Title
miRNA contributions to pediatric-onset multiple sclerosis inferred from GWAS.

Permalink
https://escholarship.org/uc/item/2ts502k9

Journal
Annals of clinical and translational neurology, 6(6)

ISSN
2328-9503

Authors
Rhead, Brooke
Shao, Xiaorong
Graves, Jennifer S
et al.

Publication Date
2019-06-01

DOI
10.1002/acn3.786

Peer reviewed
miRNA contributions to pediatric-onset multiple sclerosis inferred from GWAS

Brooke Rhead1,2, Xiaorong Shao1, Jennifer S. Graves3,4, Tanuja Chitnis5, Amy T. Waldman6, Timothy Lotze7, Teri Schreiner8, Anita Belman9, Lauren Krupp10, Benjamin M. Greenberg10, Bianca Weinstock-Guttman11, Gregory Aaen12, Jan M. Tillema13, Moses Rodriguez13, Janace Hart14, Stacy Caillier14, Jayne Ness15, Yolanda Harris15, Jennifer Rubin16, Meghan S. Candeel7, Mark Gorman18, Leslie Benson18, Soe Mar19, Ilana Kahn20, John Rose21, T. Charles Casper22, Hong Quach1, Diana Quach1, Catherine Schaefer23,24, Emmanuelle Waubant1, Lisa F. Barcellos1,2,23 & on behalf of the US Network of Pediatric MS Centers

1Division of Epidemiology, School of Public Health, University of California, Berkeley, California
2Computational Biology Graduate Group, University of California, Berkeley, California
3Department of Neurology, University of California, San Francisco, California
4Department of Neurosciences, University of California, San Diego, California
5Partners Pediatric Multiple Sclerosis Center, Massachusetts General Hospital for Children, Boston, Massachusetts
6Division of Neurology, Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania
7Blue Bird Circle Multiple Sclerosis Center, Baylor College of Medicine, Houston, Texas
8Children’s Hospital Colorado, University of Colorado, Denver, Colorado
9Lorie Center for Pediatric MS, Stony Brook Children’s Hospital, Stony Brook, New York
10Department of Neurology and Neurotherapeutics, University of Texas Southwestern, Dallas, Texas
11Pediatric Multiple Sclerosis Center, Jacobs Neurological Institute, SUNY Buffalo, Buffalo, New York
12Pediatric MS Center at Loma Linda University Children’s Hospital, Loma Linda, California
13Mayo Clinic’s Pediatric MS Center, Rochester, Minnesota
14University of California, San Francisco, Regional Pediatric MS Center Neurology, San Francisco, California
15University of Alabama Center for Pediatric–onset Demyelinating Disease, Children’s Hospital of Alabama, Birmingham, Alabama
16Division of Pediatric Neurology, Northwestern Feinberg School of Medicine, Chicago, Illinois
17Division of Pediatric Neurology, University of Utah, Primary Children’s Hospital, Salt Lake City, Utah
18Boston Children’s Hospital, Boston, Massachusetts
19Pediatric–onset Demyelinating Diseases and Autoimmune Encephalitis Center, St. Louis Children’s Hospital, Washington University School of Medicine, St. Louis, Missouri
20Children’s National Medical Center, Washington, District of Columbia
21Department of Neurology, University of Utah School of Medicine, Salt Lake City, Utah
22Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, Utah
23Kaiser Permanente Division of Research, Oakland, California
24Research Program on Genes, Environment and Health, Kaiser Permanente, Oakland, California

Correspondence
Lisa Barcellos, Division of Epidemiology, School of Public Health, University of California, Berkeley, 308D Stanley Hall, MC#3220, Berkeley, CA 94720-3220. Tel: 510-642-7814; Fax: 510-642-5163; E-mail: lbarcellos@berkeley.edu

Funding information
This work was supported in part by the NIH NINDS: R01NS071463 (Pt: Waubant), R01NS049510 (Pt: Barcellos), F31NS096885 (Pt: Rhead); NIH NIEHS: R01ES017080 (Pt: Barcellos), NIH NIAID: R01AI076544 (Pt: Barcellos), NIH NIAID: R01AI076544 (Pt: Barcellos), the National MS Society HC 0165 (Pt: Casper), and Race to Erase MS (Pt: Waubant).

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)
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. 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.

MS is thought to result from a complex interplay of genetic, epigenetic, and environmental risk factors. 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. One study specifically compared miRNA expression levels in ped-MS cases to pediatric controls, and 12 upregulated and one downregulated miRNA were reported. 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. Target sites generally lie in the 3’ untranslated regions (3’ UTRs) of miRNAs, but binding in other regions is known to occur. 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. These miRNA SNPs can disrupt normal gene regulatory functions by affecting miRNA expression levels and processing, but SNPs in the target-binding sites of miRNAs 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. 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. 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, 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 were enrolled through the U.S. Network of Pediatric MS Centers. Additional adult cases with onset prior to age 18 were recruited retrospectively from the Kaiser Permanente Northern California (KPNC) membership. All cases were confirmed to have MS using established diagnostic criteria.

Pediatric controls were enrolled through the U.S. Network of Pediatric MS Centers. 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 female adult controls (n = 268) with no prior history of autoimmune disease who were recruited as part of previous studies 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.
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 Project29 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 HLA-DRB1 and 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—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 tool35 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 database18 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

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

© 2019 The Authors. Annals of Clinical and Translational Neurology published by Wiley Periodicals, Inc on behalf of American Neurological Association.
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; 18 1063 miR-SNPs experimentally supported by crosslinking, ligation, and sequencing of hybrids (CLASH) experiments, including in noncanonical binding sites and non-protein-coding genes; 16 516 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. 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).

**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

| 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-naive 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              | Decreased in T cells of patients treated with IFN-β6 |
| hsa-miR-197 | CD109, TSEN2            | Decreased in T cells of ped-MS patients treated with IFN-β6 |
| hsa-miR-200c | SLC35B4                | hsa-miR-200c increased in white matter7; hsa-miR-200a and hsa-miR-200b decreased in B cells5; hsa-miR-200a decreased in whole blood8 |
| hsa-miR-21  | C11orf70, PLAA          | Increased in white matter7; decreased in peripheral blood of ped-MS cases14 |
| hsa-miR-3128 | CBL, SCLY               | Increased in peripheral blood of ped-MS cases14 |
| hsa-miR-3168 | PRSS12                 | Increased in peripheral blood of ped-MS cases14 |
| hsa-miR-3605 | ARL6IP6                | Increased in peripheral blood of ped-MS cases14 |
| hsa-miR-4277 | ZNF286B                | Increased in peripheral blood of ped-MS cases14 |
| hsa-miR-4294 | SLC37A4                | Increased in peripheral blood of ped-MS cases14 |
| hsa-miR-4498 | NCS1, RAB35             | Increased in peripheral blood of ped-MS cases14 |
| hsa-miR-4649 | HYOU1                  | Increased in peripheral blood of ped-MS cases14 |
| hsa-miR-587  | PRKRR1, UTP18           | Increased in peripheral blood of ped-MS cases14 |
| hsa-miR-599  | PAPPA                   | Increased in peripheral blood of ped-MS cases14 |
| hsa-miR-608  | ADPRH, CD109, CIITA, COX10, CYB561D1, EHD2, GAST, GLS2, HYOU1, NTSR1, PFH19, PIWIL3, PXN, SNAI1, SYN2BP, TFAP4, ZSCAN20 | Increased in peripheral blood of ped-MS cases14 |
| hsa-miR-744  | TANC2                   | Increased in peripheral blood of ped-MS cases14 |
| hsa-miR-875  | EIF5A2, ERP29           | Increased in peripheral blood of ped-MS cases14 |

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 × 10⁻⁵, 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.
miRNAs were also differentially expressed in adults, but found that six of the 13 confirmed ped-MS-associated ERP29 folding and secretion in the ER. HYOU1 is thought to play an important role in protein homeostasis and plays a role in maintaining 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, 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). A follow-up to the ped-MS expression study yielded two receptor-mediated signaling pathways with a more evident relationship with ped-MS. Five genes in the histamine H1 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 is involved in inflammatory responses that act through four different receptors, H1- H4. It is thought that the proinflammatory effects of histamine act through H1 receptors. Many of the same genes are also involved in the 5-HT2 type receptor mediated signaling pathway. 5-HT2 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-HT2 class of hormone receptors is expressed on several immune cell types. 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. 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.

Acknowledgments

The authors thank Jorge Oksenberg for assisting with processing and DNA extraction of patient and control samples, Hans Christian von Buedingen for assisting with cell sorting for the miRNA analysis, and Shelly Roalstad for assisting with data collection. This work was supported in part by the NIH NINDS: 1R01NS071463 (PI: Waubant), R01NS049510 (PI: Barcellos), F31NS096885 (PI: Rhead); NIH NIEHS: R01ES017080 (PI: Barcellos), NIH NIAID: R01AI076544 (PI: Barcellos), the National MS Society HC 0165 (PI: Casper), and Race to Erase MS (PI: Waubant).

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.

References

1. Chitnis T, Glanz B, Jaffin S, Healy B. Demographics of pediatric-onset multiple sclerosis in an MS center population from the Northeastern United States. Mult Scler J 2009;15:627–631.
miRNA Contributions to Pediatric MS from GWAS

B. Rhead et al.

2. Renoux C, Vukusic S, Mikaeloff Y, et al. Natural history of multiple sclerosis with childhood onset. N Engl J Med 2007;25:2603–2613.

3. Belman AL, Krupp LB, Olsen CS, et al. Characteristics of children and adolescents with multiple sclerosis. Pediatrics 2016; 138:1–8.

4. Gorman MP, Healy BC, Polgar-Turcsanyi M, Chitnis T. Increased relapse rate in pediatric-onset compared with adult-onset multiple sclerosis. Arch Neurol 2009;66:54–59.

5. Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. Nat Rev Neurol 2016;13:25–36.

6. Gandhi Roopali. miRNA in multiple sclerosis: search for novel biomarkers. Mult Scler J 2015;21:1095–1103.

7. Huang Q, Xiao B, Ma X, et al. MicroRNAs associated with the pathogenesis of multiple sclerosis. J Neuroimmunol 2016;295–296:148–161.

8. Yang Q, Pan W, Qian L. Identification of the miRNA–mRNA regulatory network in multiple sclerosis. Neuror Res 2017;39:142–151.

9. Groen K, Matthy VE, Lea RA, et al. Erythrocyte microRNA sequencing reveals differential expression in relapsing-remitting multiple sclerosis. BMC Med Genomics 2018;11:1–12.

10. Reger K, Healy BC, Paul A, et al. Identification of MS-specific serum miRNAs in an international multicenter study. Neurol Neuroimmunol Neuroinflammation 2018;5:e491.

11. Selmaj I, Cichalewska M, Nameicinska M, et al. Global exosome transcriptome profiling reveals biomarkers for multiple sclerosis. Ann Neurol 2017;81:703–717.

12. Teymoori-Rad M, Mozghani SH, Zarei-Ghobadi M, et al. Integrational analysis of miRNAs data sets as a plausible missing linker between Epstein-Barr virus and vitamin D in relapsing MS patients. Gene. 2019;689:1–10.

13. Venkatesha S, Dudics S, Song Y, et al. The miRNA expression profile of experimental autoimmune encephalomyelitis reveals novel potential disease biomarkers. Int J Mol Sci 2018;19:3990.

14. Liguori M, Nuzziello N, Liciulli F, et al. Combined microRNA and mRNA expression analysis in pediatric multiple sclerosis: An integrated approach to uncover novel pathogenic mechanisms of the disease. Hum Mol Genet 2018;27:66–79.

15. Dragomir MP, Knutsen E, Calin GA. SnapShot: unconventional miRNA functions. Cell 2018;174:1038–1038.e1.

16. Helwak A, Kudla G, Dudnakeova T, Tollervey D. Mapping the human miRNA interactome by CLASH reveals frequent noncanonical binding. Cell 2013;153:654–665.

17. Latini A, Cicacce C, Novelli G, Borgiani P. Polymorphisms in miRNA genes and their involvement in autoimmune diseases susceptibility. Immunol Res 2017;65:811–827.

18. Liu C, Zhang F, Li T, et al. MirSNP, a database of polymorphisms altering miRNA target sites, identifies miRNA-related SNPs in GWAS SNPs and eQTLs. BMC Genom 2012;13:661.

19. Bhattacharya A, Ziebarth JD, Cui Y. PolymiRTS database 3.0: linking polymorphisms in microRNAs and their target sites with human diseases and biological pathways. Nucleic Acids Res 2014;42(D1):86–91.

20. de Almeida RC, Chagas VS, Castro MAA, Petzl-Erler ML. Integrative analysis identifies genetic variants associated with autoimmune diseases affecting putative microRNA binding sites. Front Genet 2018;9:1–13.

21. Sakaue S, Hirata J, Maeda Y, et al. Integration of genetics and miRNA–target gene network identified disease biology implicated in tissue specificity. Nucleic Acids Res 2018;46:11898–11909.

22. Okada Y, Muramatsu T, Suits N, et al. Significant impact of miRNA-target gene networks on genetics of human complex traits. Sci Rep 2016;6:1–9.

23. Gianfrancesco MA, Stridh P, Shao X, et al. Genetic risk factors for pediatric-onset multiple sclerosis. Mult Scler J 2017;24:1825–1834.

24. Krupp LB, Tardieu M, Amato MP, et al. International Pediatric Multiple Sclerosis Study Group criteria for pediatric multiple sclerosis and immune-mediated central nervous system demyelinating disorders: revisions to the 2007 definitions. Mult Scler J 2013;19:1261–1267.

25. Polman CH, Reingold SC, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 revisions to the McDonald criteria. Ann Neurol 2011;69:292–302.

26. Cruz GI, Shao X, Quach H, et al. Increased risk of rheumatoid arthritis among mothers with children who carry DRB1 risk-associated alleles. Ann Rheum Dis 2017;76:1405–1410.

27. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet 2007;81:559–575.

28. Chen CY, Pollack S, Hunter DJ, et al. Improved ancestry inference using weights from external reference panels. Bioinformatics 2013;29:1399–1406.

29. The 1000 Genomes Project Consortium, Auton A, Abecasis GR, et al. A global reference for human genetic variation. Nature 2015;526:68–74.

30. Howie B, Fuchsberger C, Stephens M, et al. Fast and accurate genotype imputation in genome-wide association studies through pre-phasing. Nat Genet 2012;44:955–959.

31. Shima T, Hosomichi K, Inoko H, Kulski JK. The HLA genomic loci map: expression, interaction, diversity and disease. J Hum Genet 2008;65:811–827.

32. Liu C, Zhang F, Li T, et al. MirSNP, a database of polymorphisms altering miRNA target sites, identifies miRNA-related SNPs in GWAS SNPs and eQTLs. BMC Genom 2012;13:661.
34. Kozomara A, Griffiths-Jones S. MiRBase: annotating high confidence microRNAs using deep sequencing data. Nucleic Acids Res 2014;42(D1):68–73.
35. Tyner C, Barber GP, Casper J, et al. The UCSC Genome Browser database: 2017 update. Nucleic Acids Res 2017;45(D1):D626–D634.
36. Quinlan AR, Hall IM. BEDTools: a flexible suite of utilities for comparing genomic features. Bioinformatics 2010;26:841–842.
37. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc 1995;57:289–300.
38. IMSGC. The Multiple Sclerosis Genomic Map: role of peripheral immune cells and resident microglia in susceptibility. bioRxiv 2017;2017:1–43.
39. Mi H, Huang X, Muruganujan A, et al. PANTHER version 11: expanded annotation data from Gene Ontology and Reactome pathways, and data analysis tool enhancements. Nucleic Acids Res 2017;45(D1):D183–D189.
40. Kirby T, Ochoa-Repáraz J. The gut microbiome in multiple sclerosis: a potential therapeutic avenue. Med Sci 2018;6:69.
41. O’Leary NA, Wright MW, Brister JR, et al. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Res 2016;44:D733–D745.
42. van Kasteren SI, Overkleeft H, Ovaa H, Neefjes J. Chemical biology of antigen presentation by MHC molecules. Curr Opin Immunol 2014;26:21–31.
43. Stone S, Lin W. The unfolded protein response in multiple sclerosis. Front Neurosci 2015;9:1–11.
44. Nuzziello N, Vilardo L, Pelucchi P, et al. Investigating the role of MicroRNA and transcription factor co-regulatory networks in multiple sclerosis pathogenesis. Int J Mol Sci 2018;19:1–18.
45. Wang AL, Rao VR, Chen JJ, et al. Role of FAM18B in diabetic retinopathy. Mol Vis 2014;20:1146–1159.
46. Jadidi-Niaragh F, Mirshafiey A. Histamine and histamine receptors in pathogenesis and treatment of multiple sclerosis. Neuropharmacology 2010;59(3):180–189.
47. Herr N, Bode C, Duerschmied D. The effects of serotonin in immune cells. Front Cardiovasc Med 2017;4:1–11.
48. Arellano G, Ottum PA, Reyes LI, et al. Stage-specific role of interferon-gamma in experimental autoimmune encephalomyelitis and multiple sclerosis. Front Immunol 2015;6:1–9.

**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.