The Analysis of SNPs' Function in miR-21 and miR146a/b in Multiple Sclerosis and Active Lesions: An In Silico Study

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ABSTRACT: Multiple sclerosis (MS) is a central nervous disorder caused by several factors. Studies have recently shown that non-coding RNA such as miRNA could participate in MS initiation, progression, and active lesion. This study aims to theoretically analyze the potential impact of single-nucleotide polymorphisms (SNPs) on mir-21 and mir-146a/b, which has been previously demonstrated as MS microRNA signature. To fulfill this purpose, the SNPs were investigated for functionality through several online tools, including miRNA-SNP, SNP2-TFBS, RBP-Var, and RNAfold. Furthermore, SNPs of miR-21 and miR-146a/b that exist in pre-miRNA, mature miRNA, and promoter area were extracted; moreover, miRNA and RNA-binding protein interactions were analyzed. This article presented a list of validated SNPs that could affect the expression or function of miR-21 and miR-146a/b for the future practical study of MS and active lesions.

KEYWORDS: miR-21, miR-146a, miR-146b, single-nucleotide polymorphism (SNP), multiple sclerosis (MS), RNA-binding protein

Introduction

Multiple sclerosis (MS) is an autoinflammatory disease of the central nervous system (CNS) that leads to neurodegeneration and demyelination. Individuals' genetic background and environmental triggers, including viral infections, smoking, and vitamin D deficiency, predispose patients for autoimmune response against self-antigens. Although outstanding research have been done in this field so far, more studies are required to elucidate the pathogenesis.[1–3]

Plaques or lesions and focal areas of myelin loss within the CNS are the pathological hallmarks of MS. Lesions are distributed throughout the CNS and are more tended to happen in optic nerves, subpial spinal cord, and brainstem, cerebellum, and juxtacortical and periventricular white matter regions. Destruction of minor myelin proteins happens quickly, within 1 to 3 days, and the existence of minor myelin protein destruction causes macrophages to denote early active lesions or demyelination.[4]

All these factors probably could affect the molecular activity, proliferation, differentiation, and apoptosis of cells. Small non-coding RNA, microRNA (miRNA), influences a

wide range of cell activities.[2] miRNAs, approximately 22 nucleotides, are involved in the post-transcriptional regulation of genes. miRNAs play an essential role in regulating gene expression; they are also involved in differentiation and apoptosis. miRNAs exert their effect through complex production. RNA-induced Silencing Complex (RISCs) destroy mRNA strands for the silencing of gene expression. The Drosha enzyme converts pri-miRNA with a hairpin structure containing approximately 100 nucleotides into 1 or more precursor miRNAs called pre-miRNAs. Then, pre-miRNA, which contains 70 nucleotides, is transported to the cytoplasm by RNA-binding protein (RBP), Exportin-5. In the cytoplasm, the Dicer enzyme processes pre-miRNA into mature miRNA consisting of 20 to 24 nucleotides, which cleaves the double-stranded miRNA precursor into 2 strands.

miRNA and Argonaute protein make up the RISC complex. Mature miRNA can identify the target through a particular sequence called a seed, thus preventing or even destroying its translation. A single miRNA can target numerous mRNAs and can have a pivotal effect on cellular functions. Previous studies of miRNAs in various diseases have identified signature microRNAs linked with diagnosis, staging, progression, prognosis, and response to treatment.[5] Aberrant expression of miRNA has been observed in various autoimmune diseases

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such as MS, type 1 diabetes (T1D), spontaneous systemic autoimmunity, systemic lupus erythematosus (SLE), and other types of neurodegenerative diseases such as Alzheimer's disease and Parkinson disease.[6–9] In this article, the role of microRNAs as a signature in MS is discussed. We also investigated the effect of a few signature miRNAs and their potency in causing MS.

As it has been proved, polymorphisms in genes could probably affect the gene function, expression, splicing, stability, protein interaction, and interaction with non-coding RNAs.[6] Polymorphisms—would also influence the microRNAs -likewise genes. Muñoz-San Martín et al investigated 28 potential microRNAs in 46 patients with MS by TaqMan assays and qPCR.[10] They asserted that miR-21 and miR-146a/b were the signature miRNAs in MS and their elevation had a direct relationship with the number of active lesions.[10] On the one hand, miR-21 and miR-146a/b upregulation could cause MS; on the other hand, some single-nucleotide polymorphisms (SNPs) could affect miRNAs in many ways. This study aims to investigate SNPs of miR-21 AND miR-146a/b and evaluate their potential impression on MS by an in silico study. Finally, we also compare our findings with available experimentally confirmed results.

Material and Methods

This theoretical study was approved by the ethics committee of the IR.HUMS.REC.1400.299 University of Medical Sciences. Therefore, we have adapted the data from a study titled, "Analysis of miRNA Signatures in CSF Identifies Upregulation of miR-21 and miR-146a/b in Patients With Multiple Sclerosis and Active Lesions." miR-21, miR-146a/b, and their common targets were chosen for this study.

Finding SNPs in miRNA Functional Areas

We were able to investigate the potential effect of SNPs through the relevant databases available at the An-yuan Guo's Bioinformatics Lab Web site (http://bioinfo.life.hust.edu.cn/guo_lab#!/), especially the miRNASNPV3 database (http://bioinfo.life.hust.edu.cn/miRNASNP#!/), to delve into the potential impact of SNPs on miRNA maturation and function. The miRNASNP database consists of 5 major modules, including SNPs in pre-miRNAs of humans and other organisms, SNP-induced gain and loss of miRNA targets, seed regions, and 3'UTR of target mRNAs20.[11]

Finding SNPs in miRNA Promoter Areas

miRNAs have several promoters; all microRNA promoters engaged in MS in one way or more have been extracted. Promoter areas were identified using the Ensembl genome browse. The identified promoter areas were checked using the UCSC Genome Browser (https://genome.ucsc.edu/) via the variation section, dbSNP 153 Track Settings, and Maximum display mode; subsequently, SNPs in the promoter area of microRNAs were extracted. The potential role of SNPs in transcription factor binding (TFB) was analyzed using the SNP2TFBS Web interface (https://ccg.epfl.ch/snp2tfbs/). Position weight matrix (PWM) calculation has been applied to the human genome assembly GrCH37/hg1 from the curated JASPAR CORE 2014 vertebrate motif database to extract the data as mentioned earlier. Changes leading to alteration of transcription factor binding areas were identified using SNPViewer, which can, through its RSID identifier, search for SNP.[12]

Interaction of miRNAs and RBP

In the next step, the RBP-Var database was used to account for the effect of SNPs on protein affinity pattern and RNA binding and delve into post-transcriptional interaction and regulation of miRNA (miRNA function, maturation, and transportation from the nucleus to cytoplasm). A variety of tools, including CLIPdb, starBase, RBPDB, dbSNP v142, RADAR, GEO, CISBP-RNA, DARNED, miRanda, miR-NASNP, TargetScan, MuTher, SCAN, seeQTL, GTEx, Harvard, and dsQTL Browser, were used to provide the data sources required in the RBP-Var. To select and distinguish the cis-motifs conserved in RBP-RNA interaction (motif matches) in the transcriptome, RBP-Var uses all positional weight matrices of 2 databases CISBP-RNA RBPDB in the AURA database. In this route, all potential k-mers are aligned with the transcriptome using MAST in the MEME suite, a motif discovery algorithm, to suggest the final motif mapping with its default parameters, a match score > 0 ($P < .0001$). All SNPs involved in the miRNA gene (related to pri-miRNA, pre-miRNA, and mature miRNA) were taken into account and inserted into the search box of the dbSNP archive. We implemented the RNAfold Web server (http://rna.tbi.univie.ac.at/cgibin/RNAWebSuite/RNAfold.cgi), which can predict locally optimal secondary structures of single-stranded RNA or DNA sequences to calculate miRNAs MFE and estimate the impact of SNPs on the local miRNA secondary structure. The difference between WT and ALT alleles is considered ΔG. The RNAfold result is a string presentation of the structure and folding energy written in the standard output stream. With the -p option, a postscript file containing the matrix also creates the possibility of base pairing (ViennaRNA package 2.0; Figure 1).

Association of miRNAs in the Pathogenesis of MS

To confirm which miRNAs are involved in MS's pathogenesis, we used Human Disease MicroRNA Database 3.0 (HMDD v3.0) (http://www.cuilab.cn/hmdd) as a valid database that provides experiment-supported data on microRNA linkages and human disease, and we marked them for correlating with MS's conditions (HMDD v3.0: a database for experimentally supported human microRNA-disease associations).
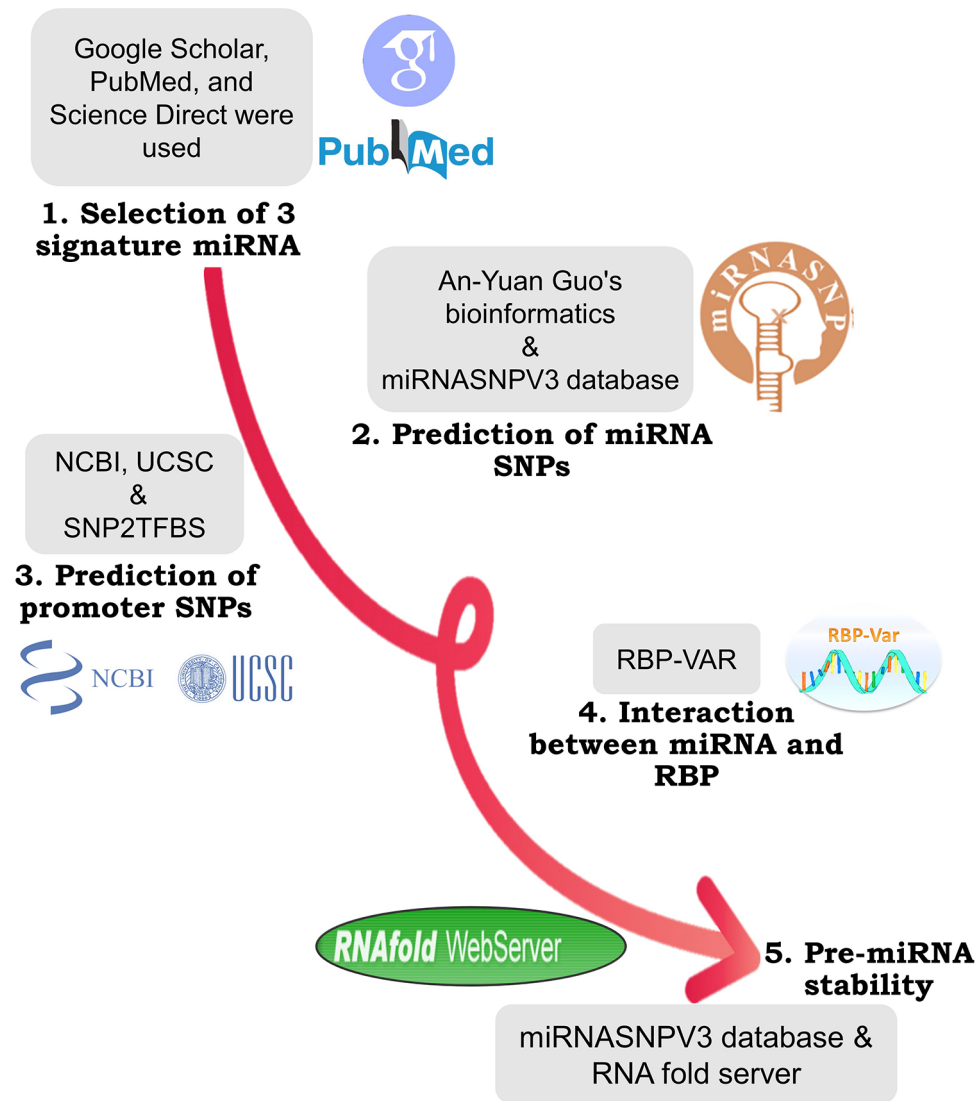
Figure 1. This figure depicts Web sites and databases which were used in this study.
miRNA indicates micro-RNA; SNP, single-nucleotide polymorphism.

Results

Selection of miRNAs

This research aimed to analyze variation in miRNA signatures such as miR-21 and miR146a/b in cerebrospinal fluid and blood samples among patients with MS and active lesions. Accordingly, the abovementioned microRNA signatures and their targets were gathered from the relevant databases. Basic information, including precursor ID, accession number, Genome position, host gene, and mature miRNA for these microRNAs, are shown in Table 1. Table 2 contains tissue type, miRNA target genes, and their expression level. We also predict all probable targets through the miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/) Web site and are presented in Table 1.

Finding SNPs in functional motifs of miRNAs

In this step, the SNPs in miRNA genes were computationally analyzed. miRNA SNP3.0, the SNP database in miRNA, was used to analyze SNPs in functional motifs of miRNAs. Finally, the server implements 2 target prediction tools, TargetScan, and miRmap, to predict miRNA target loss and gain.

If one miRNA's target of wild-type allele gene displays in both servers, but not in either, the mutant allele is recognized as "lost." Conversely, if one wild-type target allele was not observed in both servers but observed in either of the mutant alleles, then the mutant allele is considered "gain." The investigation of the SNP's functional effect on pre-miRNA processing (for mature miRNA production) was conducted via ΔG calculation, which was the difference between minimal free energy (MFE), calculated by RNAfold online server, of wild-type and SNP miRNA. Moreover, SNPs' accurate position and wild-type alleles were displayed at the pre-miRNA, mature miRNA, or seed sequences (Table 3).

An analysis of SNPs' effects on microRNA secondary structure stability was performed. Figure 2A and B is 2 examples of a calculation of miRNA stability. RNAfold predicted the

Table 1. Basic information related to 3 miRNAs that are deregulated in MS and active lesion.

MICRORNAS	GENOME POSITION	HOST GENE	MATURE MIRNA
hsa-mir-21	chr17:59841266-59841337[+]	VMP1: Exonic	hsa-mir-21-3p
			hsa-mir-21-5p
hsa-miR-146a	chr5:160485352-160485450[+]	Intergenic	miR-146a-3p
			miR-146a-5p
hsa-miR-146b	chr10:102436512-102436584[+]	Intergenic	miR-146b-3p
			miR-146b-5p

Table 2. List of miRNA target genes correlated with MS.

MICRORNA	TARGET	METHOD	EXPRESSION	REFERENCES
miR-146a/b	*IRAK1* EGFR	TaqMan and qPCR	Upregulation	Munõz-San Martín et al[10]
	IRAK1, TRAF6	TaqMan	Upregulation	Fenoglio et al[13]
miR-21	Bcl-2, CD69, and the T-cell receptor	TaqMan	Upregulation	Fenoglio et al[13]
miR-21-5p	*SOCS6*	TaqMan	Downregulation	Sanders et al[14]

Abbreviations: Bcl2, BCL2 apoptosis RegulatorCD69; *EGFR*, epidermal growth factor receptor; *IRAK1*, interleukin 1 receptor–associated kinase; MS, multiple sclerosis; *TRAF6*, TNF receptor–associated factor 6; *SOCS6*: suppressor of cytokine signaling 6.
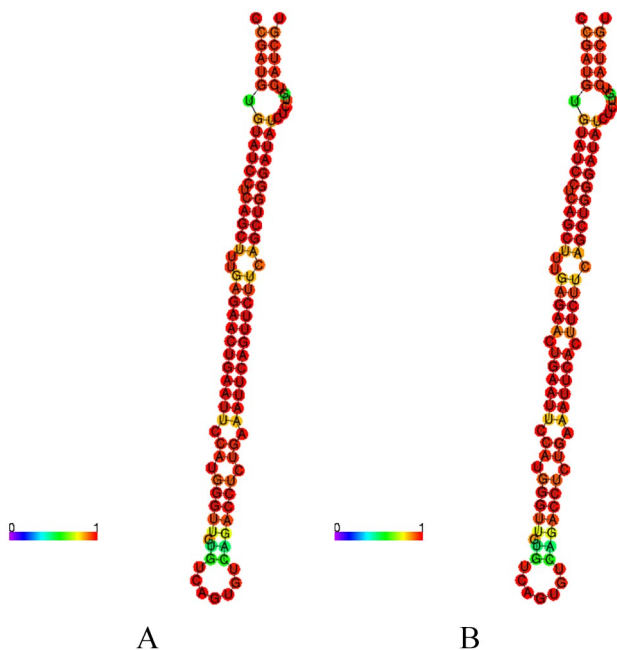
Figure 2. (A) Pre-miRNA-146a with wild-type allele G at the position of 70. The optimal secondary structure with minimum free energy of −39.00 kcal/mol is drawn by an RNAfold online server. (B) Pre-miRNA-146a mutant allele C at the position of 70. The optimal secondary structure has minimum free energy of −32.80 kcal/mol drawn by an RNAfold online server.

structure of the hairpin pre-miR-21 and pre-miR-146a and the minimum free energy (MFE) of the thermodynamic group (ΔG): (1) with wild-type allele and (2) with mutant allele.

Therefore, the pre-miRNA structure is expected to be more thermodynamically stable with less MFE.

rs2910164 was predicted in hsa-miR-146a-3p with −2.8 ΔG in the seed element to be a possible SNP at this site among all SNPs predicted for pre-miRNA, mature, and seed region of 3 microRNAs. In the second rank, rs944564808 in mature form with −2.3 ΔG was estimated to affect hsa-miR-146b-5p function.

In silico investigation of SNPs that occur in miRNA promoter genes

Furthermore, SNPs' impact was investigated on promoter regions of signature miRNAs that target genes directly involved in MS and active lesions. Putative TF binding sites from the human genome assembly GrCH37/hg1 (for wild-type allele) and 1000 Genomes project (for a mutant allele with MAF ≥ 0.001) that merged were calculated via PWM calculation (PWM score) from the curated JASPAR CORE 2014 vertebrate motif database. These SNPs affect miRNA expression level—increase, decrease, or no effect. The location of the SNPs, their specific numbers, and their effects are given in Table 4. As shown in Table 4, miRNAs have several promoter regions, each of which has multiple SNPs. Nevertheless, not all of them affect expression. The Scorediff column describes the difference in PWM scores between alternating (mutant) and reference (wild-type) alleles. So, a positive score means a more significant PWM score in the alternating allele. In addition, all 9 SNPs that are listed in the third and fourth columns of Table 4 may affect

Table 3. Data collected from miRNASNPv3 shows miR-21 and miR146a/b SNPs, their frequency, position, allele, region, and enthalpy. Finally, the effect of SNPs on microRNA expression is shown.

MATURE MIRNA	SNPS ID	POSITION	REF/ALT	FREQ.	REGION	ΔG	EXPRESSION CHANGES
hsa-miR-21-3p	rs772365223	chr17:59841316	C/G	–/–	Seed	0	Mild
	rs540457553	chr17:59841317	C/T	–/–	Seed	1.7	Mild
	rs1332902069	chr17:59841318	A/G	–/–	Seed	0.7	Mild
	rs1360004766	chr17:59841319	G/T	1/–	Mature	6	Down
	rs1314650731	chr17:59841321	C/T	1/–	Mature	1.8	Mild
	rs747325963	chr17:59841322	G/C	1/–	Mature	4	Down
	rs747325963	chr17:59841322	G/A	1/–	Mature	3.9	Down
	rs1161253978	chr17:59841325	G/A	–/–	Mature	−0.4	Mild
hsa-miR-21-5p	rs779350466	chr17:59841283	G/A	–/–	Mature	4.1	Down
	rs1182837899	chr17:59841288	A/T	1/–	Mature	0	Mild
	rs1253814784	chr17:59841290	G/A	–/–	Mature	5.9	Down
has-miR-146a	rs76149940	chr10: 104196269	C/T		pre-miRNA	1.9	Mild
hsa-miR-146a-3p	rs1323710653	chr5:160485408	C/T	–/–	Mature	2.6	Down
	rs2910164	chr5:160485411	C/G	0.3158/0.6842	Seed	−2.8	Up
	rs772931224	chr5:160485420	A/G	–/–	Mature	0.7	Mild
	rs1343966428	chr5:160485421	G/C	–/–	Mature	6	Down
	rs529455292	chr5:160485429	G/T	1/–	Mature	5.1	Down
hsa-miR-146a-5p	rs772652917	chr5:160485372	T/A	–/–	Mature	0.3	Mild
	rs1423068484	chr5:160485386	C/A	–/–	Mature	2.3	Down
	rs780488034	chr5:160485387	A/T	1/–	Mature	1.3	Mild
	rs780488034	chr5:160485387	A/G	1/–	Mature	0.7	Mild
	rs997376292	chr5:160485388	T/C	–/–	Mature	0	Mild
	rs997376292	chr5:160485388	T/A	–/–	Mature	−0.4	Mild
	rs1400440934	chr5:160485389	G/T	–/–	Mature	1.3	Mild
	rs1395832667	chr5:160485390	G/A	–/–	Mature	1.9	Mild
miR-146b	rs76149940	chr10:102436512	C/T		pre-miRNA	1.9	Mild
	rs201978234	chr10: 104196337	C/A		pre-miRNA	2.9	Down
hsa-miR-146b-3p	rs1367942461	chr10:102436557	G/A	–/–	Mature	−1.2	Mild
	rs1425832655	chr10:102436558	C/T	–/–	Seed	0.1	Mild
	rs944616447	chr10:102436564	G/A	1/–	Seed	6.5	Down
	rs1467375133	chr10:102436569	C/A	1/–	Mature	3.8	Down
	rs768018523	chr10:102436577	G/C	1/–	Mature	5.2	Down
hsa-miR-146b-5p	rs944564808	chr10:102436520	T/C	1/–	Mature	−2.3	Up
	rs762226942	chr10:102436522	A/C	–/–	Seed	1.3	Mild
	rs762226942	chr10:102436522	A/G	–/–	Seed	1	Mild
	rs1161834919	chr10:102436542	G/A	–/–	Mature	−0.9	Mild

Abbreviation: SNP, single-nucleotide polymorphism.

Table 4. List of SNPs located in the promoter region and their effect on transcription factor binding performed by SNP2TFBS Web server.

MIRNA	PROMOTER REGIONS	MORE PWM SCORE ON ALT (SCOREDIFF+) MISSING IN REF	MORE PWM SCORE ON REF (SCOREDIFF−) MISSING IN ALT	NEUTRAL
hsa-miR-146a	Chr5: 160,478,800-160,479,001	rs140226561	–	–
has-miR-146b	Chr10: 102,431,200-102,437,801	rs11398443 rs114914917	rs11592718 rs114914917 rs1536309	rs114914917 rs1572530 rs74878980 rs1536309 rs76149940 rs373098722
	Chr10: 102,438,200-102,438,401	–	–	–
hsa-mir-21	Chr17: 59,836,200-59,847,801	rs552584953 rs572747163	rs141255741	rs7218748 rs62081825
	Chr17: 59,848,800-59,849,401	–	–	–

Abbreviations: Alt, any other allele found at that locus; PMW, position weight matrices, a positive score implies a higher PWM score in the alternate allele; Ref, the allele in the reference genome; SNPs, single-nucleotide polymorphisms.

Table 5. Catalog of SNPs in miRNAs and their impact on miRNA-RBP interaction pattern provided by RBP-Var2 database.

MIRNA'S NAME	SNP'S NAME	CHROMOSOME LOCATION	RBP	RBP-VAR SCORE
hsa-miR-21	rs540457553	Chr17: 57918677-5718678	AGO, AGO1, AGO2, DGCR8, LIN28B, PTBP1, WDR33	α
hsa-miR-146a/b	rs76149940	Ch10: 104196268-104196269	AGO, PTPB1	β

Abbreviations: SNP, single-nucleotide polymorphism; RBP, RNA-binding protein.
α: Minimal possibility to affect RBP binding.
β: Likely to affect RBP binding, RNA secondary structure.

miRNA expression via affecting transcription factor–binding sites for the transcription factor to bind.

According to the results, SNPs are located in the gene's promoter of the mir-21 (rs552584953 and rs572747163) and the miR-146a (rs140226561), which could give rise to the loss of the original transcription factor recognition site (loss of function). On the contrary, the rs141255741 polymorphism in the hsa-miR-21 promoter probably generates the novel transcription factor recognition site (gain of function). The rs7218748 and rs62081825 polymorphisms in hsa-miR-21 are predicted to have a neutral effect on the motif-identifying transcription factors, that is, the promoter. Similarly, rs7218748 and rs62081825 polymorphisms in hsa-miR-21 are predicted to neutrally affect the transcription factor identification site's motif, that is, the promoter. In silico investigation of SNPs' effect on their interaction with RBPs showed that because miRNA-RBP interactions play a vital role in facilitating miRNA processing, it can be one of the most critical factors influencing functional miRNA levels.[19]

RNA-binding proteins identify RNAs with a specific sequence and bind to them to participate in various processes. Thus, the interaction between miRNAs and RBPs is another issue that is affected by SNPs.

As indicated in Table 5, the most affected RBPs are the AGO family and PTBP1. The AGO family is expressed ubiquitously and binds to miRNAs or siRNAs to guide post-transcriptional gene silencing by destabilizing the mRNA or translation repression. The PTBP1 has a role in pre-mRNA splicing. Moreover, rs76149940 is more likely to impact microRNA attachment to the AGO family and PTBP1 than rs540457553, due to their RBP-Var score.

Finally, as the result of HMDD 3.0 demonstrates, all 3 microRNAs have a 100% role in MS pathogenicity.

Discussion

Emerging data confirmed that microRNAs play a vital role in the initiation and progression of neurological disease and may be potential therapeutic targets for these diseases (MicroRNA Therapeutics in Neurological Disease).

According to a system biology study that introduced miR-21, miR-146a, and miR-146b as miRNA signatures, the *IRAK1* and *EGFR* genes were identified as a common targets for 3 microRNAs. The upregulation of the 3 mentioned miRNAs was correlated with multiple sclerosis and active lesions.[10] Distinguishing functional SNPs in genes and analyzing their effects on phenotypes may present the pivotal key to knowing the potential influence of such alterations. In other words, the expression of a miRNA and SNPs of miRNA should be considered to determine a biomarker or target for the therapy and diagnosis.[20] Despite many studies on the association of
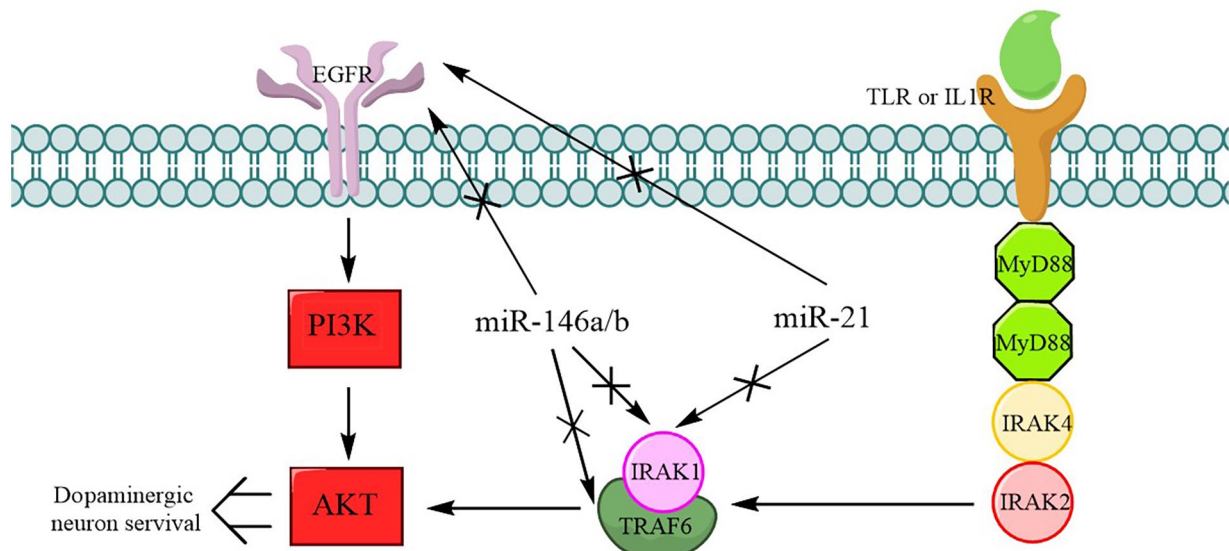
Figure 3. The probable pathway of miR-21 and miR-146a/b. This schematic picture represents a conclusion from previous studies and suggests that miR-21 and miR-146a/b could lead to dopaminergic neuron survival.[15-18] The picture is drawn by ChemDraw 16.00.

polymorphisms with some diseases, the SNPs' effect on molecular mechanisms is not completely elucidated.[21,22] Specialists have developed many efficient bioinformatics tools and important algorithms to enlighten the potential effects of SNPs.[23] This study analyzed genetic variations in miRNA signatures such as miR-21 and miR146a/b among patients with MS and active lesions.

Due to some obscure reasons, there is a conflict between expression levels of miR-21 and miR-146a/b in previous studies. One probable cause of matter could be found in Table 4, where the results present functional SNPs in the promoter area. The miR-146a variation changes its transcription levels and makes it susceptible to several neurological diseases, particularly MS.[24] Here, we also briefly used theoretical methods to introduce and predict SNPs' effect on all miRNA motifs by somehow influencing their structure, expression, and function. Allelic imbalances lead to gradual adaptation in gene expression; the consequences can be accumulated with age and lead to neurodegenerative disease. Cis-acting SNPs alter the microRNA-mediated regulation of human brain–expressed transcripts.[25] Some miRNAs have a tissue-specific or evolutionary stage expression pattern and may maintain tissue identity and function.[26] Generally, each miRNA can regulate various target genes and the polymorphisms in their target-binding site can have 3 outcomes, which include (1) neutral, (2) gain of novel targets, or (3) the loss of the original target.[6,27] Thus, a change in the binding site of miRNAs to their target genes or proteins alters the miRNA function; moreover, it alters their expression pattern by affecting the promoter area.[6,27,28] Labib et al showed that genotyping miRNA-146a polymorphism (rs2910164) and its target gene *IRAK1* (rs3027898) was significantly correlated with susceptivity, clinical manifestations, and progressive condition in patients with systemic lupus erythematosus (SLE) and MS. Hence, it seems possible that rs2910164 polymorphism is involved in patients' susceptibility to SLE and

MS.[29] The previous publication revealed that interleukin-1 receptor–associated kinase 1 (IRAK1) could act as a potential target of miR-146a.[30] The IRAK1 was found to be an important molecule in the Toll-like receptor (TLR)-4-myeloid differentiation primary response 88 (MyD88) signal transduction pathway. Following integrating with MyD88, the IRAK1 is phosphorylated and subsequently forms a complex with TRAF6. This complex, after that, activates nuclear factor (NF)-κB kinase (IKKβ) and induces the activation and translocation of the NF-κB transcription factor into the nucleus. Activated NF-κB promotes the production and secretion of a large extent of inflammatory cytokines.[31] To illustrate more, mir-146a is engaged in the signalling pathway of TLR4, and IRAK1 is a regulatory target of mir-146a. Once pathogens or antigen-antibody complexes activate TLR, it activates the associated adaptor protein IRAKI by the downstream MyD88-dependent signalling pathway, leading to TRAF6, IKKβ, and eventually NF-κB (Figure 3).[32] Epidermal growth factor receptor (EGFR) is a member of the tyrosine kinase receptor and contributes to various roles ranging from embryogenesis to the growth and development of numerous tissues. It has been stated that EGFR plays neurotrophic functions in both the central and peripheral nervous systems. Due to EGFR's central role in the maintenance and progression of the nervous system, modification in its signalling leads to the onset of several neurological disorders.[33] Another study that functionally examined the effect of rs2910464 polymorphism in the Chinese population revealed that this polymorphism was not associated with epilepsy.[34] According to genome-related studies, approximately 90% of disease-associated SNPs occur in non-coding regions. However, the functional validation of these non-coding remains mostly unexplained.[35] Variants placed in non-coding regions could affect gene expression through the changes within TBF affinity. Furthermore, their specific corresponding regulatory motifs may significantly be correlated with susceptibility to

diseases. The SNPs that affect transcription factor binding affinity can exert microRNA expression in 3 types of outcomes, including (1) being neutral, (2) leading to primary (original) TFs which cannot bind to specific sites, or (3) being different and leading to a novel transcription factor that binds to altered TFBS. The results in Table 4 show the catalogue of regulatory SNPs that affect transcription factor–binding sites for transcription factor affinity. Li et al[24] evaluated the association between 2 SNPs (rs2910164 and rs57095329) in hsa-mir-146 and MS in the Chinese population and ultimately found rs2910164 to be functional. Their results are in line with our findings presented in Table 4. Therefore, before investigating the possible impact of SNPs on genes in an experiment, the functional analysis should be conducted in silico using robust bioinformatics tools to assess their functionality.

Moreover, as the results in Table 5 elucidate, these SNPs not only affect the microRNA expression and faction, but it is also able to be disruptive to microRNA processing. Both rs540457553 and rs76149940 affect the attachment of RBPs, playing a vital role in this process, such as the AGO[36] family and DGCR8.[37] These findings could provide a set of data for a functional study examining the effect of polymorphisms in regulating gene expression in various diseases and understanding how distinct genome sequences cause unique differences between individuals. However, although such bioinformatics studies could determine that these substitutions may affect miRNA levels and their function, laboratory studies are needed to confirm the practical outcome of this SNP candidate.

Conclusion

Altogether, we used several tools to analyze the functional effect of SNPs on miRNAs, which have been theoretically validated as an MS signature biomarker. To capture a perfect molecular picture of the effects of these SNPs, we examined multiple aspects of the mined SNP's effect on miRNA and function for comprehensive research, including structural stability, pre-miRNA processing level, and miRNA-target interplay, transcript level, and miRNA-RBPs interaction. This research theoretically presented a panel of candidate underlying SNPs in various functional elements of the miRNA gene that could be reviewed for future experimental study in MS disease control.

Author Contributions

MM and HB wrote the manuscript. MM, HB, HA, MA, AAN, and PM collected the data. PM revised the literature and contributed to the conception and design of the study. Eventually, all authors contributed to the critical revision, edition, and final approval of the manuscript.

Ethical Approval

This research was approved by the ethics committee of Hormozgan University of Medical Science (Ethical code: IR.HUMS.REC.1400.299).

Guarantor

PM is guarantor of this article.

Informed Consent

Not applicable.

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