

The genetics of multiple sclerosis: review of current and emerging candidates

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Abstract: Multiple sclerosis (MS) is a complex disease in which environmental, genetic, and epigenetic factors determine the risk of developing the disease. The human leukocyte antigen region is the strongest susceptibility locus linked to MS, but it does not explain the whole heritability of the disease. To find other non-human leukocyte antigen loci associated with the disease, high-throughput genotyping, sequencing, and gene-expression studies have been performed, producing a valuable quantity of information. An overview of the genomic and expression studies is provided in this review, as well as microRNA-expression studies, highlighting the importance of combining all the layers of information in order to elucidate the causes or pathological mechanisms occurring in the disease. Genetics in MS is a promising field that is presumably going to be very productive in the next decade understanding the cross talk between all the factors contributing to the development of MS.

Keywords: multiple sclerosis, genetics, gene expression, microRNA

Introduction

Multiple sclerosis (MS) is one of the most common causes of neurological disability in young adults, affecting more than 2.1 million people in the world. MS is a complex disease, meaning that the causes of the disease are several and are not fully understood. It is widely accepted that MS is a demyelinating disorder in which the immune system attacks the myelin, and a clear neurodegeneration component exists. Among the factors proposed to play a role in the complex interaction network that leads to MS, we can undoubtedly find genetic ones.

The first genetic factor related to the disease was the human leukocyte antigen (HLA) locus in the 1970s. This locus is located in the short arm of chromosome 6, in a region called major histocompatibility complex (MHC). The genes inside this region encode highly polymorphic cell-surface glycoproteins that are key components of the immune system. Since that first discovery, a great deal of effort has been made on understanding how this mechanism works.¹ Nowadays, it is clear that HLA by itself cannot explain the whole genetic component of MS. Moreover differences by genetic load² or sex³ have been reported, highlighting the complexity of how the HLA locus exerts its influence in the disease.

The new genomic tools arriving during the last decades confirmed the association of the HLA class II haplotype DRB1*15:01–DQA1*01:02–DQB1*06:02 with MS. More genetic factors had to be discovered, and that idea drove the research to association studies aimed at finding non-HLA genetic factors.

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Association studies

The search for non-HLA genetic factors started with a hypothesis-based candidate-gene approach. During this period, hundreds of studies were performed in these genes, some trustworthy and some more controversial.

Few conclusions about candidate genes can be ascertained from this period, but these studies were the first steps towards understanding that this kind of approach requires big collaborative projects to obtain reliable data. Moreover, these studies demonstrated the importance of taking into account the population background.^{4,5}

Classic linkage studies were not the best approach in a disease like MS, due to the lack of big families and the fact that the expected genetic factors should have a modest effect in the phenotype in a multigenic way. With the arrival of new technologies, more ambitious projects were undertaken: the Genome-Wide Association Studies (GWAS). GWAS involved a hypothesis-free strategy that screens the whole genome by tagging linkage disequilibrium blocks. This approach uses single-nucleotide polymorphism (SNP) data created from the HapMap project⁶ as reference to scan the whole genome and identify parts of the genome associated with the disease. It has less power than linkage studies, but better resolution, being the best approach to find differences in a multigenic disease such as MS, in which we expect that common variants are the ones contributing to the susceptibility of the disease.

Due to the number of statistical tests that will be run, these studies need huge samples to reach enough statistical power. Briefly, in these studies the *P*-value should cross a restrictive threshold (usually established at 10^{-7} or 10^{-8}) to obtain a list of strong candidate genes that can be, finally, replicated in small populations. Some of the replications have failed, and in some cases the association has been controversial. On the other hand, pathway-based analysis has been proposed as an approach to rescue SNPs not passing the restrictive significance requirements of GWAS,⁷ but that might have an influence on the susceptibility combined with other SNPs. Applying this idea, Baranzini and colleagues identified for the first time several neural pathways as having a potential role in MS susceptibility by using a network-based approach in an elegant manner.⁸

Before August 2011, several GWAS were performed, confirming the previously known HLA DRB*1501 as having the strongest association with MS and identifying at least 14 other regions associated with the disease containing several genes.⁹⁻¹³ Moreover, some meta-analyses were

carried out with these data, adding regions to the list of associations.^{14,15}

However, the information coming from the different association studies is not always overlapping and/or validated. MS is a heterogeneous disease, with a wide spectrum of phenotypes. This reality affects the homogeneity of the groups created for the studies, adding noise to the data. A good and standardized clinical characterization of the patients is critical in any association genetic study. In this sense, a biomarker being able to distinguish different patient groups would help in a better classification, and so, refined data would be available. Moreover, the projects were looking for several (maybe hundreds) of tiny differences, each of them contributing in a modest way.

In August 2011, *Nature* published a seminal study performed by the joint efforts of the International Multiple Sclerosis Genetics Consortium and the Wellcome Trust Case Control Consortium 2.⁹ A total of 465,434 SNPs were studied in 9772 cases and 17376 controls, and after a refined analysis a list of non-HLA candidate genes related to MS was presented (Table 1).⁹ Most of the previously reported genes were confirmed and 29 new associations were described. These genes led to the conclusion that cell-mediated immune mechanisms play a primary role in the disease, and opened new ways to understand the disease based on immunological pathways and the misregulation of the immune system. From the publication of this seminal study to date, several groups have started to validate some of these results in their own populations. As a result, new candidates, such as *ANKRD55*,¹⁶ have emerged to join the last suggested list.

Functional studies should follow all these genetic studies in order to investigate the link between the genetic background and the pathophysiology of the disease. As an example of this, one of the genes listed in the GWAS, *TNFRSF1A*, has been functionally studied, with the conclusion that the MS-associated allele directs the expression of a novel form of the tumor necrosis factor (TNF)-R1 protein, which can block TNF and mimics the effect of TNF-blocking drugs.¹⁷ More functional studies that would get closer to the genetic data and clinical practice should come in the next few years to give medical relevance to the genetic discoveries.

The next revolution in the tools to study genetics came from the “-omics” techniques. Next-generation sequencing goes a step further in understanding how susceptibility is affected by candidate genes. The whole sequence of the genes will surely provide new data on how changes on DNA could affect protein function and relate them to MS.

Table 1 Non-HLA candidate genes found to be associated with multiple sclerosis

Chr	rsID	Putative gene of interest	Risk allele
1	rs4648356	<i>MMEL1</i>	C Previously identified
1	rs11810217	<i>EV15</i>	A Previously identified
1	rs1335532	<i>CD58</i>	A Previously identified
1	rs1323292	<i>RGS1</i>	A Previously identified
1	rs7522462	<i>KIF21B</i>	G Previously identified
3	rs2028597	<i>CBLB</i>	G Previously identified
3	rs2293370	<i>TMEM39A</i>	G Previously identified
3	rs2243123	<i>IL12A</i>	G Previously identified
5	rs6897932	<i>IL7R</i>	G Previously identified
5	rs4613763	<i>PTGER4</i>	G Previously identified
6	rs13192841	<i>OLIG3</i>	A Previously identified
8	rs1520333	<i>IL7</i>	G Previously identified
10	rs3118470	<i>IL2RA</i>	G Previously identified
10	rs1250550	<i>ZMIZ1</i>	A Previously identified
11	rs650258	<i>CD6</i>	G Previously identified
12	rs1800693	<i>TNFRSF1A</i>	G Previously identified
12	rs12368653	<i>CYP27B1</i>	A Previously identified
12	rs949143	<i>MPHOSPH9</i>	G Previously identified
16	rs7200786	<i>CLEC16A</i>	A Previously identified
16	rs13333054	<i>IRF8</i>	A Previously identified
17	rs9891119	<i>STAT3</i>	C Previously identified
19	rs8112449	<i>TYK2</i>	G Previously identified
20	rs2425752	<i>CD40</i>	A Previously identified
1	rs11581062	<i>VCAM1</i>	G Novel independent
2	rs12466022	No gene	C Novel independent
2	rs7595037	<i>PLEK</i>	A Novel independent
2	rs17174870	<i>MERTK</i>	G Novel independent
2	rs10201872	<i>SPI40</i>	A Novel independent
3	rs11129295a	<i>EOMES</i>	A Novel independent
3	rs669607	No gene	C Novel independent
3	rs9282641	<i>CD86</i>	G Novel independent
5	rs2546890	<i>IL12B</i>	A Novel independent
6	rs12212193	<i>BACH2</i>	G Novel independent
6	rs802734	<i>THEMIS</i>	A Novel independent
6	rs11154801	<i>MYB</i>	A Novel independent
6	rs17066096	<i>IL22RA2</i>	G Novel independent
6	rs1738074	<i>TAGAP</i>	G Novel independent
7	rs354033	<i>ZNF746</i>	G Novel independent
8	rs4410871	<i>MYC</i>	G Novel independent
8	rs2019960b	<i>PVT1</i>	G Novel independent
10	rs7923837	<i>HHEX</i>	G Novel independent
12	rs10466829	<i>CLECL1</i>	A Novel independent
14	rs4902647	<i>ZFP36L1</i>	G Novel independent
14	rs2300603	<i>BATF</i>	A Novel independent
14	rs2119704	<i>GALC</i>	C Novel independent
18	rs7238078	<i>MALT1</i>	A Novel independent
19	rs1077667	<i>TNFSF14</i>	G Novel independent
19	rs874628	<i>MPVI7L2</i>	A Novel independent
19	rs2303759	<i>DKK1</i>	C Novel independent
20	rs2248359	<i>CYP24A1</i>	G Novel independent
22	rs2283792	<i>MAPK1</i>	C Novel independent
22	rs140522	<i>SC02</i>	A Novel independent
4	rs228614	<i>NFKB1</i>	G Novel independent

(Continued)

Table 1 (Continued)

Chr	rsID	Putative gene of interest	Risk allele
11	rs630923	<i>CXCR5</i>	C Novel independent
16	rs2744148	<i>SOX8</i>	G Novel independent
17	rs180515	<i>RPS6KB1</i>	G Novel independent
20	rs6062314	<i>TNFRSF6B</i>	A Novel independent

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Using exome sequencing, rare variants in one of the genes suggested by the 2011 GWAS, *CYP27B1*, have been found in MS families.¹⁸ This variant causes an arginine-to-histidine change at position 389 (R389H) of the protein and leads to complete loss of enzyme activity, resulting in lower levels of calcitriol. These results suggest that low levels of calcitriol may play a key role in the interaction of genetic and environmental factors associated with MS. However in a recent paper, Ban et al studied this association in 495 multiplex families, 2092 single affected families, 4954 cases, and 3583 controls, and were unable to find evidence supporting this association.¹⁹ Recently, using a similar approach, a family of four generations and a validation cohort of 2104 MS trio families have been studied. The authors found a rare variant in the *TYK2* gene of modest effect on MS risk affecting a subset of patients (0.8%).²⁰

An ambitious approach using these -omics techniques tried to elucidate the genetic and epigenetic load in MS by studying two monozygotic twins discordant for the disease. Whole-genome sequence, messenger RNA (mRNA) transcriptome, and the epigenome of CD4⁺ lymphocytes were studied.²¹ However, no new information on variants related to MS was revealed, pointing to other epigenetic characteristics, or in this concrete case to a strong environmental cause.

The immune component is undeniable in MS, and has been widely supported by experimental data (reviewed by Nylander and Hafler).²² In the last few years, several GWAS in other autoimmune diseases began to identify risk alleles, and the existence of a number of shared genes became evident.²³ Taking into account the possibility of sharing susceptibility genes by chance, the statistical evidence supports the idea of the so-called “autoimmunome” network, whose genes would give a predisposition for autoimmune disease. Sophisticated bioinformatic tools, such as iCTNet (Integrative Complex Traits Network), a plugin for Cytoscape²⁴ (one of the mostly used software packages for network visualization), have been developed²⁵ to help researchers in the understanding of these networks. This tool allows the visualization of

multiple relations between diseases, genes, proteins, organs, and therapeutic drugs as a network.

As an example of this immune component, an antigen called KIR4.1 has been reported recently as one of the targets of the autoimmune response in MS patients, based on evidence that antibodies against this protein have been found in serum of 46.9% of MS patients.²⁶ However, these results have to be validated to be included in the MS paradigm, since this is the only work describing this antigen as target of the immune response in MS.

The GWAS revolution gave us exciting results, but maybe there is more to tell in this story. The Encyclopedia of DNA Elements (ENCODE) Project Consortium,²⁷ with the aim of identifying all functional elements of the genome, has been able to assign a biochemical function to 80% of the genome. As a global conclusion from this project, we could say that what used to be called “junk DNA” due to our lack of knowledge about its function must be renamed because of the huge quantity of information these regions have about regulation and modification of the coding genes.

Regions of transcription, transcription-factor association, chromatin structure, and histone modifications have been systematically mapped, providing new information about the organization and regulation of our genes and genome.²⁸ Interestingly, it was found that SNPs reported to be associated with disease by GWAS are enriched within noncoding functional elements. This observation suggests that GWAS data in MS should be revisited. GWAS are based on informative SNPs that are representative of regions of the genome. The candidate genes selected in the last GWAS are protein-coding genes inside the region defined by the informative SNP, but nonprotein-coding genes should also be considered as candidate genes, given that they may regulate the transcription events of the surrounding regions.

In fact, in the last decade, an increasing number of articles have described noncoding RNAs as an important piece of the gene-expression regulatory network, and their implication in several neurodegenerative and autoimmune diseases has been described (see below). In that sense, several studies highlight the importance of microRNAs (miRNAs; a noncoding family of small RNAs) in the etiology of MS, given that they regulate the expression levels of specific mRNA. Therefore, future projects combining both miRNA and gene expression might be of great interest to better understand the pathophysiology of the disease and treatment effects.

Besides noncoding RNAs, epigenetic alterations have been characterized in many diseases, including MS (reviewed by Huynh and Casaccia).²⁹ For instance, promoter methylation

status regulates the expression of several genes involved in MS pathogenesis, such as *PAD2*, *SHP-1*, and *IL17A*. *PAD2* is overexpressed in MS patients' brain and peripheral blood due to hypomethylation of its promoter.³⁰ Reduced *SHP-1* expression has been found in peripheral blood mononuclear cells (PBMCs) from MS patients compared to healthy donors, and abnormally high methylation of its promoter could contribute to this deregulation.³¹ The expression of interleukin (IL)-17 cytokine, which has been related to MS pathophysiology,³² is regulated by promoter methylation and an intergenic enhancer.³³ Moreover, an inefficient histone deacetylation has been related to an impaired remyelination in old mouse brains,³⁴ and in MS patients increased acetylation has been detected, associated with high levels of transcriptional inhibitors of oligodendrocyte differentiation.³⁵

In addition, an interaction of all these epigenetic mechanisms is being depicted. DNA methylation³⁶ and histone deacetylation³⁷ regulate the expression of miRNA, which in turn, can also regulate DNA methylation.³⁸

To make the picture more complex, recently another genetic marker has been related to pediatric MS, the copy-number variation (CNV). In this work, ten new CNVs not overlapping with any CNV regions previously reported in the database of genomic variants were discovered.³⁹ Despite not having found any causative variants, this study showed useful data suggesting that CNVs could be another character to take into account in MS.

Expression studies

Microarray analysis of gene expression has been used to explore several aspects of the pathological mechanisms involved in MS, providing the MS community with a huge quantity of data and valuable new discoveries.

Large-scale gene-expression studies performed on the brains of both MS patients and experimental autoimmune encephalomyelitis (EAE) mice have revealed genes and pathways implicated in the pathophysiology of the disease in the central nervous system (CNS).

In 1999, Whitney et al measured the expression of 5000 genes in the normal white matter and two acute lesions of brain tissue obtained in autopsy from a 46-year-old male MS patient.⁴⁰ They found 62 differentially expressed genes (DEGs), 29 of them upregulated in acute lesions. These 29 were genes implicated in cell adhesion, structure and transport, myelin formation, cell growth, signaling, cell cycle, and homeostasis and immunity.

In 2001, Ibrahim et al analyzed the expression of 11,000 genes in the spinal cord of C57BL/6 EAE mice

immunized with myelin oligodendrocyte glycoprotein 35–55.⁴¹ Mice were killed at 16 days (disease onset) and 22 days after immunization (peak of disease), and RNA was isolated from homogenized spinal cords. A total of 213 genes were found to be differentially expressed when compared to control mice, 100 of them consistently throughout the disease. The 213 genes were implicated in antigen processing and presentation, immunity, extracellular matrix, cell adhesion and matrix degradation, signal transduction, transcription, cell structure, movement, and secretion, functions in the CNS, cell division, and death.

The same year, Chabas et al confirmed in the spinal cord of EAE mice the elevated expression of osteopontin they had previously found in a large-scale sequencing experiment of cDNA libraries obtained from plaques of MS patients.⁴² Osteopontin-deficient mice proved to be resistant to progressive EAE.

In 2003, Graumann and colleagues performed a microarray experiment on 18 white-matter samples obtained from postmortem brains of nine MS patients and seven control cases in order to describe the earliest gene-expression changes that lead to lesion. They compared the expression of 3528 genes in normal-appearing white matter from patients and normal white matter from the corresponding brain areas of the control cases and found an upregulation of genes involved in cellular homeostasis and neural protective mechanisms triggered in ischemic preconditioning. Several HIF-1 α and hypoxia-induced genes, phosphatidylinositol 3-kinase/Akt pathway genes, and genes involved in preconditioning pathways were found to be overexpressed in normal-appearing white matter from patients.⁴³ The same year, Arnett et al studied changes in gene expression related to demyelination and remyelination in mice.⁴⁴ They analyzed the expression of 6000 genes in the corpus callosum of mice during diet with cuprizone (which causes demyelination), and after putting them back onto a normal diet (which allowed remyelination) in normal mice and mice lacking *TNF- α* (*TNF α ^{-/-}*). Then, they checked for gene-expression differences between demyelination and remyelination and between successful remyelination vs unsuccessful remyelination in *TNF α ^{-/-}* mice. The most prominent alterations happened in immunological genes: MHC genes were found strongly upregulated in microglia and astrocytes during demyelination, remyelination, and *TNF- α* stimulation. MHC-null mice showed delayed remyelination and demyelination.

In 2005, Camelo et al studied the effect of the histone deacetylase inhibitory drug trichostatin (TSA) in the CNS of EAE mice.⁴⁵ Trichostatin has been shown to ameliorate

disability in the relapsing phase of EAE. They isolated RNA from the spinal cords of normal mice, EAE mice, and TSA-treated EAE mice, and measured the expression of 12,426 genes with microarrays. They found that TSA induced the expression of antioxidant, anti-excitotoxicity, and proneural growth and differentiation genes. In contrast, the drug inhibited target genes of the proapoptotic E2F transcription factor pathway.

Finally, in 2006 Dutta et al conducted a whole-genome gene-expression experiment in postmortem motor cortex samples from six MS patients and six healthy controls.⁴⁶ They measured the expression of 33,000 genes, and 555 DEGs were identified between the nonlesion motor cortex from patients and the tissue from controls. Of the 555 genes, 488 were underexpressed and 67 overexpressed in the MS samples and Expression Analysis Systematic Explorer software classified them as being related to oxidative phosphorylation, synaptic transmission, cellular transport, the MHC, antigen presentation, antigen processing, and translational initiation. Among others, 26 mitochondrial genes and several presynaptic and postsynaptic genes of gamma-aminobutyric acidergic transmission were found to be downregulated.

Microarray studies have also been carried out on peripheral blood of MS patients with the aim of elucidating the pathogenic molecular and cellular mechanisms acting in the immune system.

In 2003, Bomprezzi et al analyzed the expression of 7500–9000 genes in PBMCs of 27 MS patients and 19 controls.⁴⁷ Applying a classification algorithm, they found more than 1000 gene pairs that could distinguish MS patients from healthy controls and came up with a final list of 53 discriminatory genes. From the biological roles of the genes in the list, they concluded that the activation of autoreactive T cells is of primary importance in MS. The following year, Achiron et al published a microarray study of PBMCs obtained from 26 MS patients and 18 healthy controls.⁴⁸ An 1109-gene signature was identified irrespective of disease state or immunomodulatory treatment. The signature comprised genes implicated in T-cell activation and expansion, inflammation, and apoptosis. Another transcriptional signature of 721 genes distinguished MS patients in relapse from those in remission and contained genes involved in cellular recruitment, epitope spreading, and escape from regulatory immune surveillance.

In 2008, Arthur and colleagues performed a whole-genome expression experiment on whole blood of ten MS patients in relapse, another ten patients in remission, and ten healthy controls.⁴⁹ They found 989 genes to be upregulated in patients in relapse. They found 1525 DEGs in patients in

relapse when compared to healthy controls. Of these, 989 were upregulated and 536 downregulated, *ALOX5* and *TGFβ1* showing the most significant overexpression. When comparing patients in remission with controls, 1317 genes were found to be deregulated: 655 upregulated and 662 downregulated. *ALOX5* was overexpressed also in remission. Among all differentially expressed genes, three had already been associated with MS: *TGFβ1*, *CD58*, and *DBC1*.

In summary, microarray studies on MS have provided valuable information on molecular pathways and cellular mechanisms implicated in the disease. The studies performed in the CNS of both MS patients and EAE mice have revealed deregulated genes involved in cell adhesion/structure/movement, signaling/signal transduction, cell cycle/growth/division/death, immunity, and antigen presentation and processing in two or more of the studies reviewed, highlighting the importance of both immune-mediated attack and a healing response of the neural/glial tissue in the pathophysiology of the disease in the CNS. On the other hand, the studies performed on peripheral blood have consistently shown a central role of T-cell activation, expansion, and inflammation, with the data coming from the DNA studies mentioned at the beginning.

However, as shown by a systematic review of gene-expression studies in MS, the reproducibility of the results between the different studies has been low; not that much at the level of molecular pathways or biological processes, but certainly for the lists of differentially expressed genes. Seven microarray studies on peripheral blood of MS patients were included in this systematic review, and from the 2017 unique genes, only 229 (11.35%) were found to be differentially expressed in MS in the same direction in two or more studies.⁵⁰

Several factors have contributed to the low reproducibility of microarray results at the differentially expressed gene level in multiple sclerosis. (1) Gene expression at the mRNA level is a very dynamic process influenced by a huge amount of variables. Moreover, interindividual variability even among healthy subjects used as reference contributes to noise. (2) Besides being a complex disease (where many genetic and environmental factors contribute to its development), MS is a very heterogeneous disease with great differences in disease progression and response to treatment between patients. The general view is that the term MS is probably an umbrella covering a whole set of different molecular pathologies converging at the clinical level. (3) Differences in starting material (PBMC vs leukocytes vs whole blood; white matter vs grey matter) and in the microarray platform

used (Affymetrix, Illumina, Agilent) have contributed to low reproducibility of the results. (4) During the past decade, several algorithms have been developed for both the normalization and summarization of raw data (robust multichip algorithm, MAS5, PLIER, etc) and to obtain differentially expressed gene lists (fold-change thresholds, multiple-testing corrected *t*-test, rank product, significance analysis of microarrays, linear models for microarray data, etc). Moreover, the filtering step performed for reducing noise and relieving multiple-testing stringency has been applied in different ways in the studies. Although the most widely used filtering method implicates the elimination of low-variable genes, the removal of genes with no significant expression is also used. The problem resides in the criteria to decide whether a gene is expressed or not, which depends on the algorithm used for summarization and normalization of data. Each of the different combinations of the alternative approaches at each of the analysis steps extracts a different part of the information contained in the data, and so gives a different deregulated gene list as an output.

Two main strategies will have to be used in the future to overcome the problem of reproducibility of whole-genome expression analysis results: (1) finding criteria to create molecularly homogeneous patient groups, and (2) establishing minimum standard procedures for experimental design and data analysis. The aforementioned GWAS results could be used in the future to cluster patients based on common genetic background linked to predisposition to develop MS. Besides, more effort in the direction established by the MicroArray Quality Control (MAQC) project started by the FDA needs to be made in order to reach a consensus on the minimum set of standard procedures to follow in whole-genome expression studies (<http://www.fda.gov/ScienceResearch/BioinformaticsTools/MicroarrayQualityControlProject/default.htm>).

miRNAs in MS

One of the most widely studied types of noncoding RNAs are miRNAs. They are 20–24 nucleotide-long, single-stranded RNAs that regulate the expression of target mRNAs at posttranscriptional level. miRNAs have a role in almost all biological processes, like development, cell differentiation, proliferation, and cell death, and also in several pathological events like cancer, neurodegeneration, or autoimmunity.^{51–54} In the last years, several works have studied miRNA expression in MS patients in a variety of tissues (peripheral blood, brain, and CSF) and in an EAE animal model (Table 2). All these groups found alteration in miRNA-expression levels

Table 2 Summary of microRNA (miRNA)-expression studies carried out both in patients and in an experimental autoimmune encephalomyelitis (EAE) model

Paper	Sample	Tissue	Technique	Number of analyzed miRNA	Dysregulated miRNA [†]	Experimentally validated target	Function
Blood							
Otaegui et al ⁶⁰	DC: 4 relapsing MS, 9 remitting MS, 8 HC. VC: 4 relapsing MS, 3 remitting MS, 7 HC 20 RRMS, 19 HC	PBMCs	qPCR (TLDA)	364	miR-18b, miR-599, miR-96		
Keller et al ⁶¹		Whole blood	Microarrays	866	miR-145, miR-186, miR-664, miR-20b, miR-422a, miR-142-3p, miR-584, miR-223, miR-1275, miR-491-5p miR-326	Ets-1	Promote TH-17 differentiation
Du et al ⁵⁵	25 relapsing MS, 18 remitting MS, 42 HC, 11 NMO	Peripheral blood leucocytes	qPCR	7			
Lindberg et al ⁶²	DC: 8 RRMS, 10 HC. VC: 15 RRMS, 10 HC	CD4 ⁺ T cells, CD8 ⁺ T cells, B cells	qPCR (TLDA)	365	CD4 ⁺ : miR-485-3p, miR-376a, miR-497, miR-193a, miR-126, miR-17-5p, miR-34a, CD8 ⁺ : miR-629, miR-30a-3p, miR-149, miR-497, B cells [‡] : miR-497, miR-92, miR-135b, miR-153, miR-189, miR-422a miR-17, miR-20a		
Cox et al ⁶³	59 RRMS, 37 HC	Whole blood	Microarrays and qPCR	733			
De Santis et al ⁶⁴	DC: 12 MS, 14 HC. VC: 10 MS, 10 HC	CD4 ⁺ CD25 ⁺ T cells	Microarrays	723	miR-29c, miR-107, miR-210, let-7i, miR-15a, miR-19a, miR-19b, miR-138-2*, miR-324-3p, miR-301a, miR-338-5p, miR-22, miR-512-3p, miR-564, miR-886-3p, miR-106b, miR-29a, miR-93, miR-489, miR-148a, miR-590-5p, miR-223, miR-221 miR-128, miR-27b, miR-340	miR-128 and miR-27b → BMI, miR-340 → IL-4	Inhibit TH2 cell differentiation and contribute to proinflammatory TH1 response
Guerau-de-Arellano et al ⁶⁵	5 PPMS, 12 RRMS, 5 SPMS, 16 HC	CD4 ⁺ T cells	qPCR (TLDA)	667			
Fenoglio et al ⁶⁶	16 relapsing MS, 6 SPMS, 7 PPMS, 19 HC	PBMCs	qPCR	5	miR-21, miR-146a, miR-146b		

(Continued)

Table 2 (Continued)

Paper	Sample	Tissue	Technique	Number of analyzed miRNA	Dysregulated miRNA [†]	Experimentally validated target	Function
Boneschi et al ⁶⁷	DC: 7 RRMS, 6 SPMS, 6 PPMS, 14 HC. VC: 5 RRMS, 2 SPMS, 3 PPMS, 10 HC 4 MS, 4 HC	PBMCs	Microarrays and qPCR	1145	let-7g, miR-150		
Siegel et al ⁶⁸		Plasma	Microarrays	>900	miR-614, miR-572, miR-1979, miR-648, miR-422a, miR-1826, miR-22, miR-155		
Paraboschi et al ⁶⁹	10 MS, 10 HC	PBMCs	Microbead-based technology	22			
Sievers et al ⁷⁰	DC: 10 untreated RRMS, 10 NTZ treated RRMS, 10 HC. VC: 30 RRMS, 7 HC	B cells	Microarrays and qPCR	1059	miR-106b, miR-19b, miR-181a		
Brain							
Junker et al ⁵⁷	20 MS, 9 HC	Active and inactive white matter MS brain lesions	qPCR	365	miR-155, miR-130a, miR-30a-3p, miR-23a, miR-199a*, miR-152, miR-146b, miR-650, miR-326	miR-155, miR-326 and miR-34a → CD47	Downregulation of CD47 in brain resident cells promotes macrophage phagocytosis of myelin
CSF							
Haghikia et al ⁷¹	17 RRMS, 30 SPMS, 6 PPMS, 39 OND	CSF	qPCR	760	miR-922, miR-633, miR-181c		
EAE							
Ponomarev et al ⁷²	4–5 mice	Peripheral macrophages and microglia	qPCR	31	miR-124	C/EBP- α	Transform macrophages from an activated into a quiescent phenotype. Administration of miR-124 during preclinical stage prevented disease symptoms
Murugaiyan et al ⁵⁸	8 mice	CD4 ⁺ T cells (spleen, lymph node, CNS)	qPCR	1	miR-155		Induce expression of cytokines needed for TH1 and TH17 cell response
Zhu et al ⁵⁶	2 mice	Inflammatory lesions	qPCR	641	miR-23b, miR-30a, miR-146a, miR-214	miR-23b → TAB2, TAB3, IKK	Repress autoimmune inflammation
Lescher et al ⁷³	20 EAE, 10 WT mice	Spinal cord	qPCR (TLDA)	586	miR-155, miR-326, miR-142-3p, miR-146a, miR-146b, miR-21, miR-184, miR-19a		
Guan et al ⁵⁹	WT, CD44 KO mice	CD4 ⁺ T cells	Microarrays and qPCR	609	let-7e	IL-10	Modulation of TH differentiation and development of EAE

Note: [†]Only validated miRNAs or the most remarkable ones in each study have been listed. [‡]Not validated

Abbreviations: DC, discovery cohort; VC, validation cohort; OND, other neurological diseases; PPMS, primary progressive multiple sclerosis; SPMS, secondary progressive multiple sclerosis; HC, healthy controls; EAE, experimental autoimmune encephalomyelitis; WT, wild-type; KO, knockout; CNS, central nervous system; CSF, cerebrospinal fluid; NTZ, Natalizumab; PBMC, peripheral blood mononuclear cells; qPCR, quantitative polymerase chain reaction; TLDA, TaqMan Low Density Arrays.

in MS patients compared to healthy controls, and therefore an implication for miRNAs seems to be evident. Yet little overlap is observed among different studies, probably due to the difference in the miRNA-profiling technology, the complexity of the tissue being studied, and the relatively small sample size of all studies (Table 2).

Several works have been able to establish a concrete function for some miRNAs related to MS. For instance, Du and colleagues found that miR-326 was upregulated specifically in MS patients with relapse (not in remitting patients) and more prominently in CD4⁺ T cells compared to CD8⁺ T cells and non-T cells.⁵⁵ Then, they demonstrated that miR-326 expression in CD4⁺ T cells promotes T-helper (TH)-17 differentiation by targeting *Ets-1*, a known negative regulator of TH-17 cells. EAE pathology was milder in mice receiving an miR-326 inhibitor, and in fact downregulation of this miRNA was observed in a group of patients having received glucocorticoid treatment for the relapse. As the authors discuss, all these observations suggest that miR-326 is involved in the acute phase of the disease, and furthermore is under treatment effect. Another study also described a link between IL-17-associated inflammation with miRNA regulation. Researchers observed a downregulation of miR-23b in several autoimmune diseases or their animal models (EAE included) due to high expression of IL-17.⁵⁶

Another noteworthy study analyzed miRNA-expression profiles in active and inactive MS lesions. Using luciferase assays, the researchers found that miR-155, miR-326, and miR-34a targeted CD47 3'UTR.⁵⁷ The authors proposed that the overexpression of these three miRNAs in MS brains promotes the downregulation of CD47 on brain resident cells, thereby triggering macrophage phagocytosis of the myelin. miRNAs are also involved in the differentiation of CD4⁺ T cells, as reported by studies in animal models. These demonstrated that miR-155 and let-7e shift CD4⁺ T cell polarization towards a TH-1 and TH-17 phenotype (Table 2).^{58,59}

All these works show how miRNAs are involved in the misregulated immune system in MS and may help us to better understand the mechanisms of the pathology. At the same time, they offer a new research field to find another piece of the MS pathophysiology puzzle.

Concluding remarks

To cut a long story short, the genetic history in MS during the last 40 years shed light on the etiology of the disease, highlighting the importance of the collaborative projects, and

without any doubt, bringing out the complexity of MS. In the future, we will talk about MS gene panels for resequencing containing around 100–150 genes, but with this information we will be covering only part of the genetic component. The study of epigenetic factors such as methylation, CNV, and posttranscriptional modifications, which in some way are the hinge that links genetic background and environment or lifestyle, are some of the challenges for the next decade. Furthermore, validation studies, meta-analysis and even additional GWAS are being performed, and thus in coming years we might see the discovery of new candidate genes not only related to the risk but also to the evolution of the disease and to the drug response.

On the other hand, learning from other complex diseases like Parkinson's, we might see in the future a scenario where small groups of MS patients have a clear genetic component that explains the disease. Next-generation sequencing techniques and family studies will probably clarify this picture. The past has taught us that these challenges should be tackled by joint efforts, and that in this way the next decade will possibly come with promising and exciting results in the field of MS genetics.

Disclosure

The authors report no conflicts of interest in this work.

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