, and, in addition, it provides interesting protection against severe MS. We have revealed that the effects of *HDAC7* are involved in multiple transcriptional programs, including Foxo1, Foxp3, STAT3, MEF2D, and Bcl6 (45).

## Feroptosis Pathway

Ferroptosis, a novel iron-dependent programmed cell death pathway, has recently been shown to be involved in neurodegenerative diseases. Many publications demonstrated that microglial resistance to ferroptosis due to the rapid loss of oligodendrocytes and demyelination (46-48). Furthermore, ferroptosis has been reported to participate in neuroinflammation and neuronal cell death following acute brain injury, which may be responsible for the pathogenesis. Ferroptosis-associated factors and signaling pathways are now considered biomarkers and therapeutic targets of NDD. Proinflammatory microglia display anti-ferroptosis effects of neuroinflammation. Therefore, the exact role of ferroptosis in MS is unclear (49-52).

Supportingly, a new study on EAE mice restored anti-ferroptosis gene expression, decreased inflammation-induced neuronal loss, and improved clinical outcomes. In a similar vein, G9a inhibition increased neuronal anti-ferroptotic gene expression in human neuronal cultures while reducing it in MS brain tissue. G9a is found to be a crucial transcriptional enhancer of neuronal ferroptosis and a potential therapeutic target for fighting inflammation-induced neurodegeneration (53).

## Micro RNAs (miRNA)

The second epigenetic mechanism associated with MS is the microRNAs (miRNA). The miRNAs are the non-coding RNAs, including 21-24 nucleotides, and regulate the expression of DNA by signaling pathways to the differentiation, apoptosis, or proliferation of the cells (54,55). The miRNAs play important roles in gene silencing by

degrading target mRNA sequences and preventing their translation into proteins. It has also been suggested that specific miRNAs are highly upregulated in active MS lesions (56). In the most recent investigations on the expression levels of serum miRNAs, several miRNAs were not only up or down-regulated in MS patients, but also the association was observed between PPMS and SPMS and between relapsing and remitting phases of the disease in RRMS as well (57). These studies highly support the role of miRNAs as biomarkers of MS. Moreover, their potential contribution to MS pathology was correlated with the disease severity and response to treatment (58,59).

In a study in 2017, nine miRNAs were demonstrated as comparisons between different phases of the disease (relapsing-remitting and progressive)(60). On the other hand, miR-27a-3p and miR 376b.3p expression levels were notably different in RRMS compared to SPMS; also, some miRNAs demonstrated linkage with disease progression (miR-27a-3p the most significant), and some with the expanded disability status of the disease (miR.199a.5p the most)(61) . In addition, disability progression index was remarkably correlated with increased levels of miR-24-3p. On the other hand, annual relapse rates in PPMS and RRMS were significantly associated with miR-128- 3p (62). Some researchers present different miRNA expressions even in two stages of the remission phase. Accordingly, miR-301a and miR155 were higher in the post-acute compared to the stable phase (63).

Evidence has defined the role miR-96 by targeting the exact genes that take part in immunological pathways by releasing interleukins and WNT. Notably, miR-96 showed significant levels, especially in remission of MS, while miR-18b and miR-599 played significant roles in relapse (64).

MiRNA-associated epigenetic studies have shown significant results regarding the association of hypermethylated genes with special miRNAs. Similarly, T-cell activation genes are also up-regulated in MS whole blood mRNAs (65). Lower miRNA-21 levels and concomitant up-regulation of the target genes in $CD4^+$ T-cells were also detected. Moreover, levels of important neuro-steroids were suppressed in the white matter of MS patients, which were all in support of dysregulated miRNA levels in MS (66) . In a DNA methylation analysis on $CD4^+$ T-cells from the patients with RRMS, secondary progressive-MS (SPMS), and healthy controls, significant differences were observed in the methylation status of VMP1/miR-21 locus, and the level of methylation in patients with RRMS was remarkably higher than SPMS or controls. Also, there was a significant negative correlation between age and the levels of mature miR-21 in $CD4^+$ T-cells. When compared to SPMS, the miR-21 level was significantly lower in the other subtype, RRMS (67).

Follicular helper T cells do not transmit demyelinating disease in mice and are unlikely to have a pathological effect in MS patients (68). It is not yet known whether follicular helper T cells play a role in MS. MHC class I-restricted $CD8^+$ cells have been found in MS brain lesions, but also in patients with infections and other brain diseases, so there is no conclusive evidence of their involvement in MS (69).

The study by Hansen *et al*. in 2022 on peripheral helper T cells recognized two practically specific Tph cell populations and a regulatory partner, Tpr cells. No differences in blood frequency, cytokine generation, or the ability to cooperate with B cells were found between control and MS patients. Together with comparable CNS migration potential, we found both enhanced Tph cell populations in the CSF, and amazingly, the extensive recurrence of intracortical Tph cells in the reference group was in contrast to the MS patients (70).

To Identify characteristic miRNAs, genetic studies suggested that epigenetic changes in the CNS take part in the pathogenesis of MS. For example, low levels of the miR-191 affect distinct pathways, including FZD5, BDNF, WSB1, and SOX4. By the way, the products of these pathways start the inflammation in the CNS and destroy the myelin repair systems (71).

Similarly, MiR-125a-3p could slow the myelination process through dysregulation of some genes in MS patients (72). Eight miRNAs target the gene SOCS6 (suppressor of cytokine signaling 6), and interestingly, the expression of these miRNAs is up-regulated in SPMS $CD4^+$ T cells. SOCS6 was previously shown to have a negative role in the activation of T cells (59). The expression of miR-223 was demonstrated to target the genes such as signal transducer and activator of transcription 3 (*STAT3*) and Arginase-1 (*ARG1*).

**Table 1.** DNA methylation status in associated genes with MS

| Gene Name | Chromosome | Gene Location | Methylation Status | Explanation | References |
|---|---|---|---|---|---|
| ***MICA*** | 6 | 6p21.33 | Hypo-methylation | | (27) |
| ***MICB*** | 6 | 6p21.33 | Hypo-methylation | | (27) |
| ***HLA-DRB1*** | 6 | 6p21 | Hypo/hypermethylation | In specific subtype of RRMS but in PPMS mostly included hypermethylation | (27, 28, 31, 170) |
| ***HLA-DRB5*** | 6 | 6p21.32 | Hyper-methylation | in specific subtype of RRMS | (28) |
| ***HLA-DRB6*** | 6 | 6p21.32 | Hyper-methylation | | (28) |
| ***PM20D1*** | 1 | 1q32.1 | Hyper-methylation | | (28) |
| ***MORN1*** | 1 | 1p36.33 | Hyper-methylation | notably in MS associated genes | (27) |
| ***RUNX3*** | 1 | 1p36.11 | Hyper-methylation | | (36) |
| ***MMEL1*** | 1 | 1p36.32 | | | (170) |
| ***EVI5*** | 1 | 1p22.1 | | | (170) |
| ***CD58*** | 1 | 1p13.1 | | | (170) |
| ***RGS1*** | 1 | 1q31.2 | | | (170) |
| ***LCLAT1*** | 2 | 2p23.1 | Hypo-methylation | | (27) |
| ***PDCD1*** | 2 | 2q37.3 | Hyper-methylation | | (27) |
| ***MUC4*** | 3 | 3q39 | Hypo-methylation | | (27) |
| ***CD86*** | 3 | 3q13.33 | | | (170) |
| ***ANKRD55*** | 5 | 5q11.2 | | | (170) |
| ***AHRR*** | 5 | 5p15.33 | Hypo-methylation | | (27) |
| ***ARSB*** | 5 | 5q14.1 | Hypo-methylation | | (27) |
| ***PCBD2*** | 5 | 5q31.1 | Hypo-methylation | | (27) |
| ***TGFBI*** | 5 | 5q31.1 | Hyper-methylation | | (27) |
| ***PCDHB13*** | 5 | 5q31.3 | Hyper-methylation | | (27) |
| ***PCDHB15*** | 5 | 5q31.3 | Hypo-methylation | | (27) |
| ***NEUROG1*** | 5 | 5q31.1 | | | (36) |
| ***L3MBTL3*** | 6 | | | | (170) |
| ***HCG4P6*** | 6 | | Hyper-methylation | | (28) |
| ***RNF39*** | 6 | 6p22.1 | Hyper-methylation | | (28) |
| ***KIF25*** | 6 | 6q27 | Hypo-methylation | | (27) |
| ***CSGALNACT1*** | 8 | 8p21.3 | Hyper-methylation | | (27) |
| ***ERICH1*** | 8 | | Hyper-methylation | | (28) |
| ***CDKN2A*** | 9 | 9p21.3 | Hyper-methylation | CDKN2A (Cyclin-dependent Kinase Inhibitor 2A) | (36) |
| ***ADARB2*** | 10 | 10p15.3 | Hyper-methylation | | (27) |
| ***IL2RA*** | 10 | 10p15.1 | | | (170) |
| ***Intergenic*** | 10 | | | | (170) |
| ***LDHAL6A*** | 11 | 11p15.1 | Hypo-methylation | | (27) |
| ***CORO1B*** | 11 | 11q13.1 | Hyper-methylation | | (27) |
| ***USP35*** | 11 | 11q14.1 | Hypo-methylation | | (27) |
| ***FUT4*** | 11 | 11q21 | Hypo-methylation | | (27) |
| ***ERC1*** | 12 | 12p13.3 | Hypo-methylation | | (27) |
| ***TNFRSF1A*** | 12 | | | DLEU1 (deleted in lymphocytic leukemia 1) | (170) |
| ***DLEU1*** | 13 | | | | (170) |
| ***TCRA*** | 14 | 14q11.2 | Hyper-methylation | | (27) |
| ***PACS2*** | 14 | 14q32.33 | Hypo-methylation | | (27) |
| ***IL32*** | 16 | 16p13.3 | Hyper-methylation | | (27) |
| ***SOCS1*** | 16 | | | SOCS1(suppressor of cytokine signaling 1) | (36) |
| ***SOCS6*** | 18 | 18q22.2 | | SOCS6 (suppressor of cytokine signaling 6) This gene has previously been reported to negatively regulate T cell activation by promoting ubiquitin-dependent proteolysis | (59) |
| ***CLEC16A*** | 16 | 16p13.13 | | | (170) |
| ***MAZ*** | 16 | 16p11.2 | | | (170) |
| ***SHMT1*** | 17 | 17p11.2 | | | (170) |
| ***KCTD11*** | 17 | 17p13.1 | Hypo-methylation | | (27) |
| ***C17orf108*** | 17 | 17q11.2 | Hyper-methylation | | (27) |
| ***ARHGAP27*** | 17 | 17q21.31 | Hypo-methylation | | (27) |
| ***NPLOC4*** | 17 | 17q25.3 | Hyper-methylation | | (27) |
| ***SBNO2*** | 19 | 19p13.3 | Hypo-methylation | | (27) |
| ***GNG7*** | 19 | 19p13.3 | Hypo-methylation | | (27) |
| ***ERG*** | 21 | 21q22.2 | | | (170) |
| ***C21orf56*** | 21 | 21q22.3 | Hypo-methylation | | (27) |
| ***RIBC2*** | 22 | 22q13.31 | Hypo-methylation | | (27) |

**Table 1. Continued**

| | | | | | |
|---|---|---|---|---|---|
| ***C22orf43*** | 22 | | Hyper-methylation | | (30) |
| ***LOC285830*** | | | Hyper-methylation | | (30) |
| ***NAPEPLD*** | 7 | 7q22.1 | Hyper-methylation | N-Acyl Phosphatidylethanolamine D | (30) |
| ***NHLH2*** | 1 | 1p13.1 | Hyper-methylation | Nascent Helix Loop Helix 2 | (30) |
| ***PLCH1*** | 3 | 3q25.31 | Hyper-methylation | Phospholipase C, Eta 1 | (30) |
| ***SERPINA9*** | 14 | 14q32.13 | Hyper-methylation | Serpin Peptidase Inhibitor, Member 9 | (30) |
| ***SLFN13*** | 17 | 17q12 | Hyper-methylation | Schlafen Family Member 13 | (30) |
| ***TMEM132B*** | | | Hyper-methylation | Transmembrane Protein 132B | (30) |
| ***TTLL3*** | | | Hyper-methylation | Tubulin Tyrosine Ligase-Like 3 | (30) |
| ***WDR81*** | 17 | 17p13.3 | Hyper-methylation | WD Repeat Domain | (30) |
| ***AKNA*** | | | Hypo-methylation | AT-Hook Transcription Factor | (30) |
| ***EBPL*** | 13 | 13q14.2 | Hypo-methylation | Emopamil Binding Protein-like | (30) |
| ***FLJ42709*** | | | Hypo-methylation | | (30) |
| ***HERC6*** | | | Hypo-methylation | HECT Domain Containing E3 UBL Member 6 | (30) |
| ***OR52M1*** | | | Hypo-methylation | Olfactory Receptor, Family 52, Subfamily M1 | (30) |
| ***SFRP1*** | 8 | 8p11.21 | Hypo-methylation | Secreted Frizzled-Related Protein 1 | (30) |
| ***MLLT4*** | 6 | 6q27 | Hypo-methylation | Myeloid/Lymphoid or Mixed-Lineage Leukemia | (30) |
| ***PPIF*** | 10 | 10q22.3 | Hypo-methylation | Peptidylprolyl Isomerase F | (30) |
| ***SNRNP40*** | 1 | 1p35.2 | Hypo-methylation | Small Nuclear Ribonucleoprotein 40kDA | (30) |
| ***MEF2A*** | 15 | 15q26.3 | Hypo-methylation | Myocyte Enhancer Factor 2A | (30) |
| ***PMEPA1*** | 20 | 20q13.31 | Hypo-methylation | Prostate Transmembrane Protein, Androgen Induced 1 | (30) |
| ***ABCA4*** | 1 | 1p22.1 | Hypo-methylation | ATP-Binding Cassette, Subfamily A, Member 4 | (30) |
| ***ADAMTS12*** | 5 | 5p13.3-p13.2 | Hypo-methylation | ADAM Metallopeptidase TS12 | (30) |
| ***AHRR*** | 5 | 5p15.33 | Hypo-methylation | Aryl-Hydrocarbon Receptor Repressor | (30) |
| ***BEST3*** | 12 | 12q15 | Hypo-methylation | Caspase 7, Apoptosis-Related Cysteine Peptidase | (30) |
| ***CASP7*** | 10 | 10q25.3 | Hypo-methylation | Caspase 7, Apoptosis-Related Cysteine Peptidase | (30) |
| ***CCL4L2*** | 17 | 17q12 | Hypo-methylation | Chemokine (C-C Motif) Ligand 4-Like 2 | (30) |
| ***CPXM2*** | 10 | 10q26.13 | Hypo-methylation | Carboxypeptidase X (M14 Family), Member 2 | (30) |
| ***FBXW8*** | 12 | 12q24.22 | Hypo-methylation | F-Box and WD Repeat Domain Containing 8 | (30) |
| ***MEIS1*** | 2 | 2p14 | Hypo-methylation | Meis Homeobox 1 | (30) |
| ***MGMT*** | 10 | 10q26.3 | Hypo-methylation | O-6-Methylguanine-DNA Methyltransferase | (30) |
| ***MYO7A*** | 11 | 11q13.5 | Hypo-methylation | Myosin VIIA | (30) |
| ***PKP2*** | 12 | 12p11.21 | Hypo-methylation | Plakophilin 2 | (30) |
| ***PQLC1*** | | | Hypo-methylation | PQ Loop Repeat Containing 1 | (30) |
| ***PSD3*** | 8 | 8p22 | Hypo-methylation | Pleckstrin and Sec7 Domain Containing 3 | (30) |
| ***SCN4B*** | 11 | 11q23.3 | Hypo-methylation | Sodium Channel, Voltage-Gated, Type IV B | (30) |
| ***SDK2*** | 17 | 17q25.1 | Hypo-methylation | Sidekick Cell Adhesion Molecule 2 | (30) |
| ***SMYD3*** | 1 | 1q44 | Hypo-methylation | SET and MYND Domain Containing 3 | (30) |
| ***TGFBI*** | 5 | 5q31.1 | Hypo-methylation | Transforming Growth Factor, Beta-Induced | (30) |
| ***TMEM165*** | 4 | 4q12 | Hypo-methylation | Transmembrane Protein 165 | (30) |
| ***PON1*** | 7 | 7q21.3 | Hypo-methylation | Paraoxonase 1 | (30) |
| ***HDLBP*** | 2 | 2q37.3 | Hypo-methylation | High-Density Lipoprotein Binding Protein | (30) |
| ***MKKS*** | 20 | 20p12.2 | Hypo-methylation | McKusick-Kaufman Syndrome | (30) |
| ***TRIM26*** | 6 | 6p22.1 | Hypo-methylation | Tripartite Motif Containing 26 | (30) |
| ***TRPS1*** | 8 | 8q23.3 | Hypo-methylation | Trichorhinophalangeal Syndrome 1 | (30) |
| ***MGP*** | 12 | 12p12.3 | Hypo-methylation | Matrix Gla Protein | (30) |
| ***AJAP1*** | 1 | 1p36.32 | Hypo-methylation | Adherens Junctions Associated Protein 1 | (30) |
| ***C1orf106*** | 1 | 1q32.1 | Hypo-methylation | Chromosome 1 open reading frame 106 | (30) |
| ***DSE*** | 6 | 6q22.1 | Hypo-methylation | Dermatan Sulfate Epimerase | (30) |

**Table 1. Continued**

| | | | | | |
|---|---|---|---|---|---|
| ***EIF2C2*** | 8 | 8q24.3 | Hypo-methylation | Eukaryotic Translation Initiation Factor 2C, S 2 | (30) |
| ***NFASC*** | 1 | 1q32.1 | Hypo-methylation | Neurofascin | (30) |
| ***RASA3*** | 13 | 13q34 | Hypo-methylation | RAS P21 Protein Activator 3 | (30) |
| ***SDK1*** | 7 | 7p22.2 | Hypo-methylation | Sidekick Cell Adhesion Molecule 1 | (30) |
| ***SHISA2*** | 13 | 13q12.13 | Hypo-methylation | Shisa Family Member 2 | (30) |
| ***SOLH*** | 16 | 16p13.3 | Hypo-methylation | Calpain 15 or Small Optic Lobes Homolog | (30) |
| ***SORBS2*** | 4 | 4q35.1 | Hypo-methylation | Sorbin and SH3 Domain Containing 2 | (30) |
| ***TAGLN3*** | | 3q13.2 | Hypo-methylation | Transgelin 3 | (30) |
| ***TM9SF1*** | | | Hypo-methylation | Transmembrane 9 Superfamily Member 1 | (30) |
| ***TOP1MT*** | 8 | 8q24.3 | Hypo-methylation | Topoisomerase (DNA)I, Mitochondrial | (30) |
| ***L1PA2* subfamily: *Alu, LINE-1* and *SAT-α* repetitive elements** | | | Hypermethylation | LINE-1 (Long interspersed nuclear element-1) | (28,29) |
| **proximal promotor of *interleukin-2 receptor-α*** | 10 | 10p15.1 | Hypomethylation | In MS patients with subsequent higher levels of gene expression in T-cells | (31) |
| ***IGF2*** | 11 | 11p15.5 | | | (32) |
| ***CRABP1*** | 15 | 15q25.1 | | | (32) |
| ***CACNA1G*** | 17 | 17q21.33 | | | (32) |
| ***APLP2*** | 11 | 11q24.3 | Demethylation | APLP2 (amyloid beta (A4) precursor-like protein 2) | (129) |
| ***SLC25A11*** | 17 | 17p13.2 | Demethylation | SLC25A11 (solute carrier family 25, member 11) is related with TET3 enzyme | (129) |
| ***ATP6AP2*** | X | Xp11.4 | Demethylation | ATP6AP2 (ATPase, H+ transporting, lysosomal accessory protein 2) is related with TET3 enzyme | (129) |
| ***PAD2* promoter** | | | Demethylation | The PAD2 promoter of the PAD2 enzyme has an over-expression in MS patients. | (36) |

As proven in MS patients, the STAT3 and ARG1 expression were decreased compared with healthy controls (73). In addition, potential targets of miR-223 are STAT1, Forkhead Box O (FOXO1), and FOXO3. Besides, it has been proven that FOXP3 and RORC are upregulated in $CD4^+$ T cells in the remitting phase. The expression of RORγt, the master transcription factor of Th17, is upregulated in the relapsing phase (74,75). BCL2, previously known as miR-15a/16-1 target gene, is defined to be respectively overexpressed in RR-MS patients (76). miR-141 and miR-200a showed significant augmentation in the relapsing phase of the disease. On the other hand, the expression of target genes of these miRNAs displayed remarkable down-regulation in the relapsing phase. Meanwhile, both miRNAs play a role in T helper cell differentiation pathways by activating TGF-β, mTOR, and JAK/STAT (77).

Demonstrating a correlation between these biochemical results and clinical parameters, different pathways have been suggested. miR-199a and miR-142-3p may be crucial for MS by targeting pivotal susceptibility genes, in particular, KRAS and IL-7R (78).

A hypothesis proposes that extracellular exosomes, transferring microRNAs by exosomes, maybe the linkage between the gut microbiota and the host autoimmune diseases. Transferred let-7i can cause a decreased expression of insulin-like growth factor 1 receptor (IGF1R) and transforming growth factor β receptor 1 (TGF-BR1) and also play a role in the inhibition of T cell differentiation (79).

T cells and B cells are affected in the pathogenesis of MS, especially in the relapsing phase, in which B cells act by producing matrix metallopeptidase-9 (MMP-9) that disrupts the blood-brain barrier (BBB). Down-regulation of miR-320a leads to over-expression of MMP-9 protein in B cells of MS patients and acts in the pathogenesis of the disease by increased blood-brain barrier permeability (80). Additionally, in erythrocytes, different expressions of erythrocyte miRNAs were shown by using RT-qPCR (specially miR-30b-5p and miR-3200-3p) in RRMS patients in comparison with healthy controls (81).

As with any other autoimmune disease, MS

**Table 2.** Alterations in miRNA expression levels in MS

| MicroRNA | Cells/tissues expressed | Up/Down-regulation | Function and details | References |
|---|---|---|---|---|
| **miR-let-7a-5p, miR-let-7b-5p** | | Up-regulation | Regulate stem cell differentiation, T cell activation | (31,130,131) |
| **miR-let-7d** | | Up-regulation | Correlated with the cytokine interleukin-1B. | (132) |
| **miR-let-7g, let-7g-3p** | | Down/Up-regulation | | (31,133,134) |
| **miR-7-1-3p** | | Down-regulation | In Relapsing remitting multiple sclerosis (RRMS) CIS/RRMS | (135) |
| **miR-9-5p** | | Up-regulation | | (55) |
| **miR-15a** | White matter, CD4+ T cells | Up/Down -regulation | Up-regulation in White matter. Down –regulation in CD4+ T cells in RRMS patients | (56) |
| **miR-15b** | CD4+ T cells Th17 | Down-regulation | In primary progressive MS Suppresses Th17 Differentiation by targeting O-linked N-acetylglucosamine (O-GlcNAc) transferase | (136-138) |
| **miR-15b-5p** | | Up-regulation | In RRMS patients. | (43) |
| **miR-16-1** | | Down-regulation | In RRMS patients | (56,139) |
| **miR-16-2-3p** | | Up-regulation | In CIS/RRMS patients. | (135) |
| **miR-17** | CD4+ T cell | Down-regulation | CD4+ T cell activation and | (15) |
| **miR-17, miR-17-5p** | CD4+ T cells, CD41 | Up-regulation | CD4+ T cell activation and proliferation. In relapsing-remitting (RRMS) | (61,62) |
| **miR-18b** | BBB | Down-regulation | | (47) |
| **miR-18b** | | Up-regulation | | (140) |
| **miR-19a, miR-19b** | CD4+, CD25, CD127+ | Up-regulation | Silencing the effects of the TGF-β signaling pathway. In relapsing phase. | (31,141,142) |
| **miR-20a-5p** | CD4+ T cell | Down-regulation | CIS/RRMS | (48,135,143) |
| **miR-20b** | | Down-regulation | | (31,144) |
| **miR-21-5p** | CD4+ T cells | Up/Down-regulation | Up-regulation especially in remission phase of multiple sclerosis patients and down regulation in secondary progressive MS (SPMS) | (42,67,145,146) |
| **miR-23a, miR-23a-3p** | | Up/Down-regulation | Up-regulation significantly in RRMS an down-regulation in primary progressive MS | (43,136,137) |
| **miR-24** | | Down-regulation | | (139) |
| **miR-24-3p** | | Up-regulation | In RRMS and PPMS | (45) |
| **miR-25, miR-25–3p** | CD4+, CD25, CD127+ | Up-regulation | | (44,131,141) |
| **miR-26a** | Th17 | Up-regulation | Specially in in relapsing phase By affecting the differentiation of Th17 cells also targeting TGF-β signaling pathway | (147,148) |
| **miR-26b-5p** | CD4+ T cells | Down -regulation | In secondary progressive MS | (42) |
| **miR-27a, miR-27a-3p** | CD4+ T cells | Up-regulation | promoting differentiation of Th17 cell. In relapsing phase MS compared to remitting phase and controls. Linked to disease progression. | (44,149) |
| **miR-27b** | | Down -regulation | In both remission and in non-stimulated MS compared to healthy controls. | (140) |
| **miR-29b-3p** | CD4+ T cells | Down -regulation | In secondary progressive MS. | (42,150) |
| **miR-30a** | Th17 | Down-regulation | Inhibits differentiation of T cells into Th17 by targeting interleukin-21 receptor (IL-21R). | (151) |
| **miR-30a-5p, miR-30b-5p, miR-30c** | Erythrocyte, CD4+ T cells | Up-regulation | Specially relapsing-remitting MS (RRMS) | (31,43,66,142, 146) |
| **miR-34a** | CD4+ T cells | Up-regulation | In relapsing phase of MS. | (142) |
| **miR-92a-1** | | | Comparisons between RRMS vs. SPMS and controls. | (130) |
| **miR-93** | CD4+, CD25, CD127+ | Up-regulation | | (141) |
| **miR-96** | | Up-regulation | | (47) |
| **miR-98** | | Up-regulation | | (140) |
| **miR-99b-5p** | | Up-regulation | | (131) |
| **miR-101-5p** | | Up-regulation | in RRMS | (152) |
| **miR-106a, miR-106a-5p** | Th17 cells | Down-regulation | in relapsing phase of MS patients | (55,146) |
| **miR-106b** | CD4+, CD25, CD127+ | up-regulation | | (141) |
| **miR-125a-5p** | BBB | Down-regulation | Down-regulation in MS patients. Influences the brain endothelial tightness and immune cell efflux from the Blood brain barrier (BBB) | (153) |

**Table 2. Continued**

| | | | | |
|---|---|---|---|---|
| **miR-125a-5p** | | up-regulation | | (104,134,154) |
| **miR-125a-3p** | | up-regulation | Inhibits maturation of oligodendroglia cells | (131) |
| **miR-126-3p, miR-126-5p** | | up-regulation | In RRMS patients. | (67,152) |
| **miR-127-3p** | | Up-regulation | In progressive MS patient | (43) |
| **miR-132** | | Up-regulation | | (59) |
| **miR-137** | | Down-regulation | | (139) |
| **miR-141** | CD4+ T cells Th17 | Up-regulation | In relapsing phase of MS patients compared to remitting and control groups. | (57) |
| **miR-142-3p** | CD3+, CD4+ | Up/Down-regulation | Up regulation in CD3 by the action of IL-1 β - mediated synaptic dysfunctions and Down-regulation specially in SPMS | (42,58,144,155) |
| **miR-145** | | up-regulation | In RRMS vs. SPMS and controls | (130,132,143, 144) |
| **miR-146a, miR-146a*G/G** | | up-regulation | Only in females it was associated with a higher risk of developing relapsing–remitting MS (RRMS) | (31,68,145,156) |
| **miR-146b-5p** | | up-regulation | In relapsing remitting MS (RRMS) | (67,134,145) |
| **miR-148b-3p** | | Down-regulation | In pediatric MS | (131) |
| **miR-150** | | Up-regulation | in MS patients and especially in patients with lipid-specific oligoclonal IgM bands (LS_OCMB+). In patients with CIS who converted to MS. | (60,146) |
| **miR-150** | | Down-regulation | | (133) |
| **miR-152** | | Down-regulation | | (134) |
| **miR-155** | CD4+ Th1 and Th17, B-cell, CD14+ monocytes, CD68+ cells | Up-regulation | Promotes the development of inflammatory Th17/Th1 cell subsets. In RRMS. | (46,59,157-160) |
| **miR-155-5p** | CD4+ T cells | Down-regulation | Specially in SPMS | (42,46) |
| **miR-181** | | Down-regulation | | (139) |
| **miR-181a-5p, miR-181b, miR-181c** | CD4+ T cells, macrophages | Up-regulation | Differentiation of T helper cell and activation of macrophages. In MS patients and specially in clinically isolated syndrome (CIS) conversion to RRMS. | (131,161,162) |
| **miR-182–5p** | | Up-regulation | | (131) |
| **miR-185–5p** | | Up-regulation | In RRMS remission phase. | (67,131) |
| **mir-186, miR-186–5p** | | Up-regulation | | (144,154) |
| **miR-191** | | Down-regulation | | (51) |
| **miR-191-5p** | | up-regulation | In RRMS and PPMS. | (45) |
| **miR-196a-5p** | | up-regulation | | (67) |
| **miR-197** | | Down-regulation | | (134) |
| **miR-199a, miR-199a -3p, miR.199a.5p** | CD4+ T cell | Up-regulation | In the remitting phase. By acting on differentiation pathways of Th17 cell. correlation with disability. | (44,58,134,142,146) |
| **miR-199b-3p** | | Down-regulation | | (140) |
| **miR-200a** | CD4+ T cells Th17 | Up-regulation | In relapsing phase of MS patients compared to remitting and control groups. | (48) |
| **miR-200a** | | Up-regulation | | (134) |
| **miR-210** | | Up-regulation | | (140) |
| **miR-214** | CD4+ T cell | Down-regulation | In relapsing phase of MS compared to remitting phase. Acting on Th17 cell differentiation | (149) |
| **miR-219** | Central nervous system (CNS) | Down-regulation | Suggested to be participant in maturation of myelin-forming cells. | (163) |
| **miR-221–3p** | | Up-regulation | | (131,154) |
| **miR-223** | CD4+ T-cells | Up-regulation | In primary progressive MS suppressed T cell proliferation and cytokine production | (53,137,144) |
| **miR-223-3p** | CD4+ T-cells | Up-regulation | Only in men, significantly in RRMS | (43,136) |
| **miR-300** | | Down-regulation | | (31) |
| **miR-301a** | | Down-regulation | Significantly in RRMS | (46) |
| **miR-320a, miR-320b** | B cells | Down-regulation | In MS patients decrease of miR-320a acts by an increased level of MMP-9 protein expression and contribute the permeability of BBB | (44,65,131) |
| **miR-326** | CD4+ T cells Th-17 | Up-regulation | Acts on TH-17 differentiation by targeting Ets-1(a negative regulator of TH-17) and has a correlation with the disease severity. In RRMS patients Specially in in relapsing phase. | (46,164) |

**Table 2. Continued**

| | | | | |
|---|---|---|---|---|
| **miR-328** | | Up-regulation | | (31,46,146) |
| **miR-342-3p** | | Up-regulation | In relapsing-remitting MS (RRMS) | (43) |
| **miR-345-5p** | | Up-regulation | | (31) |
| **miR-363-3p** | | Down-regulation | | (31,134) |
| **miR-365** | | Down-regulation | | (146) |
| **miR-370-3p** | | Up-regulation | In progressive MS patient | (43) |
| **miR-379-5p** | | up-regulation | Only in men. In RRMS relapse phase. | (67) |
| **miR-379-5p** | | Down-regulation | | (31) |
| **miR-409-3p** | | Up-regulation | In progressive MS patient | (43) |
| **miR-422a** | | Up-regulation | | (144) |
| **miR-432-5p** | | Up-regulation | In progressive MS patient | (43) |
| **miR-433-3p** | | Up-regulation | In progressive MS patient | (43) |
| **miR-448** | CD4+ T cells Th17 | Up-regulation | Induction of Th17 response correlated with the disease severity | (165) |
| **miR-450b-5p** | | Down-regulation | | (31) |
| **miR-451a** | | Up-regulation | In relapsing-remitting MS (RRMS) | (43) |
| **miR-454** | | | In RRMS vs. SPMS and controls | (130) |
| **miR-485-3p** | | Up-regulation | Mostly in S/PPMS groups | (43) |
| **miR.486.5p** | B cells | Down-regulation | | (28) |
| **miR-491-5p** | | Up-regulation | | (144) |
| **miR-494** | | Down-regulation | | (140) |
| **miR-499a** | | | | (156) |
| **miR-548ac** | CD58+ | | Correlation to Drosha and its cofactor DGCR8. | (166) |
| **miR-572** | White matter | Down-regulation | Overall, the expression was reduced in MS patients but also upregulated in SPMS and in RRMS during relapse. Suggested to be participant in CNS remyelination. | (40) |
| **miR-580** | | Down-regulation | | (31) |
| **miR-584** | | Up-regulation | | (144) |
| **miR-590** | CD4+ T cells Th17 | Up-regulation | Facilitate Th17 differentiation and pathogenicity by inhibiting Tob1, a suppressor of Th17 differentiation | (167) |
| **miR-599** | B cells | Down-regulation | | (47) |
| **miR-629** | | Up-regulation | | (134) |
| **miR-633** | | Up-regulation | Differentiates relapsing-remitting from secondary progressive MS | (161) |
| **miR-645** | | Up-regulation | | (146) |
| **miR-652–3p** | | Up-regulation | | (131) |
| **miR-664, miR-664a-3p** | | Up-regulation | | (31) |
| **miR-942–5p** | | Up-regulation | | (131) |
| **miR-1206** | | Down-regulation | | (31) |
| **miR-1275** | | Up-regulation | | (144) |
| **miR-3200-3p, miR-3200-5p** | Erythrocyte | Up-regulation | | (66) |
| **circ_0005402** | Leucocytes | Down-regulation | Circular RNAs a novel family of non-coding RNAs. Down-regulation is seen in MS patients. | (168) |
| **circ_0035560** | Leucocytes | Down-regulation | circular RNAs a novel family of non-coding RNAs. Down-regulation is seen in MS patients. | (168) |

is more common in females; therefore, applying gender stratification to miRNA studies also displayed a gender specificity. miR-223-3p and miR-379-5p were upregulated only in men, which influenced maintaining a stable MS course (82). In addition, some miRNA levels were not significantly different between male patients and controls; they displayed different levels in female patients (The most combination of MIR499A*C/T + MIR196A2*C)(85). Also, relapsing-remitting MS (RRMS) is mostly common in some specific genotypes in women (83). In another study comparing MRI findings of MS patients, it was shown a variable microRNA evidence between lesions and atrophy measures (84). In a study by Zailaie *et al.* 11 miRNAs that were upregulated in MS patients: miR-145, miR-376 c-3p, miR-128-3p, miR-191-5p, miR-26a-5p, miR-320a, miR-486-5p, miR-320b, miR-25-3p, miR-24-3p, and miR-140-3p. Conversely, eight molecules were downregulated: miR-572, miR-15b, miR-331-5p, miR-23a, let-7 c-5p, miR-16, miR-24, miR-137, and miR-181.

In terms of their potential usefulness, miR-145, miR-223, miR-128-3p, and miR-191-5p showed high sensitivity and specificity (85).

MS patients had a higher expression of miR-

150-5p and miR-155-5p, while miR-15a-3p and miR-34c-5p were lower; they also accessed these miRNAs among different types of MS, and down-regulation of miR-20a-5p, -33a-3p, and -214-3p and upregulation of miR-149-3p were seen associated with remission phase of MS patients (86). Although the maximum association was found with miR-126.3p and miR-200c.3p. These microRNAs are up-regulated during the remission phase of the disease (82,87).

In a most recent study in 2022, the expression of miR-146a and miR-155 was markedly observed in RRMS compared with the control group. miR-146a may be associated with vit. D deficiency and pathological disorders, and miR-155 may be associated with attack frequency (88).

In another study between RRMS patients with healthy controls. RRMS had significantly higher plasma concentrations of miR-34a and -125a-5p, whereas CTR had significantly higher plasma concentrations of miR-146a-5p. For miR-155, no significant difference was noted. During the 12-month follow-up, two patients experienced a clinical relapse due to increasingly severe disability. After 12 months of follow-up, there were no significant differences in circulating levels of miRNAs between patients with MRI activity and those without. In addition, patients who developed leukopenia during the 12-month follow-up period did not have significantly different circulating inflammatory-miR levels at baseline (89).

Immunoglobulins that can pass through BBB, free light chains (FLCs), kappa (KFLC), and lambda (LFLC) are associated with the disease, and as could be found in the serum and the CSF, they are available biomarkers. As proven, the KLFC in MS patients is increased so that it can be used as a high-sensitivity biomarker of MS (89-92).

Similarly, the other potential biomarkers of MS are known to be Inflammatory cytokines. Latest studies provided elevated IL-12B, CD5, eotaxin-1, MIP-1a, and CXCL9. Moreover, some of them, like (CCL11 and CCL20) were associated with progression and severity (93).

## Histone Acetylation

Another mechanism in epigenetic modifications is histone acetylation. Histones are known as proteins in the nuclei and act in folding the double-stranded chain of the DNA. Different reversible changes in the histones include acetylation and deacetylation. Acetylation at the lysine residues of the protein by histone acetyltransferases (HAT) plays a role in changing the expression of the gene and changes transcriptional factors binding sites on the gene. On the other hand, deacetylation mechanisms due to histone deacetylases (HDAC) accelerate histone methylations and inhibit transcription (14,94-96). Histone acetylation was dominantly seen in the white matter of the frontal lobes of aged patients with chronic MS. Also, in a subset of MS samples, an increased immune reactivity for acetylated histone H3 was found in nuclei of mature oligodendrocytes (97). Histone deacetylation is an important change in the CNS of MS patients and EAE mice with myelin repair, as HDAC1 and HDAC2 showed dysregulation in myelin synthesis and in the repair process of demyelinated sights. Also, *HDAC* genes showed an association with brain volume loss in the clinical phase of MS (76,97-99).

Decreased histone acetylation and increased DNA methylation in oligodendrocyte lineage cells would enhance myelin repair, which has been proposed as supportive for MS. On the other hand, the same epigenetic process in T-cells would augment their pro-inflammatory phenotype, which could exacerbate disease severity (100).

## Interaction of Environmental Factors and Epigenetics of MS

Different factors are known to have relevance with exact epigenetic changes in the pathogenesis of MS, such as vit. D (histone modification), cigarette (DNA methylation), EBV (miRNAs), and short-chain fatty acids from gut microbiota (histone modification). A possible cause of myelin damage and axonal loss might be due to oxidative stress, occurred by a decrease in antioxidant levels (25).

### Role of Vitamin D (vit. D) in MS

According to several studies, migration after the age of fifteen may put the migrant at risk of developing MS, similar to those living in the primary home country. However, in-migration before that age, the risk of MS would be the same as in the second country (2). Moreover, studies

on the worldwide distribution of MS incidence indicated a significant increase in northern and southern latitudes of the earth. Therefore, one of the causative factors for this pattern of disease distribution could be sunlight exposure, which has been reported to have a protective role in MS development and could be suggestive of the role of Vit. D as well (101). While the well-known act of Vit. D is calcium homeostasis; the other role of this element on immune regulation is determined to reflect on regulatory T-cell functions by direct effects on T-helper 1 (Th1) or Th2 cells (102).

Recent findings potentially support the role of Vit. D as an important environmental factor for MS (103). Higher serum levels of 25(OH) Vit. D has a protective effect on MS risk but not on the clinical course or the severity of the disease (104). One of the explanations for the role of vitamin D in the etiology of MS is the extensive genomic binding regions of the nuclear vitamin D receptor (VDR). The active form of Vit. D (1,25(OH)2D3 or calcitriol) acts like a transcription factor (TF) that influences multiple other TFs and co-regulators and also binds to regulatory hotspots in the genome (105,106). Proving the role of Vit. D in other studies, the results have shown conserved vit. D and especially the active form of it (1,25(OH)2D3) stimulates the expression of HLA-DRB1*1501 (107). Also, in the parts of the genome that are associated with autoimmune diseases, there are special changes in the VDR binding sites of genes (108). DNA methylation of VDR promotor, at exon 1c, could act as a gene regulator. Accordingly, higher levels of methylation have been observed concomitantly with 6.5-fold higher mRNA levels in RRMS patients (109).

Although different parts of the DNA have been proposed to be associated with Vit. D, there are also some other factors that could dynamically alter the epigenetic landscapes through binding to certain DNA regions and TFs (110). Interestingly, Vit. D could explain the month-of-birth effect on MS incidence as well. Therefore, decreased MS rate in November-born babies could be interpreted as a result of lower maternal 25(OH)D levels or lower sun exposure in the first twelve weeks of life in those babies (111). However, the role of Vit. D alone was not sufficient enough to explain the different incidence rates in males and females because, in different studies, decreased risk of MS has been associated with 25(OH)D, which was found similar in both sexes, as similarly found among the American population (112,113).

The role of Vit. D in MS incidence was found to be remarkably different in Latin America (LATAM) in comparison with European or Anglo-Saxon populations. Notably, whereas the low level of Vit. D is known as a risk factor for MS; it's not extensible for the tropical countries in LATAM, such as Mexico and Brazil. In these countries, the levels of Vit. D were the same in both patients and controls. The data of LATAM cases showed very different results in disease frequencies, the course of disease, and response to treatments compared to other regions. Different results may be due to the broad heterogeneity known in Latin America. Overall, this is another fact that indicates the important role of genetics and epigenetics in the pathogenesis of the disease (114). Vit. D also influences disease progression and long-term disability outcomes (115). In a cross-sectional study, the use of Vit. D supplements was associated with better physical and mental quality of life (116). However, long-term follow-up studies have shown that low serum Vit. D levels are correlated with more severe disability outcomes. Some of the genes coding Single nucleotide polymorphisms (SNPs) are considered to be associated with Vit. D metabolism. In the previous study, there has been no association between SNPs and the severity of the disease (117). Because clinical trials have not provided meaningful evidence, the exact role of Vit. D in controlling disease activity remains still unresolved (118).

**Body Mass Index (BMI)**

A prospective study in the US recording females' body size (at the ages of 5, 10, and 20 years), adulthood height, and weight at the age of 18 indicated over 2-fold increased risk of MS in obese women at the age of 18, compared to women with normal BMI. The results of this study suggested that obesity in late adolescence or young adulthood could be a more effective risk factor for MS than in childhood or adulthood (119).

Besides, Vit. D could somehow influence the role of BMI in the etiology of MS as well, as there have been reports of lower levels of this vitamin in obese patients (120).

To describe the role of BMI in multiple scle-

rosis, studies showed different types of DMA ceramide-dependent hypermethylation in specific genes of MS patients, which changes the monocyte count. Also, MS patients with high BMI have shown a more severe clinical appearance of the disease (121,122).

**Smoking**

Cigarette smoking is one of the most studied environmental risk factors of MS. Not only active smoking but also exposure to smoke, either passively or secondary, have been associated with an increased risk of developing MS, progression of disease, and clinical disability (123). Smoking plays an important role in the DNA methylation status and modeling of the methylation levels of a CpG site in the *AHRR* gene, which remarkably interacts with smoking load. It has also been reported that methylations would increase the expression level of AHRR in MS patients after smoking (124).

**Epstein Barr Virus (EBV) and Infectious Mononucleosis**

There is a hygiene hypothesis suggesting a double-edged sword effect for viruses in the pathophysiology of MS disease. Acquiring the infection in late childhood or adulthood would make the individuals more susceptible to MS, but it may confer immunologic protection if acquired in infancy or early childhood. According to this theory, exposure to infectious agents in early childhood affects the development of Th1 pro-inflammatory cellular immune response and leads to low MS risk. However, this idea has not been implicated with any particular pathogen (125). Supporting the hygiene hypothesis, EBV infection and infectious mononucleosis in older ages have significantly enhanced the risk of MS. However, as a paradox, those who completely escaped EBV infection conferred very low MS risk (126).

Recent studies in 2022 empowered the role of Epstein-Barr virus infection in the prevalence of autoimmune diseases, including MS (117).

**Human Endogenous Retroviruses and the Varicella Zoster Virus (VZV)**

The human endogenous retrovirus (HERV) sequences have been integrated into our genomes in several loci (127). HERV antigens are predominantly expressed on monocytes and B cells, which have been directly associated with MS and the disease activity (128,129) . The other virus suggested to be associated with MS is the varicella-zoster virus (VZV). By the way, in Mexico, VZV was the most frequent virus detected in relapses of MS patients (130).

**Gut Microbiota and Parasites**

The studies on animal models suggested that bacteria in the gut take part in the development of T-cells and cause MS-like neuro-inflammations (131). Despite the lack of human data to support the hypotheses of gut microbiota as a risk factor of MS, there have been studies proving that parasitic infections play a role in suppressing symptoms of MS, affecting T-cells and reducing proinflammatory cytokines by the mechanism of histone modifications (123,132).

**Shift Work and Melatonin**

Shift work during adolescence was observed to be correlated with an increased risk of MS (133). Considering the influence of night awakening on melatonin oscillations, some studies suggest the role of melatonin in inhibiting the differentiation of T-cells into pathogenic TH17 cells in vitro. But in the EAE exams, melatonin had therapeutic effects on neuro-inflammatory diseases (134). Regardless of the contrasting findings, melatonin may be responsible for shift work as a risk factor of MS.

## Epigenetics and New Therapeutic Approaches for MS

Epigenetic changes, as noted before, are reversible, and this makes them suitable targets for pharmacological therapies. Epigenetic therapy is known as the use of drugs to correct epigenetic defects (135). There are different kinds of epigenetic drugs, including DNA methyl transferase inhibitors (DNMTi), which have been involved in DNA damage repairs such as 5-aza-deoxycytidine, histone deacetylase inhibitors (HDACi) such as valproic acid or trichostatin, histone acetyltransferase inhibitors (HATi), histone methyltransferase inhibitors (HMTi), and drugs targeting microRNAs (miRNAs)(136-139). The results of microRNA analysis suggest that they have the potential to be used as biomarkers before and during the

treatment of MS patients. Various drugs considered to take part based on research findings on microRNAs (140). Among these drugs, DNMTi and HDACi were more commonly of investigation interest. The studies were mostly conducted on animal models of MS and experimental autoimmune encephalomyelitis (EAE), in which the effect of HDACi has been noticeable in mice and rats (141). Additional studies showed that valproic acid (VPA) in combination with thyroid hormone had benefits by restricting the pathogenic T-cells in rats, and VPA attenuated the disease severity and duration in EAE mice (136,142).

VPA was demonstrated to enhance CD4 Th1 and Th17 and also macrophages through down-regulating mRNA expression of the pro-inflammatory cytokines, such as interferons, tumor necrosis factor (TNF)-a, IL-1, and IL-17 in the spinal cord. In addition, it has increased the expression of IL-4, which is known as an anti-inflammatory cytokine (139). The HDACi trichostatin drug, used in the relapsing phase of EAE in mice, has reduced the spinal cord inflammation and demyelination axonal loss and, therefore, reduced the disability (141).

## Glatiramer Acetate

Moreover, the level of brain-derived neurotrophic factor (BDNF) has been reduced in serum and CSF of RRMS patients compared to controls, which has been increased during the post-relapse phase in patients, and it was remarkably reversed by induction of the MS drug Glatiramer acetate (143,144). In another study, SIRT1, the histone deacetylase, was found to be impressed by glatiramer acetate treatment, which seems to be a biomarker in order to evaluate response to treatment in MS (145).

## Natalizumab and Fingolimod

As discussed above, the level of miRNAs would increase the expression of IL-17a, TNFα, and IFN γ. Furthermore, in the patients with a high level of miR-155, IgG titers were increased significantly. Also, in the patients who were treated with Natalizumab, miR-155 and miR-26a were down-regulated. Therefore, miRNAs may be used as biomarkers to determine the efficacy of treatment as well (146). Moreover, while the CSF level of miR-150 decreased following treatment with Natalizumab, the plasma level decreased as well. As this level was increased following treatment with Fingolimod, it is suggestive of the role of immune cells as a source of miR-150 (146).

MiR-17-5p, which was previously proven in autoimmunity, was up-regulated in CD41 cells of MS patients. Further studies demonstrated that miR-17-5p affects the expression of genes of phosphatase, tensin, and phosphatidyl-inositol-3-kinase (147). On the other hand, miR-17 down-regulation was associated with the upregulation of its target genes, such as *PTEN*, *BIM*, *E2F1*, and *p21*. This miRNA was downregulated by Natalizumab therapy and upregulated during relapse (146). Further studies indicated modified expression of in miR-125a-5p, let-7c, miR-642, miR-320, miR-320b and miR-629, after 1 to 6-month of therapy with Natalizumab (147).

MiRNA levels in natalizumab-treated patients have been measured in several clinical studies. miR-155, miR-132, miR-146a,z, and miR-26a are recommended as one of the starting drugs for patients with active phase RRMS by the American Academy of Neurology. It was treated with natalizumab, and after six months of treatment, the levels of these miRNAs were reduced. $CD8^+$ T-cell activation and $CD4^+$ T-cell proliferation are induced by miR-155 (148,149).

## Dimethyl Fumarate

The FDA approved dimethyl fumarate, an oral medication, in 2013 to treat RRMS. Further studies in 2021 reported reduced levels of miR-125a-5p, miR-146a-5p, and miR-155 after prescription of dimethyl fumarate treatment. By inhibiting the release of proinflammatory cytokines and chemokines from endothelial cells, miR-125a-5p reduces neuroinflammation and improves blood-brain barrier integrity (89).

Several adverse effects, including progressive multifocal leukoencephalopathy (PML), were associated with dimethyl fumarate, diroximel fumarate (150,151), and fingolimod, which had moderate benefits for the patients (152).

Ofatumumab (153) and ublituximab, an SC-administered anti-CD20 mAb (154), confer modest protection against MS. In addition, rituximab, an anti-CD20 antibody, which has been shown to provide protection against RRMS (155,156), has been discontinued due to patent expiration.

The nematode Auanema freiburgensis was treated with the HDAC class I inhibitor butyrate and valproic acid, and the broad-spectrum HDAC inhibitor TSA increased histone 3 and 4 acetylation (157). Recent research has revealed the transmission of epigenetic or epigenetic information not based on DNA sequence, from yeast to humans over generations (158).

Detecting cell-free circulating DNA (cfDNA) released from target organs is a new method in epigenetic therapies. As determined in 2016, higher cfDNA levels in the relapse phase of MS patients may help to distinguish cellular debris of target tissue from normal debris (159). While the latter study has been performed based on cell type-specific DNA methylation, further cfDNA-based studies were done by using myelin-producing oligodendrocytes-specific markers (160).

The next method, the miRNA 'sponge method,' was used as a tool to probe miRNA functions. Using vectors encoding these sponges into cultured cells, the sponges could selectively bind to endogenous miRNAs and allow translation of the target mRNAs on the exact region of the genome (161). As another method, receptor-coated nanoparticles or micro-vesicles provided a specific cell type-guided delivery in peripheral immune cells (162,163). In the study by Meijer *et al.* has shown that BACH1 and STAT1 are transcription factors that have been described to participate in gene regulation of oligodendrocyte progenitor cells (OPCs). Furthermore, single-nucleotide polymorphisms (SNPs) interfered with these regions in mouse and human OLG when examined for treatment with enhanced activated interferon-gamma (IFN-g)(53). These overlaps could add new therapeutic targets for future studies.

## Expert Opinion Section

The HDACi and DNMTs inhibitors may result in side effects due to their global action and low specificity (164,165). Therefore, specific epigenetic therapy that acts only on certain pathogenic loci without affecting the other regions is required. Recent studies support the ability of the CRISPR-dCas9 system to play a role in transcription targeting histone PTMs and DNA methylation (166-169). So far, these studies are only in preclinical stages and there is still an open way to reach safe clinical outcomes.

Based on different strong evidence on the role of epigenetics on MS and DNA Methylation Status in Associated Genes with MS **(Table 1)**, including hypo – hyper methylations on the specific LOCs and Alterations in miRNA Expression Levels in MS **(Table 2)**. Lately, drugs that act on the methylation status of genes have been used in the treatment of MS. In the case of these, DNA methylation changes differ significantly in the different phases of MS and even patterns of the disease relapsing-remitting, progressive or secondary progressive MS. For example, *HLA-DRB5* Hyper-methylation in specific subtype of RRMS or miR-155-5p Down-regulation $CD4^+$ T cells Specially in SPMS. As an opinion personalized gene therapy based on HLA typing of the gene on the exact laking LOCs may open a new way to treatment of the patients.

## Conclusion

Although after decades of studies on MS, the certain cause of this neurodegenerative, disabling disease remains under a shadow of doubt, an increasing number of valuable data has been provided on the role of genetics and environmental factors in the pathophysiology of this disease. Furthermore, specific epigenetic changes found in MS patients are suggested to be a link between genetics, lifestyle, and environmental factors. Most of all, there is valuable evidence of DNA methylation changes and histone acetylation in MS patients on the special loci of the genome in this disease. Due to the reversibility of epigenetics, it seems to be a good pathway to MS treatment. Accordingly, as the pathogenesis of the disease is believed to be interacted by these changes in the epigenome, epigenetic therapies will act on the exact region of the genome. However, more studies are still needed on epigenetic changes, and as the epigenetic drugs have been used only in preclinical trials, more trials are required for the common use of these medications in MS.

## Conflict of Interest

The authors declare to have no conflict of interest.

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