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RESEARCH ARTICLE

miRNA contributions to pediatric-onset multiple sclerosis inferred from GWAS

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Abstract

Objective: Onset of multiple sclerosis (MS) occurs in childhood for approximately 5% of cases (pediatric MS, or ped-MS). Epigenetic influences are strongly implicated in MS pathogenesis in adults, including the contribution from microRNAs (miRNAs), small noncoding RNAs that affect gene expression by binding target gene mRNAs. Few studies have specifically examined miRNAs in ped-MS, but individuals developing MS at an early age may carry a relatively high burden of genetic risk factors, and miRNA dysregulation may therefore play a larger role in the development of ped-MS than in adult-onset MS. This study aimed to look for evidence of miRNA involvement in ped-MS pathogenesis. **Methods**: GWAS results from 486 ped-MS cases and 1362 controls from the U.S. Pediatric MS Network and Kaiser Permanente Northern California membership were investigated for miRNA-specific signals. First, enrichment of miRNA-target gene network signals was evaluated using MIGWAS software. Second, SNPs in miRNA genes and in target gene binding sites (miR−SNPs)

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were tested for association with ped-MS, and pathway analysis was performed on associated target genes. **Results**: MIGWAS analysis showed that miRNA-target gene signals were enriched in GWAS ($P = 0.038$) and identified 39 candidate biomarker miRNA-target gene pairs, including immune and neuronal signaling genes. The miR-SNP analysis implicated dysregulation of miRNA binding to target genes in five pathways, mainly involved in immune signaling. **Interpretation**: Evidence from GWAS suggests that miRNAs play a role in ped-MS pathogenesis by affecting immune signaling and other pathways. Candidate biomarker miRNA-target gene pairs should be further studied for diagnostic, prognostic, and/or therapeutic utility.

Introduction

Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the central nervous system and a leading cause of neurological disability in young adults. MS is typically diagnosed between the ages of 20 and 40, but it is estimated that up to 5% of all cases experience their first symptoms before the age of 18.[1,2] While pediatric-onset MS (ped-MS) and adult MS presentation largely overlap, disease course in children is almost exclusively relapsing-remitting, with a higher relapse rate, and a longer time to development of secondary progressive MS and disability.[2–4]

MS is thought to result from a complex interplay of genetic, epigenetic, and environmental risk factors.[5] MicroRNAs (miRNAs) are epigenetic factors that have been investigated in MS, and over 170 miRNAs have been found to be differentially expressed in various tissues in either adult-onset MS or experimental autoimmune encephalomyelitis (EAE) in mice.[6–13] One study specifically compared miRNA expression levels in ped-MS cases to pediatric controls, and 12 upregulated and one downregulated miRNA were reported.[14] miRNAs are short (~22 nucleotides) noncoding RNAs that usually downregulate gene expression by binding to specific sequences of messenger RNA (mRNA) transcripts, targeting them for degradation and blocking protein translation, though different miRNA functions have also been reported.[15] Target sites generally lie in the 3' untranslated regions (3' UTRs) of mRNAs, but binding in other regions is known to occur.[16] Because each miRNA can target hundreds of genes, and any gene can be regulated by multiple miRNAs, they have the potential to influence entire networks of genes at once.

Single-nucleotide polymorphisms (SNPs) in and around miRNA genes have been associated with a number of autoimmune diseases, including MS.[17] These miRNA SNPs can disrupt normal gene regulatory functions by affecting miRNA expression levels and processing, but SNPs in the target-binding sites of mRNAs can also impact the normal function of miRNAs. Strategies to examine target gene SNPs in addition to miRNA gene SNPs have recently been developed and have implicated specific miRNAs and target genes in the development of autoimmune and other diseases.[18–21]

This study examined evidence of miRNA involvement in ped-MS susceptibility in two ways. First, genome-wide association study (GWAS) results were tested for enrichment of signals in miRNA-target gene networks utilizing MIGWAS software.[21,22] Second, a miR-SNP association study was performed, and pathway analysis was used to characterize target genes harboring miR-SNPs associated with ped-MS. We define miR-SNP as a SNP that is either (1) located in a gene that codes for a miRNA, or (2) located in a miRNA-binding site of a target gene.

Methods

Study participants

The study participants have been previously described,[23] but now include additional ped-MS cases with same inclusion/exclusion criteria. Additionally, only participants who were genotyped using an Illumina BeadChip array were included in this study. Briefly, patients with onset of MS or clinically isolated syndrome (CIS) with two silent MRI lesions suggestive of early MS before the age of 18 ($n = 432$) were enrolled through the U.S. Network of Pediatric MS Centers.[3] Additional adult cases with onset prior to age 18 ($n = 68$) were recruited retrospectively from the Kaiser Permanente Northern California (KPNC) membership. All cases were confirmed to have MS using established diagnostic criteria.[24,25]

Pediatric controls ($n = 208$) were enrolled through the U.S. Network of Pediatric MS Centers.[3] Adult controls ($n = 894$) without a diagnosis of MS, optic neuritis, transverse myelitis, or demyelinating disease confirmed through electronic medical records were recruited from KPNC and enrolled. A second set of previously described[26] female adult controls ($n = 268$) with no prior history of autoimmune disease who were recruited as part

of the University of California San Francisco Mother-Child Immunogenetic Study were also included.

Data collection

DNA from participants was purified from either whole blood or saliva samples. Genotyping was performed using Illumina Infinium 660K and Human Omni Express BeadChip arrays. Genotype data were merged into a single dataset and processed using PLINK 1.9.[27] SNPs with a minor allele frequency (MAF) <1% or success rate <90% and samples with >10% failed genotype calls were removed from analysis. To reduce confounding due to population stratification, analyses were restricted to white individuals, defined as having >=80% European ancestry identified using SNP weights for European, West African, East Asian, and Native American ancestral populations.[28] Related individuals were identified, and only one randomly chosen person in any related group was retained for analysis, with the exception that cases were preferentially retained in instances where cases and controls were related. Classical multidimensional scaling (MDS) was used to visualize study population ancestry (Figure S1) and include as covariates in subsequent statistical analyses. Five outlier samples were removed from analysis. SNP imputation was performed with reference haplotypes from Phase 3 of the 1000 Genomes Project[29] using SHAPEIT2 and IMPUTE2.[30] Genotypes were called using the default hard-call threshold of 90% using PLINK. Imputed SNPs with info score <0.3, with MAF <1%, with genotype call rate <90%, or not in Hardy–Weinberg equilibrium (HWE) among controls ($P < 0.00001$) were removed. The final imputed dataset consisted of 7 570 644 autosomal SNPs, of which 42 277 lay within the major histocompatibility complex (MHC) region chr6:29570005-33377657 in GRCh37/hg19, spanning genes *GABBR1* to *KIFC1*.[31] Presence of the *HLA-DRB1*15:01* allele, the strongest genetic risk factor for MS, was determined for each participant using the tag SNP rs3135388.[32] There were 486 cases and 1362 controls in the final dataset. Study protocols were approved by all institutions, and informed consent or assent was obtained from all participants, as previously described.[23]

Statistical analyses—miRNA-target gene network enrichment in GWAS (MIGWAS)

Genome-wide association tests were performed on all autosomal SNPs outside of the MHC using logistic regression and additive genetic models in PLINK. Models included the first three MDS components to adjust for residual confounding by population stratification. Enrichment of miRNA-target gene network signals in the GWAS results was evaluated using MIGWAS software.[21,22] Briefly, MIGWAS takes GWAS *P*-values as input, selects the lowest *P*-value per miRNA and target gene, and, for each of 179 different tissues with available miRNA expression data from FANTOM5,[33] identifies *the number of miRNA-target gene pairs* that satisfy the following conditions: both the miRNA and the target gene are associated with the outcome ($P < 0.01$), there is a high binding score prediction for the pair, and the miRNA is highly and specifically expressed in the tissue. Enrichment of miRNA-target gene signal is estimated by permuting GWAS *P*-values 20 000 times and recomputing the number of miRNA-target gene pairs that satisfy the conditions to obtain an empirical null distribution of that number. A *P*-value for enrichment is then reported for each tissue, as well as an overall enrichment *P*-value that does not take tissue expression into consideration is also reported. Enrichment *P*-values of 0.05 or lower were considered significant. Candidate biomarker miRNA-target gene pairs are also reported, and are defined as pairs where both the miRNA and target gene are nominally associated with the outcome ($P < 0.01$) and the miRNA-target gene binding prediction score is in the top one percentile of all pairs.[22]

Statistical analyses—miR-SNP association

miR-SNPs were tested separately for association with ped-MS. miR-SNPs in miRNA genes were identified using version 21 high-confidence annotations from the miRBase database.[34] Coordinates for 1877 miRNA genes were converted from build 38 to build 37 using the UCSC Genome Browser liftOver tool[35] and intersected with imputed SNPs in the ped-MS dataset using BEDTools,[36] resulting in 267 miR-SNPs outside of the MHC and 8 within the MHC. miR-SNPs in predicted target-binding sites in the 3' UTRs of protein-coding genes were identified using the MirSNP database[18] and version 3.0 of the PolymiRTS database.[19] SNPs in predicted target regions from either database were intersected with the ped-MS dataset, resulting in 51 725 target region miR-SNPs outside of the MHC and 586 miR-SNPs within the MHC.

Each miR-SNP was tested for association with ped-MS using the same logistic regression models as in the GWAS analysis, with the exception that models for miR-SNPs within the MHC also included the *HLA-DRB1*15:01* tag SNP as a covariate. *P*-values in each category of association tests—miRNA gene SNPs or target gene SNPs, within or outside of the MHC—were adjusted separately for multiple hypothesis testing using the Benjamini–Hochberg procedure to control the false discovery rate.[37] A threshold of 0.05 was used to determine significance. In an effort to reduce the statistical burden of multiple hypothesis tests, several sets of candidate mir-SNPs were

considered separately: 897 miR-SNPs in the 3'UTRs of genes proximal to 200 non-MHC loci identified in the latest adult-onset MS genome-wide association study;[38] 1063 miR-SNPs experimentally supported by crosslinking, ligation, and sequencing of hybrids (CLASH) experiments, including in noncanonical binding sites and non-protein-coding genes;[16] 19 516 miR-SNPs predicted in the polymiRTS database to either create a new miRNA-binding site or disrupt a conserved miRNA-binding site, with a context+ score difference of less than −0.15 (more negative scores indicate increased confidence that miRNA binding is disrupted); and one miR-SNP in the 3' UTR of the *HLA-DRB1* gene.

Pathway analysis of target genes with mir-SNPs that was nominally associated with ped-MS at $P < 0.01$ in the main association analysis was conducted using PANTHER.[39] Statistical overrepresentation tests were performed using a background list of only protein-coding genes. Each of nine available annotation datasets in the "PANTHER," "GO," and "Reactome" pathways were tested using Fisher's Exact test with FDR multiple test correction.

Results

Characteristics of study participants are summarized in Table 1. Average age of onset for ped-MS cases was 14.3 years, and cases had more copies of the *HLA-DRB1*15:01* allele than controls, as expected. When plotted with HGDP reference populations, cases, and controls clustered together near European individuals (Figure S1).

miRNA-target gene network enrichment in GWAS (MIGWAS)

Enrichment of miRNA-target gene network signals was observed in the ped-MS GWAS results for 25 different tissues ($P < 0.05$) as well as overall, without considering tissue-specific miRNA expression ($P = 0.038$). Results are summarized in Table 2. MIGWAS identified 39 candidate biomarker miRNA-target gene pairs comprised of 16 unique miRNAs and 37 unique genes (Table 3).

Table 1. Characteristics of ped-MS case and control individuals in the miR-SNP association study

	Ped-MS cases	Controls
N	486	1362
Sex		
Female	362 (74)	1122 (82)
Male	124 (26)	240 (18)
Age of onset	14.3 (3.2)	–
Copies *HLA-DRB1*15:01* allele		
0	250 (51)	1005 (74)
1	194 (40)	334 (24)
2	42 (9)	23 (2)

Table values are mean (SD) for continuous variables or *n* (%) for categorical variables.

miR-SNP association

After adjusting *P*-values for multiple hypothesis testing, no miR-SNPs were significantly associated with ped-MS in the genome-wide analyses at FDR < 0.05. There were 255 target genes with 3' UTR miR-SNPs associated at $P < 0.01$ in the genome-wide analysis that were used as input for

Table 2. Tissues enriched for miRNA-target gene network signals ($P < 0.05$) in ped-MS GWAS results in the MIGWAS analysis

Tissue	*P*-value	Fold change	MIGWAS tissue category
Keratinized cell of the oral mucosa	0.002	4.07	gastrointestinal
Human spinal cord—adult sample	0.011	2.61	brain
Epithelial cell of amnion	0.014	2.99	fetal
Preadipocyte	0.020	2.42	fat
Amnion mesenchymal stem cell	0.020	2.74	fetal
Epithelial cell of alimentary canal	0.023	2.79	gastrointestinal
Synovial cell	0.025	2.25	joint
Epithelial cell of esophagus	0.026	2.28	gastrointestinal
Acinar cell of sebaceous gland	0.027	2.64	fat
Mast cell	0.029	2.49	immune
Nonpigmented ciliary epithelial cell	0.031	2.13	skin
Tracheal epithelial cell	0.033	2.59	lung
Smooth muscle cell of the internal thoracic artery	0.035	2.14	vascular
All (tissue-naïve test)	0.038	1.68	-
Extraembryonic cell	0.036	2.42	fetal
Human renal cortical epithelial cell sample	0.039	1.79	kidney
Pericyte cell	0.041	2.02	others
Hair follicle dermal papilla cell	0.041	2.46	skin
Mesangial cell	0.043	2.03	kidney
Keratinizing barrier epithelial cell	0.043	2.54	others
Gingival epithelial cell	0.043	1.86	gastrointestinal
CD14-positive CD16-negative classical monocyte	0.044	2.30	immune
Exocrine cell	0.045	2.42	others
Epidermal cell	0.046	2.37	others
Omentum preadipocyte	0.047	2.25	fat
Keratinocyte	0.050	2.43	skin

P-values and fold changes are for enrichment of the number of miRNA-target gene pairs associated with ped-MS (where the pair has a high predicted binding score and the miRNA is highly expressed in the tissue) compared to the empirical null distribution of the number of such pairs.

Table 3. Candidate biomarker miRNA-target gene pairs associated with ped-MS in MIGWAS

miRNA	Genes	Known miRNA expression associations in MS
hsa-miR-141	*CD80, THAP5*	
hsa-miR-197	*CD109, TSEN2*	Decreased in T cells of patients treated with IFN-β[6]
hsa-miR-200c	*SLC35B4*	hsa-miR-200c increased in white matter[7]; hsa-miR-200a and hsa-miR-200b decreased in B cells[7]; hsa-miR-200a decreased in whole blood[8]
hsa-miR-21	*C11orf70, PLAA*	Increased in white matter[7]; decreased in peripheral blood of ped-MS cases[14]
hsa-miR-3128	*CBL, SCLY*	
hsa-miR-3188	*PRSS12*	
hsa-miR-3605	*ARL6IP6*	Increased in peripheral blood of ped-MS cases[14]
hsa-miR-4277	*ZNF286B*	
hsa-miR-4294	*SLC37A4*	
hsa-miR-4498	*NCS1, RAB35*	
hsa-miR-4649	*HYOU1*	
hsa-miR-587	*PRKRIR, UTP18*	
hsa-miR-599	*PAPPA*	Increased in PBMCs[6] and decreased in B cells[7]
hsa-miR-608	*ADPRH, CD109, CIITA, COX10, CYB561D1, EHD2, GAST, GLIS2, HYOU1, NTSR1, PHF19, PIWIL3, PXN, SNAI1, SYNJ2BP, TFAP4, ZSCAN20*	
hsa-miR-744	*TANC2*	Increased in PBMCs[6]
hsa-miR-875	*EIF5A2, ERP29*	

Pairs are candidate biomarkers if both the miRNA and target gene are nominally associated with ped-MS ($P < 0.01$) and the miRNA-target gene binding prediction score is in the top one percentile of all pairs. The last column indicates previously observed MS associations in miRNA expression studies. The "hsa-" prefix in the miRNA names stands for *homo sapiens*.

pathway analyses. These genes were overrepresented in five pathways in PANTHER (Table 4). Among the candidate miR-SNP sets, only one CLASH-supported miR-SNP, rs61075345 in the third exon of *TVP23B*, was associated with ped-MS ($P = 4.59 \times 10^{-05}$, FDR = 0.047).

Discussion

In this study, evidence that miRNAs are involved in ped-MS pathogenesis was sought using two different approaches that utilized genetic data from the largest population of ped-MS cases gathered to date.

The MIGWAS method identified enrichment of miRNA-target gene networks in ped-MS GWAS results, and identified tissues in which miRNAs involved in those networks are known to be highly expressed. Tissues included gastrointestinal, brain, fetal, fat, joint, immune, lung, vascular, skin, kidney, and other tissues (Table 2). While it is true that immune and central nervous system tissues have clear roles in MS and are generally prioritized first for study, there is evidence that processes starting in other tissues may play a role in triggering MS and also warrant investigation. For instance, smoking is hypothesized to exert its effect on MS risk primarily through irritation and inflammation of lung tissue, which in turn likely trigger (possibly autoreactive) immune responses.[5] The highest enrichment observed in this ped-MS study was in a gastrointestinal tissue, *keratinized cells of the oral mucosa*, and gastrointestinal tissues were overrepresented in these results: four of the seven gastrointestinal tissues tested were enriched for miRNA-target gene network signals. Evidence that miRNA dysregulation could specifically be occurring in gastrointestinal tissues is notable because there is existing evidence of a bidirectional relationship between MS and the gut microbiome, where aberrant gut microbiomes found in MS patients contribute to a proinflammatory state, and the autoreactive immune systems of MS patients shape the gut microbiome.[40]

Several genes identified in the MIGWAS candidate biomarker target-gene pairs are involved in immune signaling and activation (Table 3) according to RefSeq annotations,[41] and are therefore particularly promising targets of future research into the role miRNAs play in ped-MS development. *CIITA* is a "master regulator" of class II HLA gene expression, and *CD80* is a T-cell membrane receptor that provides the costimulatory signal necessary for T-cell activation. *CD109* is expressed in activated T cells and regulates transforming growth factor beta signaling. *CBL* is an enzyme required for targeting substrates for degradation by the proteasome and is a negative regulator of many signaling pathways triggered by activation of cell surface receptors. *TFAP4* is a transcription factor that activates both viral and cellular genes. Two other MIGWAS genes with plausible roles in ped-MS pathogenesis affect neuronal differentiation and signaling. *GLIS2* is widely expressed at low levels in the neural tube and peripheral nervous system and is thought to promote neuronal differentiation, and *NCS1* modulates synaptic transmission and synaptic plasticity and is expressed predominantly in neurons. Three genes identified by MIGWAS are involved in protein folding and homeostasis in the endoplasmic reticulum (ER), which is notable because the ER lumen cellular component was also identified in the miR-SNP pathway analysis. The ER

Table 4. Pathways in which the 255 protein-coding genes containing miR-SNPs associated with ped-MS ($P < 0.01$) are statistically overrepresented

Pathway name (PANTHER annotation source)	# Protein-coding genes in pathway	# Expected in 255 ped-MS genes	# Found in 255 ped-MS genes	*P*-value (FDR)	Ped-MS genes in pathway
Histamine H_1 Receptor mediated signaling (PANTHER Pathway)	43	0.58	5	0.035	*GNG4, PLCB3, PLCG2, PRKCB, PRKCI*
5-HT_2 type receptor mediated signaling (PANTHER Pathway)	66	0.86	6	0.064	*PLCB3, PRKCI, PLCG2, GNG4, PRKCB, SLC18A2*
MHC Protein Complex (GO Cellular Component)	25	0.34	5	0.085	*HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-A, HLA-G*
Integral component of lumenal side of endoplasmic reticulum membrane (GO Cellular Component)	28	0.38	5	0.046	*HLA-DPB1, HLA-DQB1, HLA-DRA, HLA-A, HLA-G*
Interferon gamma signaling (Reactome Pathway)	90	1.21	9	0.014	*HLA-DPB1, TRIM14, HLA-DQB1, HLA-DRA, HLA-A, TRIM26/AFP, TRIM10, HLA-G, CIITA*

For each pathway, the total number of protein-coding genes in the pathway is given, followed by the number of those genes expected by chance to be found among the 255 ped-MS associated protein-coding genes, the actual number found, the *P*-value for statistical overrepresentation (adjusted for multiple hypothesis tests), and the list of ped-MS associated genes in the pathway.

lumen is where class I and II HLA proteins are assembled,[42] and stress in the ER caused by accumulation of misfolded proteins (the "unfolded protein response" or UPR) is associated with a number of inflammatory diseases, including MS.[43] Of the MIGWAS-identified genes, *HYOU1* is thought to play an important role in protein folding and secretion in the ER. *ERP29* localizes to the lumen of the ER and is involved in the processing of secretory proteins. *SLC37A4* regulates transport from the cytoplasm to the lumen of the ER to maintain glucose homeostasis and plays a role in calcium sequestration in the ER lumen. Collectively, the genes in the 39 miRNA-target gene pairs suggest that miRNAs could be affecting ped-MS through many mechanisms, including immune signaling and activation, neuronal differentiation and signaling, and protein folding in the ER. Finally, it is notable that expression differences in five of the 16 candidate miRNAs identified by MIGWAS, hsa-mir-197, hsa-mir-200c, hsa-mir-21, hsa-mir-599, and hsa-mir-744, have been associated with MS or EAE in previous studies,[6,7] and two of them, hsa-miR-21 and hsa-miR-3605, were differentially expressed in ped-MS cases specifically (though hsa-miR-21 failed a subsequent validation assay).[14] A follow-up to the ped-MS expression study found that six of the 13 confirmed ped-MS-associated miRNAs were also differentially expressed in adults,[44] but hsa-miR-3605 was not among them, suggesting that it could be a biomarker specific to ped-MS.

In the miR-SNP analysis, the single CLASH-supported miR-SNP associated with ped-MS resides in the *TVP23B* gene, which codes for a membrane protein associated with diabetic retinopathy.[45] It is not immediately clear how it may play a role in ped-MS pathogenesis. However, statistical overrepresentation tests of top miR-SNP hits yielded two receptor-mediated signaling pathways with a more evident relationship with ped-MS. Five genes in the histamine H_1 receptor pathway were found to have ped-MS-associated miR-SNPs (Table 4). Histamine is a ubiquitous compound in human tissues that acts as a neurotransmitter and that is involved in inflammatory responses that act through four different receptors, H_1-H_4. It is thought that the proinflammatory effects of histamine act through H_1 receptors.[46] Many of the same genes are also involved in the 5-HT_2 type receptor mediated signaling pathway. 5-HT_2 is a subtype of serotonin receptors. Similar to histamine, serotonin is a signaling molecule with wide-ranging effects that acts as both a neurotransmitter and a hormone. The 5-HT_2 class of hormone receptors is expressed on several immune cell types.[47] Our results suggest that dysregulation of genes involved in these signaling pathways by miRNAs increases ped-MS risk.

The other three pathways identified in the miR-SNP analysis each encompass many of the same genes, including several genes encoded in the MHC (Table 4). Five class I and II HLA genes associated with ped-MS are in the *MHC protein complex (GO cellular component)*. These genes code for proteins that present antigens to T cells, and variants in antigen-presenting genes are the first-documented and strongest genetic risk factors for MS.[5,38] The same five HLA genes are part of the *integral component of the luminal side of endoplasmic reticulum* (ER) *membrane (GO Cellular Component)*. Of note, the genes identified in the miR-SNP analysis are different from those identified

in the MIGWAS analysis, but both methods point to dysregulation of processes in the ER. The *interferon gamma* (IFN-γ) *signaling* pathway contains a total of nine ped-MS-associated genes. The role of IFN-γ in MS has been extensively studied, and, similar to histamine and serotonin, it can have detrimental or beneficial effects on MS depending on where and when it is active.[48] Our miR-SNP analysis findings indicate that aberrant regulation by miRNAs of genes in the MHC protein complex, genes on the inner part of the ER lumen, or genes involved in IFN-γ signaling (which are not mutually exclusive genes), could be contributing to ped-MS pathogenesis.

This study had some limitations. It is possible that the SNPs identified in the MIGWAS and miR-SNP studies do not affect ped-MS via miRNA function but instead are associated due to linkage disequilibrium with SNPs acting by other mechanisms. Another issue is that miRNA-target binding prediction is imperfect, and therefore some of the miRNAs may not actually act on the genes identified in MIGWAS, and some of the miR-SNPs tested may not in reality impact miRNA function. Because the study was restricted to a white population, results may not be generalizable, and miRNA associations that exist in other non-white populations may have been missed.

An important strength of this study is that it utilized the largest study population thus far for ped-MS, which is a rare disease, and therefore difficult to study. Furthermore, cases were ascertained by a panel of pediatric MS specialists. Only samples genotyped on Illumina microarrays were utilized, minimizing the possibility of imputation bias, and rigorous quality control of microarray data was applied. By assessing *P*-values of SNPs in miRNA and target genes at the same time, and by including miRNA expression data, MIGWAS was able to detect signals that may be missed with traditional GWAS or miR-SNP analysis.

In conclusion, this study provides evidence that ped-MS risk is influenced by miRNAs acting on immune signaling and other genes, and several miRNA-target gene pairs and specific tissues were nominated for further study. Larger studies are needed to confirm these results, and further work is needed to determine whether any miRNA-mediated disease processes are specific to the pediatric population.

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Conflicts of Interest

E. Waubant is site PI for a Novartis and Roche trial. She has volunteered on an advisory board for a Novartis trial. She is a nonremunerated advisor for clinical trial design to Novartis, Biogen-IDEC, Sanofi, Genentech, Serono, and Celgene. She has funding from the NMSS, PCORI, and the Race to Erase MS. She is the section editor for Annals of Clinical and Translational Neurology, and co-Chief editor for MS And Related Disorders. A. Waldman reports grants from NIH (NINDS) NS071463 and from NMSS during the conduct of the study, as well as funds for investigator-initiated study from Ionis Pharmaceuticals and Biogen Idec and grants from United Leukodystrophy Foundation outside the submitted work. She is a consultant for Optum and has received royalties from UpToDate. B. Greenberg has received grant funding from Chugai, Medimmune, Medday, National Institutes of Health (NIH), NMSS, Guthy Jackson Charitable Foundation, and Transverse Myelitis Association. He has received consulting fees from Novartis, EMD Serono, Celgene, and Alexion. L. Benson reports BG12 clinical trial support from Biogen, grants from Boston Children's Hospital Office of Faculty Development, travel funds from National MS Society, and personal fees from National Vaccine Compensation Program outside the submitted work. T.C. Casper reports grants from National MS Society. J. Graves reports speaking honoraria from Novartis outside the submitted work. B. Weinstock-Guttman reports grants and personal fees from Biogen, EMD Serono, Novartis, and Genentech, and personal fees from Mallinckrodt outside the submitted work.

Author Contributions

E.W. and L.F.B. contributed to the conception and design of the study. J.S.G, T.C., A.T.W., T.L., T.S., A.B., L.K., B.M.B, B.W.G., G.A., J.M.T., M.R., J.H, S.C., J.N., Y.H., J.R., M.S.C, M.G., L.B., S.M., I.K., J.R., T.C.C., H.Q., D.Q., C.S., and E.W. contributed to acquisition of data. B.R. and X.S. performed statistical analyses. B.R., E.W., and L.F.B. drafted the manuscript, and all authors reviewed, revised, and approved the final manuscript.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1. Multidimensional scaling plot of ped-MS cases (black) and controls (gray) with ≥80% European ancestry who were included in the GWAS, along with individuals from the Human Genome Diversity Project.

