Original Article

Prediction of Single-Nucleotide Polymorphisms within microRNAs Binding Sites of Neuronal Genes Related to Multiple Sclerosis: A Preliminary Study

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Abstract

Background: Different genetic variants, including the single-nucleotide polymorphisms (SNPs) present in microRNA recognition elements (MREs) within 3'UTR of genes, can affect miRNA-mediated gene regulation and susceptibility to a variety of human diseases such as multiple sclerosis (MS), a disease of the central nervous system. Since the expression of many genes associated with MS is controlled by microRNAs (miRNAs), the aim of this study was to analyze SNPs within miRNA binding sites of some neuronal genes associated with MS. **Materials and Methods:** Fifty-seven neuronal genes related to MS were achieved using dbGaP, DAVID, DisGeNET, and Oviddatabases. 3'UTR of candidate genes were assessed for SNPs, and miRNAs' target prediction databases were used for predicting miRNA binding sites. **Results:** Three hundred and eight SNPs (minor allele frequency >0.05) were identified in miRNA binding sites of 3'UTR of 44 genes. Among them, 42 SNPs in 22 genes had miRNA binding sites and miRNA prediction tools suggested 71 putative miRNAs binding sites on these genes. Moreover, *in silico* analysis predicted 22 MRE-modulating SNPs and 22 MRE-creating SNPs in the 3'UTR of these candidate genes. **Conclusions:** These candidate MRE-SNPs can alter miRNAs binding sites and mRNA gene regulation. Therefore, these genetic variants and miRNAs might be involved in MS susceptibility and pathogenesis and hence would be valuable for further functional verification investigation.

Keywords: *microRNAs, multiple sclerosis, Polymorphism, Single Nucleotide*

Introduction

Single-nucleotide polymorphisms (SNPs) may play potential roles in the pathogenesis of multifactorial diseases such as multiple sclerosis (MS).[1] MS is the most common autoimmune disease of the central nervous system (CNS).[2,3] In the past years, different studies have demonstrated that microRNAs (miRNAs) can play key roles in the pathogenesis of neurodegenerative diseases such as MS.[4] SNPs in miRNA recognition elements (MREs) located in 3'UTR of miRNA target genes may increase or decrease MRE affinity, create new elements or remove existing elements, which influence the expression of target genes.[5] Therefore, the discovery of SNPs in miRNA binding sites, as well as miRNAs, is vital for understanding the pathophysiology of MS and other complex diseases,[6] and could play functional roles in the diagnosis and treatment of MS. At this point, bioinformatic approaches allow identifying functional genetic variants within miRNA binding sites. In this study, SNPs in MREs located in the 3'UTR of MS-related neuronal Genes were investigated using *In silico* methods.

Materials and Methods

In silico analysis of neuronal genes related to multiple sclerosis

Neuronal genes related to MS were obtained from DisGeNET,[7] dbGaP (https://www.ncbi.nlm.nih.gov/gap/phegeni), Ovid (http://www.ovid.com), and DAVID Bioinformatics Resources 6.8 (https://david.ncifcrf.gov/).

Investigation of the single-nucleotide polymorphisms within 3'UTR of candidate genes

To identify the SNPs within 3'UTR of candidate genes, we used the "1000 Genome

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database" (https://www.internationalgenome.org/category/dbsnp/). Moreover, the allele's frequencies were checked, and the SNPs with the minor allele frequency (MAF) higher than 0.05 were chosen.

In silico predictions of microRNAs target binding sites

miRNA target prediction databases including miRdSNP[8] (http://mirdsnp.ccr.buffalo.edu/search.php/), MirSNP.[9] (http://202.38.126.151/hmdd/mirsnp/search/), TargetScan Human 6.2[10] (http://www. targetscan. org), miRNASNP 2.0[11] (http://www.bioguo.org/miRNASNP/search.php), and PolymiRTS 3.0.[12] (http://compbio.uthsc.edu/miRSNP/) were used to identify putative miRNA binding sites on 3'UTR of each selected gene. miRNA sequences were got from miRBase 21 (http://mirbase. org). mirRNAs' function can be affected by SNPs within MREs so the function was obtained from MirSNP.

Calculation of the binding free energy

The Gibbs binding free energy (ΔG, kCal/mol) was assessed for the wild and variant alleles using RNAcofold (http://rna.tbi.univie.ac.at/cgi-bin/RNAcofold.cgi). Then, the variation of ΔG between two alleles (i.e., ΔΔG) was calculated to assess the stability of the mRNA: miRNA duplex.[13]

Results

The neuronal genes and single nucleotide polymorphisms associated with multiple sclerosis

By researching in the different databases (DisGeNET, dbGaP, Ovid and DAVID), 57 MS-related neuronal genes were found. Next, 308 SNPs (MAF >0.05) on 3'UTR of 44 candidate genes were identified.

Single nucleotide polymorphisms predicted within the microRNAs binding site

Three hundred and eight SNPs locating on 3'UTR of these 44 genes were investigated in detail. The results indicated that 42 SNPs of 22 genes had the miRNA binding sites and 71 putative miRNAs were predicted to target them. Thirty-seven miRNAs were identified by SNPMIR, 44 miRNAs were identified by PolymiRTS 3.0, and 18 miRNAs were identified by miRNASNP v2. These SNPs were MRE-modulating (enhancing, decreasing, and disrupting) or MRE-creating. Sixteen SNPs of them could disrupt miRNA target sites, 5 SNPs could enhance, 1 SNP could decrease and 22 SNPs could create a novel miRNA target site [Table 1].

Discussion

Genetic variations have been reported to play important roles on susceptibility to common autoimmune diseases. Despite recent advances in genetics, the pathogenesis of MS remains largely unknown suggesting that further studies are needed to evaluate the genetic basis of susceptibility to this disease.[14,15] SNPs are the most prevalent types of genetic variations among individuals and associated with clinical progression of human disease, and response to treatment.[16] The SNPs within 3'UTR, which can be the binding sites for miRNA, change the ability of miRNAs binding to target genes, influence gene regulation and the susceptibility risk to MS.[17]

In this paper, the main approach was finding SNP variants that are likely to disrupt miRNAs binding target sites and consequently cause dysregulation of gene expression or creates new target sites in 3´UTR of genes and cause down-regulated gene expression. Since, Functional studies are time-consuming and expensive, bioinformatics tools have been designed to facilitate investigations into biological interactions and pathogenomics pathways.[18] Here, our emphasis was on suggesting a list of miRNAs and their target sequences in the candidate neuronal genes which have not yet been reported.

Since demyelination and degeneration of the nervous system are main parameters in the development of MS,[3] in this way, 22 genes were chosen that at least one SNP was at their MREs. 3´UTR contains highly conserved MREs that even a single-nucleotide change in these regions can disrupt the thermodynamic stability of miRNAs binding to the mRNA. Therefore, in the next step, the SNPs were investigated to define different types of change such as removing the previous element, creating a new element, increasing or decreasing MRE affinity, and then cause the difference of the free energies between the two alleles was assessed [variation of ΔG or ΔΔG, Table 1]. The higher ΔΔG indicates the bigger impact of SNP on the miRNA binding site.[13] Hence, further experiments of miRNAs with significant ΔΔG can be valuable. For example, ΔΔG between wild type allele and rs13515 variant allele in *MAPK1* gene is 48 KJ/mol, which is the highest ΔΔG in our list. After further assessments in miRDB and TarBase V.8 databases, 15 miRNAs of the total 71 identified miRNAs were reported that could downregulate the expression of target genes [these miRNAs have been marked with one asterisk (*) in Table 1].

Up and downregulation of hsa-mir-196b-5p,[19,20] hsa-mir-26a,[20,21] hsa-mir-4479,[1] has-mir-660,[22,23] has-mir-484,[24,25] has-4665-5p,[26,27] has-mir-199a-3p[20,28] in MS have been reported by previous studies. For instance, Hsa-miR-484 showed significantly different expression levels between MS and healthy controls. Hsa-miR-484 has an important function in the regulation of neural progenitor cells and its downregulation is associated with dysregulation of synaptogenesis, which leads to neurodevelopmental diseases such as epilepsy, autism, and hyperactivity.[24] In contrast, increased expression of hsa-miR-484 has been detected in MS patients' sera and may indicate the activation of neurogenesis pathways as part of an ongoing repair process. C/T substitution in 3´UTR of hsa-miR-484 target, *MBP*, creates new MRE and perhaps

Table 1: Predicted single nucleotide polymorphisms and microRNAs analyzed using microRNA target prediction databases

Gene name	dbSNP ID	Variant	miRNA	ΔΔG kcal/mol	SNPMIR	PolymiRTS 3.0	miRNASNP v2	Effect
MAPK1	rs13515	C/T	hsa-miR-592	48.02	√	√		Break
	rs13943	C/G	hsa-miR-3145-5p	6.34	√	√		Create
	rs3810608	G/A	hsa-miR-1288-3p	4.17	√	√		Break
			hsa-miR-3169	3.61	√	√		Break
			hsa-let-7b-3p	4.47	√			
			hsa-let-7f-1-3p	4.27	√			
FGF2	rs45504296	T/C	hsa-miR-6715b-3p*	13.18		√		
	rs1476215	A/T	hsa-miR-196b-5p	4.6	√	√	√	Break
	rs41278093	G/A	hsa-miR-196a-5p*	4.54	√	√	√	Break
	rs3804158	G/A	hsa-miR-26a-1-3p	4.15	√	√	√	Create
	rs7683093	C/G	hsa-miR-26a-2-3p	4.13		√	√	Create
	rs1048201	C/T	hsa-miR-3064-3p	3.82		√		Enhance
	rs6853268	T/C	hsa-miR-4711-5p	3.8		√		Create
			hsa-miR-892c-5p	3.7				
			hsa-miR-3190-3p*	3.64				
			hsa-miR-4533	3.36				
PGR	rs1046982	T/C	hsa-miR-5096	11.93	√			Decrease
	rs500760	T/C	hsa-miR-127-5p	3.32			√	
OLIG2	rs1059004	C/A	hsa-miR-6803-5p	6.32		√		
	rs13046814	T/G	hsa-miR-7110-5p	4.93		√	√	Break
			hsa-miR-2277-5p	5.71		√	√	Break
			hsa-miR-744-5p	5.41		√	√	Create
			hsa-miR-423-5p*	4.07		√		Create
			hsa-miR-3184-5p	4.01		√		
			hsa-miR-4479	3.29		√		
ABAT	rs45615432	A/G	hsa-miR-6785-3p	5.92		√		
	rs3743798	G/A	hsa-miR-1470	5.55	√	√	√	Create
	rs9456	T/A	hsa-miR-660-3p	4.14	√	√		Create
	rs7201586	C/T	hsa-miR-133a-3p	5.06	√	√		Create
	rs17674530	T/C	hsa-miR-6843-3p	3.98	√	√		Create
	rs1641032	A/G	hsa-miR-4768-3p*	4.66	√	√		Break
	rs4985000	G/C	hsa-miR-4798-5p	3.77				
			hsa-miR-483-3p*	3.76				
			hsa-miR-4795-5p	3.67				
ATP1A3	rs919390	G/C	hsa-miR-5010-5p	5.44		√		
			hsa-miR-4665-5p	4.4	√	√		Break
			hsa-miR-7111-5p	4.08		√		
NRG1	rs73672607	C/A	hsa-miR-6808-5p*	5.4		√		
MBP	rs3752069	C/T	hsa-miR-3194-3p	5.24	√	√		Create
	rs9199	T/C	hsa-miR-484	3.31	√	√	√	Create
	rs2282557	C/T	hsa-miR-2682-5p*	4.68	√	√	√	Create
			hsa-miR-34b-5p	4.68	√			Create
			hsa-miR-4769-5p*	4.65	√			Enhance
			hsa-miR-3714*	4.64	√			Create
			hsa-miR-3926	3.51	√			Create

Contd...

Table 1: Contd...

Gene name	dbSNP ID	Variant	miRNA	ΔΔG kcal/mol	SNPMIR	PolymiRTS 3.0	miRNASNP v2	Effect
IGF1	rs6217	A/C	hsa-miR-7109-3p	4.64		√		
	rs6218	A/G	hsa-miR-6757-3p	3.72	√	√		Enhance
	rs5742714	C/G	hsa-miR-362-3p	3.64	√			
			hsa-miR-4666a-3p	3.2				
JAG1	rs8708	T/C	hsa-miR-4445-3p	4.29	√			Create
NRCAM	rs78511988	T/C	hsa-miR-3613-5p	4.27		√		Break
			hsa-miR-3140-3p	4.22		√		Create
			hsa-miR-297*	4.21		√		
			hsa-miR-3149*	4.11		√		
NOS1	rs11068413	A/G	hsa-miR-1245a	4.2			√	Break
SCN8A	rs3741705	T/C	hsa-miR-3658	3.81	√			
CXCL12	rs1029153	A/G	hsa-miR-499a-3p*	3.74	√	√	√	Create
			hsa-miR-499b-3p*	3.56	√	√		Create
LINGO1	rs12438314	G/A	hsa-miR-199a-3p	3.73	√		√	Break
			hsa-miR-199b-3p	3.73	√		√	Break
			hsa-miR-3155a	3.73	√		√	Enhance
			hsa-miR-3155b	3.73	√		√	Enhance
			hsa-miR-371a-3p	3.73	√			Break
			hsa-miR-33b-3p	3.52				
TCF4	rs1261084	A/G	hsa-miR-105-3p	3.68	√	√	√	Create
MYT1	rs2427625	A/C	hsa-miR-412	3.65	√			Break
IGF2	rs7873	T/C	hsa-miR-128-1-5p	3.42		√		
MOG	rs62392951	A/T	hsa-miR-4713-3p	3.41		√		
PMP2	rs72684439	G/C	hsa-miR-4693-5p*	3.39		√		Create
THRB	rs844107	T/C	hsa-miR-6879-3p	3.38		√		
NOTCH1	rs6563	A/G	hsa-miR-3691-3p	3.26	√			Break

The miRs marked with one star (*) downregulate the expression of relevant genes. miRNA: microRNAs, SNP: Single-nucleotide polymorphism. √: Check mark

increases the affinity of hsa-miR-484 to that gene [Table 1]. Quintana *et al*. showed that the expression of miR-199a-3p along with 5 other miRNAs in cerebrospinal fluid of MS patients has down-regulated in comparison with the control group.[28] G/A substitution in MRE of the LINGO-1 gene as a target of miR-199a-3p can remove miRNA binding site. Interestingly, LINGO-1 is an important negative regulator of myelination by oligodendrocytes in the CNS and its overexpression can result in increased demyelination of axons.[29] In another experiment, Selmaj *et al*. studied the expression of miRNAs in the serum of 19 patients with relapsing remitting MS. They observed that there was a significant difference between expression levels of 4 miRNA, including has-mir-196b-5p in patients compared with the control group and the expression of these miRNAs was significantly reduced during the relapse.[19] dysregulation of 2 miRNAs of the 71 miRNAs represented in Table 1 was reported in other neurological diseases. One of them, hsa-mir-34b, is a stable microRNA in plasma of Huntington's patients that is used as a biomarker for the diagnosis of the presymptom condition in HD gene carriers.[30] Moreover, it may regulate the entry of dendritic cells into the CNS in an animal model of MS.[31] The other miRNA, has-mir-3613-5p, has shown a significantly increased expression in serum exosomes from mTLE-HS) mesial temporal lobe epilepsy with hippocampal sclerosis.[32]

Conclusions

Due to the existence of common pathways in the nervous diseases, further investigations on these two miRNAs can be done in MS. Other remaining mirRNAs and SNPs, which have not been investigated yet in MS, are proposed as potentially attractive targets in association studies or functional investigations in MS.

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Conflicts of interest

There are no conflicts of interest.

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