Evidence for a causal relationship between low vitamin D, high BMI, and pediatric-onset MS

Milena A. Gianfrancesco, PhD, MPH
Pernilla Stridh, PhD
Brooke Rhead, BS
Xiaorong Shao, MA
Edison Xu, BA
Jennifer S. Graves, MD, PhD
Tanuja Chitnis, MD
Amy Waldman, MD
Timothy Lotze, MD
Teri Schreiner, MD
Anita Belman, MD
Benjamin Greenberg, MD
Bianca Weinstock-Guttman, MD
Gregory Aaen, MD
Jan M. Tillema, MD
Janace Hart, BA
Stacy Caillier, BS
Jayne Ness, MD
Yolanda Harris, NP
Jennifer Rubin, MD
Meghan Candee, MD
Lauren Krupp, MD
Mark Gorman, MD
Leslie Benson, MD
Moses Rodriguez, MD
Soe Mar, MD
Ilana Kahn, MD
John Rose, MD
Shelly Roalstad, MSc, BS
T. Charles Casper, PhD
Ling Shen, PhD, MPH
Hong Quach, BA
Diana Quach, BA
Jan Hillert, MD
Maria Bäärnhielm, MD
Anna Hedstrom, MD, PhD
Tomas Olsson, MD, PhD
Ingrid Kockum, PhD
Lars Alfredsson, PhD

Author list continued on next page

ABSTRACT

Objective: To utilize Mendelian randomization to estimate the causal association between low serum vitamin D concentrations, increased body mass index (BMI), and pediatric-onset multiple sclerosis (MS) using genetic risk scores (GRS).

Methods: We constructed an instrumental variable for vitamin D (vitD GRS) by computing a GRS for 3 genetic variants associated with levels of 25(OH)D in serum using the estimated effect of each risk variant. A BMI GRS was also created that incorporates the cumulative effect of 97 variants associated with BMI. Participants included non-Hispanic white individuals recruited from over 15 sites across the United States (n = 394 cases, 10,875 controls) and Sweden (n = 175 cases, 5,376 controls; total n = 16,820).

Results: Meta-analysis findings demonstrated that a vitD GRS associated with increasing levels of 25(OH)D in serum decreased the odds of pediatric-onset MS (odds ratio [OR] 0.72, 95% confidence interval [CI] 0.55, 0.94; $p = 0.02$) after controlling for sex, genetic ancestry, *HLA-DRB1*15:01*, and over 100 non–human leukocyte antigen MS risk variants. A significant association between BMI GRS and pediatric disease onset was also demonstrated (OR 1.17, 95% CI 1.05, 1.30; $p = 0.01$) after adjusting for covariates. Estimates for each GRS were unchanged when considered together in a multivariable model.

Conclusions: We provide evidence supporting independent and causal effects of decreased vitamin D levels and increased BMI on susceptibility to pediatric-onset MS. *Neurology*® 2017;88:1623–1629

GLOSSARY

BMI = body mass index; **chBMI** = childhood body mass index; **CI** = confidence interval; **CIS** = clinically isolated syndrome; **EIMS** = Epidemiologic Investigation of Risk Factors for MS; **GEMS** = Genes and Environment in MS; **GRS** = genetic risk score; **HLA** = human leukocyte antigen; **GWAS** = genome-wide association studies; **HGDP** = Human Genome Diversity Project; **KPNC** = Kaiser Permanente Northern California; **MDS** = multidimensional scaling; **MS** = multiple sclerosis; **OR** = odds ratio; **RPGEH** = Research Program on Genes, Environment, and Health; **SNP** = single nucleotide polymorphism; **UCSF** = University of California San Francisco; **vitD GRS** = genetic instrumental variable for vitamin D; **wGRS** = weighted genetic risk score.

Disease onset in multiple sclerosis (MS) typically occurs between the ages of 20 and 40 years; however, approximately 5% of patients experience symptoms before the age of 18.[1–3] The exact mechanisms involved in pediatric-onset MS pathogenesis have yet to be defined; however, similar to adult MS, infection with Epstein-Barr virus, exposure to

*These authors contributed equally to this work.

From the Division of Epidemiology, School of Public Health (M.A.G., X.S., E.X., H.Q., D.Q., C.M., L.F.B.), and Computational Biology Graduate Group (B.R.), University of California, Berkeley; Department of Clinical Neuroscience and Center for Molecular Medicine (P.S.), Karolinska Institutet at Karolinska University Hospital, Stockholm, Sweden; Department of Neurology (J.S.G., E.W.) and Regional Pediatric MS Center, Neurology (J.H., S. C.), University of California, San Francisco; Partners Pediatric Multiple Sclerosis Center (T.C.), Massachusetts General Hospital for Children, Boston; Division of Neurology (A.W.), Children's Hospital of Philadelphia, PA; Blue Bird Circle Multiple Sclerosis Center (T.L.), Baylor College of Medicine, Houston, TX; Children's Hospital Colorado (T.S.), University of Colorado, Denver; Lourie Center for Pediatric Multiple Sclerosis (A.B., L.K.), Stony Brook Children's Hospital, NY; Department of Neurology (B.G.), University of Texas Southwestern, Dallas; Pediatric Multiple Sclerosis Center (B.W.-G.), Jacobs Neurological Institute, SUNY Buffalo, NY; Pediatric MS Center at Loma Linda University Children's Hospital (G.A.), CA; Mayo Clinic's Pediatric Multiple Sclerosis Center (J.M.T., M.R.), Rochester, MN; University of Alabama Center for Pediatric-onset Demyelinating Disease (J.N., Y. H.), Children's Hospital of Alabama, Birmingham; Department of Pediatric Neurology (J.R.), Northwestern Feinberg School of Medicine, Chicago, IL; Primary Children's Hospital (M.C.), University of Utah, Salt Lake City; Boston Children's Hospital (M.G., L.B.), MA; Pediatric-onset Demyelinating Diseases and Autoimmune Encephalitis Center (S.M.), St. Louis Children's Hospital, Washington University School of Medicine, MO; Children's National Medical Center (I.K.), Washington, DC; Departments of Neurology (J.R.) and Pediatrics (S.R., T.C.C.), University of Utah School of Medicine, Salt Lake City; Kaiser Permanente Division of Research (L.S., C.S., L.F.B.), Oakland, CA; Institute of Environmental Medicine (J.H., M.B., A.H., T.O., I.K., L.A.), Karolinska Institutet; Centre for Occupational and Environmental Medicine (L.A.), Stockholm County Council, Stockholm, Sweden; and Research Program on Genes, Environment and Health (C.S.), Kaiser Permanente, Oakland, CA.

Coinvestigators are listed at Neurology.org.

Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

Catherine Metayer, MD, PhD
Catherine Schaefer, PhD
Lisa F. Barcellos, PhD, MPH*
Emmanuelle Waubant, MD, PhD*
For the Network of Pediatric Multiple Sclerosis Centers

Correspondence to Dr. Waubant: emmanuelle.waubant@ucsf.edu

Supplemental data at Neurology.org

cigarette smoking, and the genetic risk factor *HLA-DRB1* are associated with pediatric-onset MS.[1,4–6]

While there is strong research demonstrating the association between low vitamin D status and increased risk of adult-onset MS,[7–9] there is a paucity of studies examining this relationship in pediatric MS. In addition, an association between childhood obesity and risk of pediatric MS has been reported,[10] though a causal relationship remains to be confirmed. Further, it remains unclear whether the association between increased obesity and MS risk is mediated by low circulating levels of vitamin D, which can be stored in adipose tissue.

Mendelian randomization uses genetic variants strongly associated with an exposure as an instrumental variable, rather than a direct measure of the exposure, to estimate the effect of the exposure on an outcome. Inherited alleles are not affected by most potential confounding variables or disease status, thus reverse causation is unlikely. Our goal was to estimate the causal association of vitamin D and body mass index (BMI) on pediatric-onset MS risk using instrumental variable analysis based on genetic variants from established large genome-wide association studies (GWAS) in 2 large datasets of cases and controls.

METHODS

US participants. Pediatric-onset MS cases were enrolled through pediatric MS centers established at University of California San Francisco (UCSF), Stony Brook, Children's Hospital of Philadelphia, Texas Children's Hospital, University of Colorado School of Medicine, University of Texas Southwestern, State University of New York Buffalo, Loma Linda University, Mayo Clinic, University of Alabama at Birmingham, Ann and Robert Lurie Children's Hospital of Chicago, University of Utah, Boston Children's Hospital, Massachusetts General Hospital, Washington University St. Louis, and Children's National Medical Center between January 2006 and December 2014. These centers are tertiary referral centers, but also serve local patients from all socioeconomic groups. Consecutive patients with onset of MS or clinically isolated syndrome (CIS) suggestive of early MS before age 18 years seen at some of these pediatric MS clinics were offered participation as previously described.[11] In addition, we utilized adult cases from Kaiser Permanente Northern California (KPNC) with reported age at onset <18 years. A separate cohort of distinct cases were enrolled in an NIH-supported case-control study (NS071463, PI Waubant) with MS or CIS onset before the age of 18, seen within 4 years of disease onset, with at least 2 silent T2-bright foci on brain and cord MRI, and ascertained by a panel of at least 2 pediatric MS experts.[12] Additional cases were provided from another MS genetic study and only patients with a disease onset prior to age 18 based on medical records were included through UCSF. Established diagnostic criteria were used for all cases (total n = 738).[3,13]

Control individuals were derived from multiple sources to increase statistical power. These included (1) adult controls recruited from the KPNC membership without a diagnosis of MS or related condition (optic neuritis, transverse myelitis, or demyelination disease) confirmed through electronic medical records (n = 1,182)[14]; (2) adult controls from the Genetic Epidemiology Research on Adult Health and Aging cohort who participated in the KPNC Research Program on Genes, Environment, and Health (RPGEH) without evidence of MS in electronic medical records (dbGaP phs000674.v2.p2; N = 10,819); (3) pediatric controls (R01 NS071463, PI Waubant; n = 70); and (4) pediatric controls recruited as part of the Northern California Childhood Leukemia Study (n = 229).[15]

Whole blood was collected, processed, and extracted for DNA using Gentra Puregene protocol or received as Guthrie Card blood samples and extracted for DNA using the QIAamp DNA micro kit for dried blood spots (Qiagen, Venlo, Netherlands). Saliva was collected for DNA extraction using Oragene (Genotek, Murrieta, CA) kits. Genome-wide profiling was performed for all pediatric-onset MS cases and a subset of controls using the Illumina (San Diego, CA) Infinium platform. The Infinium 660K BeadChip or HumanOmniExpressExom BeadChip was used to genotype each study participant. RPGEH was genotyped using custom-designed Affymetrix (Santa Clara, CA) Axiom arrays. Genotype imputation was performed using IMPUTE2 and the 1,000 Genomes phase 3 reference haplotypes. Only single nucleotide polymorphisms (SNPs) with a high imputation quality score (info score ≥0.3) were retained for analysis. We eliminated SNPs that were associated with being genotyped on a particular array, were imputed in fewer than 99% of individuals, and deviated from Hardy-Weinberg equilibrium in controls ($p < 0.000001$).

Classical multidimensional scaling (MDS) was performed to visualize population substructure and provide quantitative measures of population genetic variation. MDS was completed for all genotyped data, which was merged with the Human Genome Diversity Project (HGDP) reference. Analysis of genome-wide average proportion of alleles shared identical by state was performed, and related/identical individuals were removed. Study samples were aligned with HGDP references, and the first 3 dimensions from MDS of the HGDP European population were used. We restricted our sample to white non-Hispanic participants (European ancestry), the largest group in our dataset, to ensure a genetically homogenous sample and avoid the possibility of confounding by genetic ancestry. After restriction and excluding population outliers (identified using MDS), the final dataset comprised 394 pediatric-onset MS cases and 10,875 controls.

Swedish participants. Data were collected from 2 population-based case-control studies of incident (Epidemiologic Investigation of Risk Factors for MS [EIMS]) and prevalent (Genes and Environment in MS [GEMS]) MS. The EIMS study (2005–2014) inclusion criteria were age 16–70 years, diagnosed MS according to the to the McDonald criteria[13,16] within 2 years, and ability to understand the Swedish language. GEMS study participants were identified from the Swedish National MS registry, fulfilled the McDonald criteria, and were recruited during 2009–2011. We included cases with a reported age at onset <18 years. For both studies, controls were randomly chosen from the population register and matched to cases by sex, age at inclusion in the study, and region of residence. Two controls were matched to

each case in the EIMS study and one control per case in the GEMS study. All participants in the EIMS study were distinct from those in the GEMS study. Details of the study design have been described elsewhere.[17] The participation rate in the EIMS study was 92% for cases and 67% for controls, and in the GEMS study 82% for cases and 66% for controls. Genotyping data were available for 75% of EIMS and 91% of GEMS participants. Data for 175 cases and 5,376 controls were available for the current study.

All participants were asked to give blood samples, which were genotyped on an Illumina custom array and on OmniExpress-24. *HLA-DRB1* information was imputed with *HLA*IMP02* using genotypes in the major histocompatibility complex region from the custom array. SNPs with <2% minor allele frequency, genotyped in <98% of individuals, or not in Hardy-Weinberg equilibrium among controls ($p < 0.0001$) were removed from analysis. Individuals with >2% failed genotype calls, related individuals, or population outliers identified using the SmartPCA program were removed. Ninety of the 110 MS risk SNPs were present on the custom array. Proxy SNPs for an additional 18 MS risk SNPs were selected from the custom array ($R^2 > 0.8$, except rs28723576, which had $R^2 > 0.71$). All vitamin D SNPs were present on the custom array. Twelve BMI SNPs were taken from the custom array, and the remaining from the OmniExpress chip. Forty-three BMI SNPs were not present on the array and were imputed using MaCH 1.0 with standard settings and the Northern European 1,000 Genomes reference panel. Seventeen markers utilized the August 2009 reference panel, 25 markers the August 2010 panel, and 1 marker the July 2011 panel. The variant rs2245368 could not be imputed with high quality and was therefore omitted from the BMI GRS.

Standard protocol approvals, registrations, and patient consents. Study protocols were approved by the institutional review boards for human subjects at UCSF, Stony Brook, Children's Hospital of Philadelphia, Texas Children's Hospital, University of Colorado School of Medicine, University of Texas Southwestern, State University of New York Buffalo, Loma Linda University, Mayo Clinic, University of Alabama at Birmingham, Ann and Robert Lurie Children's Hospital of Chicago, University of Utah, Boston Children's Hospital, Massachusetts General Hospital, Washington University St. Louis, Children's National Medical Center, Kaiser Permanente Division of Research, University of California Berkeley, Regional Ethical Review Board in North Stockholm, and Karolinska Institutet. Informed consent or assent (children) was obtained for all study participants and their parents when appropriate.

Exposure assessment. A previous GWAS identified 3 SNPs strongly associated with serum vitamin D level: rs2282679, rs2060793, and rs3829251, which together explain approximately 2.8% of the variance in circulating vitamin D levels.[18] A genetic instrumental variable for vitamin D (vitD GRS) was constructed using the 3 risk variants, weighting each allele by the effect size reported in the GWAS and summing across the variants. The BMI instrumental variable was derived using β coefficients as weights for 97 variants associated with BMI (R^2 = 2.7%) identified through the largest and most recent GWAS.[19] The sum of risk alleles multiplied by the estimated effect reported of each risk allele on the phenotype was calculated as each individual's BMI genetic risk score (GRS).

We also explored whether variants specifically associated with childhood BMI (chBMI) were associated with pediatric-onset MS susceptibility. A chBMI GRS was constructed using 28 independent variants associated with pediatric/childhood BMI in the literature.[20–24] The score was unweighted, and calculated by summing the number of risk alleles across each locus. Of the 28 variants in the chBMI GRS, 11 overlapped or were highly correlated with ($R^2 > 0.6$) variants in the BMI GRS.

A weighted GRS (wGRS) that combines the weighted odds ratio (OR) from each of 110 non-MHC MS susceptibility loci identified through recent GWAS[25] was calculated for each pediatric-onset case and control by multiplying the number of risk alleles for each locus by the weight for that variant and then taking the sum across the 110 loci.[26] The weight for each locus is the natural log of the OR for each allele. Three SNPs were missing in the US study (rs201202118, rs201847125, and imm_5_141486748), and 1 SNP failed Hardy-Weinberg equilibrium, rs2744148. Five SNPs were missing from the Swedish data and adequate proxies were not found (rs4772201, rs2028597, rs4679081, imm_5_1414867748, and rs533646). Characterization of *HLA-DRB1*15:01* (0, 1, or 2 allele copies) for US participants was based on the rs3135388 tag SNP, which is highly correlated with *DRB1*15:01* alleles ($r^2 = 0.97$).[27]

Statistical analyses. Logistic regression was used to estimate the effect of BMI and vitD GRS on pediatric-onset MS case/control status, controlling for sex, wGRS, presence of any *HLA-DRB1*15:01* alleles, and genetic ancestry. Ninety-five percent confidence intervals (CIs), OR, and p values were reported. Interaction was assessed on the multiplicative scale. Meta-analysis assuming fixed effects was performed if χ^2 tests of heterogeneity demonstrated $p > 0.05$. All analyses were conducted in PLINK, STATA, or R.

RESULTS Clinical and genetic characteristics of cases and controls are shown in table 1. Mean age at onset for pediatric cases was 14.05 years (±3.30) in the US study and 14.91 (±2.67) in the Swedish study. There was a significant difference in wGRS and *HLA-DRB1*15:01* status between cases and controls in both studies ($p < 0.001$). Average GRS for each instrumental variable in cases and controls are also reported in table 1. There was no association between age at onset and any of the instrumental variables in either study population (data not shown).

In the meta-analysis of both US and Swedish studies, vitD GRS was significantly associated with a decreased risk of MS ($p = 0.02$) after adjusting for sex, *HLA-DRB1*15:01*, wGRS, and genetic ancestry (table 2). The instrumental variable analysis for the BMI GRS also demonstrated a causal association between BMI and pediatric-onset MS as represented by the BMI GRS after adjusting for covariates ($p = 0.01$). There was no significant association between chBMI GRS and pediatric-onset MS. These results were consistent with results from analyses that also included pediatric-onset cases with age at onset 18 years (total n = 415 US cases and n = 262 Swedish cases; table e-1 at Neurology.org), with meta-analysis estimates demonstrating a significant association between MS and BMI GRS (OR 1.15, 95% CI 1.04, 1.27; $p = 0.01$), vitD GRS (OR 0.75, 95% CI 0.59, 0.97; $p = 0.03$), but not chBMI GRS (OR 1.01, 95% CI 0.99, 1.04; $p = 0.31$).

Table 1 Clinical and genetic characteristics of patients with pediatric-onset multiple sclerosis and controls

	United States			Sweden		
	Pediatric-onset MS (n = 394)	Controls (n = 10,875)	*p* Value	Pediatric-onset MS (n = 175)	Controls (n = 5,376)	*p* Value
Age at onset, y	14.05 ± 3.30	—	—	14.91 ± 2.67	—	—
Female:male	3.0:1	1.6:1	0.02	2.5:1	3.2:1	0.15
wGRS	12.78 ± 0.67	12.31 ± 0.69	<0.001	12.41 ± 0.66	11.91 ± 0.69	<0.001
*HLA-DRB1*15:01*			<0.001			<0.001
0	193 (49)	7,973 (72)		57 (33)	3,806 (71)	
1–2	201 (51)	2,902 (28)		118 (67)	1,570 (29)	
vitD GRS	1.02 ± 0.28	1.04 ± 0.31	0.11	1.01 ± 0.32	1.06 ± 0.32	0.07
BMI GRS	11.51 ± 0.85	11.38 ± 0.81	0.002	11.54 ± 0.79	11.50 ± 0.82	0.49
chBMI GRS	25.45 ± 3.15	25.32 ± 3.26	0.44	26.06 ± 3.50	26.09 ± 3.32	0.93

Abbreviations: BMI = body mass index; chBMI GRS = childhood body mass index genetic risk score; vitD GRS = genetic instrumental variable for vitamin D; wGRS = weighted genetic risk score.
Table values are mean ± SD for continuous variables and n (column %) for categorical variables; *p* value is for *t* test or χ^2 test between cases and controls.

When modeled together, both the vitD and BMI GRS independently contributed to pediatric-onset MS susceptibility. Joint estimates were consistent with those found when each GRS was modeled alone in both populations. For example, in the US study, estimates from the multivariate analysis reflected OR 1.21 (95% CI 1.06, 1.37) for the BMI GRS and OR 0.74 (95% CI 0.53, 1.03) for the vitD GRS.

There was no evidence of interaction between vitD GRS or chBMI GRS and *HLA-DRB1*15:01* (data not shown); however, a significant interaction was present between BMI GRS and *HLA-DRB1*15:01* in the US study (*p* interaction 0.04). Individuals carrying 1–2 *DRB1*15:01* risk alleles demonstrated a stronger association (OR 1.39) compared to noncarriers (OR 1.05). However, this interaction was not detected in the Swedish dataset (*p* interaction 0.66).

Table 2 Meta-analysis results support a causal association between both low vitamin D and high body mass index (BMI) and pediatric-onset multiple sclerosis

Instrumental variable[a]	Odds ratio[b] (95% CI)	*p* Value
Vitamin D GRS	0.72 (0.55–0.94)	0.02
BMI GRS	1.17 (1.05–1.30)	0.01
Childhood BMI GRS	1.02 (0.79–1.33)	0.88

Abbreviation: GRS = genetic risk score.
Analyses adjusted for sex, *HLA-DRB1*15:01*, weighted GRS, and genetic ancestry derived from principal components.
[a] GRS used as instrumental variables to capture low vitamin D and high BMI (see Methods).
[b] Fixed effects meta-analysis; all χ^2 tests of heterogeneity $p > 0.05$.

DISCUSSION We report strong evidence for a causal and independent association between low serum concentrations of vitamin D and increased BMI and risk of pediatric-onset MS after adjusting for sex, ancestry, *HLA-DRB1*15:01*, and over 100 non–human leukocyte antigen (HLA) MS risk variants. While previous studies have attributed an increased risk of MS associated with BMI to lower vitamin D levels seen in obese individuals, our findings support a role for independent contributions to disease susceptibility.

Several studies have shown a protective effect of vitamin D levels on MS risk. Two prospective studies showed a significantly reduced risk of MS in those with higher 25(OH)D.[28,29] Maternal vitamin D deficiency (25[OH]D levels <12.02 ng/mL) during early pregnancy may be associated with a 2-fold risk of MS in offspring.[30] Further, recent MR studies suggest a causal effect for low 25(OH)D on adult MS risk.[8,9] Findings from our meta-analysis reveal that vitamin D represented as a GRS is significantly associated with pediatric-onset MS, with a stronger effect than in comparable adult-onset populations (OR 0.85).[9] A larger sample size or instrumental variable constructed to include additional variants may help to demonstrate an even larger magnitude of effect, as each SNP used in the vitD GRS explained ≤1.2% of the variance in serum 25(OH)D concentrations.

Our findings also suggest that increased BMI is indeed a causal risk factor for pediatric-onset MS, similar to adults.[31,32] Analogous to vitD GRS results, the BMI GRS demonstrates a stronger effect than that seen in adults (OR 1.10).[32] Interestingly, previous studies have shown that specific HLA genotypes interact with self-reported BMI during adolescence to increase the risk of MS,[33] an interaction that was also present in our study based on a BMI GRS; however,

we were unable to replicate this finding in the Swedish dataset, likely due to lower power resulting from a smaller sample size.

There are several hypotheses linking low vitamin D and high BMI to autoimmune diseases, including MS. Increased exposure to vitamin D leads to decreased production of inflammatory cytokines, a decrease in Th1 and Th17 cell differentiation, and an increase in T-regulatory cells, suggesting that low vitamin D is acting on MS risk by shifting the balance of the immune response toward a more proinflammatory state.[34–36] Obesity may also induce a Th17 response via an interleukin-6-dependent process leading to exacerbation of inflammatory diseases such as MS.[37] In addition, a Th17/Treg imbalance may lead to alteration of intestinal microbiome in obese individuals, altering the intestinal immune response, which may explain the association between increased BMI and MS.[38,39]

Our study had several strengths, including clinically well-characterized pediatric-onset MS cases with average symptom onset 20 years earlier than most adult MS cases, rigorous diagnostic criteria, and focus on a single racial group. Comprehensive methods were used for quality control of genotypes and to identify and remove population outliers prior to analysis. We also analyzed BMI and vitamin D risk scores together in multivariate modeling, and used 2 study samples to conduct a large, well-powered meta-analysis. In addition, evaluation of our genetic risk scores in a subset of participants demonstrated a significant association with childhood body size (BMI GRS: Sweden n = 1,359, R^2 = 1.3%, $p < 0.001$; US n = 723, R^2 = 0.7%, $p = 0.03$) and serum vitamin D levels adjusted for month of sample draw (vitD GRS: Sweden n = 881, R^2 = 2.5%, $p < 0.001$; US results previously demonstrated).[40] Further research involving larger sample sizes is needed to examine whether serum vitamin D levels and childhood obesity independently and directly increase the risk, severity, and progression of pediatric-onset MS.

Limitations include identification of pediatric-onset MS cases through tertiary clinics in the United States, which may not represent all MS cases with pediatric onset. While many of our cases had confirmed physician-based reports of pediatric MS (n = 130), a fraction self-reported the onset of their first disease symptoms in childhood. When the 2 patient groups were examined separately, similar effect sizes for each instrumental variable were observed (data not shown); therefore, self-report of pediatric symptoms was not likely to lead to misclassification. It is possible that individuals with a more benign disease or without access to health care may have been missed. In addition, instrumental variable analysis involves certain assumptions that we have addressed. We met required model assumptions by utilizing a vitD and BMI GRS constructed from weights derived from independent populations through large published GWAS. However, associations of genetic variants with unmeasured or unknown confounders cannot be ruled out, and our models assumed linearity of the relationship between instrumental variable and outcome. Our study included non-Hispanic white participants and small numbers of male patients, which may limit the generalizability of our findings. Larger studies are needed to determine whether sex-specific effects contribute to pediatric-onset MS.

Our results add to the growing evidence supporting an important role of genetic and environmental factors in pediatric-onset MS. A causal relationship between low vitamin D and increased BMI has implications for prevention strategies in children. The effect of both low vitamin D and obesity may involve independent predisposing genetic factors and biological pathways mediating disease onset that future studies will unravel.

AUTHOR CONTRIBUTIONS

Dr. Gianfrancesco: analysis and interpretation of the data, drafting the article, final approval of the version to be published. Dr. Stridh: analysis and interpretation of the data, drafting the article, final approval of the version to be published. B. Rhead: analysis and interpretation of the data, reviewing and revising of the article, final approval of the version to be published. X. Shao: analysis and interpretation of the data, reviewing and revising of the article, final approval of the version to be published. E. Xu: analysis and interpretation of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Graves: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Chitnis: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Waldman: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Lotze: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Schreiner: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Belman: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Greenberg: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Weinstock-Guttman: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Aaen: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Tillema: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. J. Hart: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. S. Caillier: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Ness: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Y. Harris: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Rubin: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Candee: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Krupp: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Gorman: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Benson: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Rodriguez: acquisition of the data, reviewing and revising of

the article, final approval of the version to be published. Dr. Mar: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Kahn: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Rose: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. S. Roalstad: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Casper: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Shen: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. H. Quach: analysis of the data, reviewing and revising of the article, final approval of the version to be published. D. Quach: analysis of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Hillert: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Bäärnhielm: analysis of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Hedstrom: analysis of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Olsson: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Kockum: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Alfredsson: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Metayer: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Schaefer: acquisition of the data, reviewing and revising of the article, final approval of the version to be published. Dr. Barcellos: conception and design, data acquisition, interpretation of data, reviewing and revising of the article, final approval of the version to be published. Dr. Waubant: conception and design, data acquisition, reviewing and revising of the article, final approval of the version to be published.

ACKNOWLEDGMENT

The authors thank Dr. Jorge Oksenberg for technical support and contributions to this study; Drs. Ingileif Jónsdóttir and Kári Stefánsson from deCODE Genetics for assistance with genetic data from the Swedish study; and the patients and controls for participation.

STUDY FUNDING

This work was supported in part by the NIH NINDS: 1R01NS071463 (PI Waubant), R01 NS049510 (PI Barcellos), 1F31NS093832 (PI Gianfrancesco), 1F31NS096885 (PI Rhead), NIH K23 NS069806 (PI Waldman), NIH NIEHS: R01 ES017080 (PI Barcellos), R01ES009137 (PI Metayer), P42ES004705, NIH NIAID: R01 AI 076544 (PI Barcellos), NIH RC2 AG036607 (PI Schaefer), the National MS Society HC 0165 (PI Casper), The Robert Wood Johnson Foundation, The Wayne and Gladys Valley Foundation, The Ellison Medical Foundation. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

DISCLOSURE

M. Gianfrancesco, P. Stridh, B. Rhead, X. Shao, E. Xu, J.S. Graves, T. Chitnis, A. Waldman, T. Lotze, T. Schreiner, A. Belman, B. Greenberg, B. Weinstock-Guttman, G. Aaen, J. Tillema, J. Hart, S. Caillier, J. Ness, Y. Harris, J. Rubin, M. Candee, L. Krupp, M. Gorman, L. Benson, M. Rodriguez, S. Mar, and I. Kahn report no disclosures relevant to the manuscript. J. Rose has research support from VA, NMSS, Guthy Jackson Charitable Foundation, Arrien Pharmaceuticals, Teva Neuroscience, and Biogen. He is a member of the Medical Advisory Board for the DECIDE trial, which is funded by Biogen and AbbVie. S. Roalstad, T. Casper, L. Shen, H. Quach, D. Quach, J. Hillert, M. Bäärnhielm, A. Hedstrom, T. Olsson, and I. Kockum report no disclosures relevant to the manuscript. L. Alfredsson received research support from the Swedish Medical Research Council (K2013-69X-14973-10-4), the Swedish Council for Health, Working Life and Welfare (DNR 2012-0325), and the Swedish Brain Foundation and has received speaker honoraria from Biogen Idec and TEVA. C. Metayer and C. Schaefer report no disclosures relevant to the manuscript. L. Barcellos is funded by the NIH, the National MS Society, and the Race to Erase MS. E. Waubant is funded by the NIH, the NMSS, and the Race to Erase MS. She volunteers on an advisory board for a Novartis trial. She has received honorarium or travel support from ACTRIMS, ECTRIMS, and AAN. Go to Neurology.org for full disclosures.

Received August 11, 2016. Accepted in final form January 20, 2017.

REFERENCES

1. Chitnis T, Krupp L, Yeh A, et al. Pediatric multiple sclerosis. Neurol Clin 2011;29:481–505.
2. Yeh EA, Chitnis T, Krupp L, et al. Pediatric multiple sclerosis. Nat Rev Neurol 2009;5:621–631.
3. 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 2013;19:1261–1267.
4. Mikaeloff Y, Caridade G, Tardieu M, Suissa S. Parental smoking at home and the risk of childhood-onset multiple sclerosis in children. Brain 2007;130:2589–2595.
5. Waldman A, Ghezzi A, Bar-Or A, Mikaeloff Y, Tardieu M, Banwell B. Multiple sclerosis in children: an update on clinical diagnosis, therapeutic strategies, and research. Lancet Neurol 2014;13:936–948.
6. Banwell B, Bar-Or A, Arnold DL, et al. Clinical, environmental, and genetic determinants of multiple sclerosis in children with acute demyelination: a prospective national cohort study. Lancet Neurol 2011;10:436–445.
7. Mowry EM, Krupp LB, Milazzo M, et al. Vitamin D status is associated with relapse rate in pediatric-onset multiple sclerosis. Ann Neurol 2010;67:618–624.
8. Mokry LE, Ross S, Ahmad OS, et al. Vitamin D and risk of multiple sclerosis: a mendelian randomization study. PLoS Med 2015;12:e1001866.
9. Rhead B, Bäärnhielm M, Gianfrancesco MA, et al. Mendelian randomization shows a causal effect of low vitamin D on multiple sclerosis risk. Neurol Genet 2016;2:e97.
10. Langer-Gould A, Brara SM, Beaber BE, Koebnick C. Childhood obesity and risk of pediatric multiple sclerosis and clinically isolated syndrome. Neurology 2013;80:548–552.
11. Waubant E, Mowry EM, Krupp L, et al. Common viruses associated with lower pediatric multiple sclerosis risk. Neurology 2011;76:1989–1995.
12. McDonald J, Graves J, Waldman A, et al. A case-control study of dietary salt intake in pediatric-onset multiple sclerosis. Mult Scler Relat Disord 2016;6:87–92.
13. McDonald WI, Compston A, Edan G, et al. Recommended diagnostic criteria for multiple sclerosis: guidelines from the international panel on the diagnosis of multiple sclerosis. Ann Neurol 2001;50:121–127.
14. Gianfrancesco MA, Acuna B, Shen L, et al. Obesity during childhood and adolescence increases susceptibility to multiple sclerosis after accounting for established genetic and environmental risk factors. Obes Res Clin Pract 2014;8:e435–e447.
15. Ma X, Buffler PA, Layefsky M, et al. Control selection strategies in case-control studies of childhood diseases. Am J Epidemiol 2004;159:915–921.
16. 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.
17. Hedstrom AK, Baarnhielm M, Olsson T, Alfredsson L. Tobacco smoking, but not Swedish snuff use, increases the risk of multiple sclerosis. Neurology 2009;73:696–701.

18. Ahn J, Yu K, Stolzenberg-Solomon R, et al. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet 2010;19:2739–2745.
19. Locke AE, Kahali B, Berndt SI, et al. Genetic studies of body mass index yield new insights for obesity biology. Nature 2015;518:197–206.
20. Bradfield JP, Taal HR, Timpson NJ, et al. A genome-wide association meta-analysis identifies new childhood obesity loci. Nat Genet 2012;44:526–531.
21. den Hoed M, Ekelund U, Brage S, et al. Genetic susceptibility to obesity and related traits in childhood and adolescence: influence of loci identified by genome-wide association studies. Diabetes 2010;59:2980–2988.
22. Mitchell JA, Hakonarson H, Rebbeck TR, Grant SF. Obesity-susceptibility loci and the tails of the pediatric BMI distribution. Obesity 2013;21:1256–1260.
23. Stergiakouli E, Gaillard R, Tavare JM, et al. Genome-wide association study of height-adjusted BMI in childhood identifies functional variant in ADCY3. Obesity (Silver Spring) 2014;22:2252–2259.
24. Warrington NM, Howe LD, Paternoster L, et al. A genome-wide association study of body mass index across early life and childhood. Int J Epidemiol 2015;44:700–712.
25. Beecham AH, Patsopoulos NA, Xifara DK, et al. Analysis of immune-related loci identifies 48 new susceptibility variants for multiple sclerosis. Nat Genet 2013;45:1353–1360.
26. De Jager PL, Chibnik LB, Cui J, et al. Integration of genetic risk factors into a clinical algorithm for multiple sclerosis susceptibility: a weighted genetic risk score. Lancet Neurol 2009;8:1111–1119.
27. Zivkovic M, Stankovic A, Dincic E, et al. The tag SNP for HLA-DRB1*1501, rs3135388, is significantly associated with multiple sclerosis susceptibility: cost-effective high-throughput detection by real-time PCR. Clin Chim Acta 2009;406:27–30.
28. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA 2006;296:2832–2838.
29. Salzer J, Hallmans G, Nystrom M, Stenlund H, Wadell G, Sundstrom P. Vitamin D as a protective factor in multiple sclerosis. Neurology 2012;79:2140–2145.
30. Munger KL, Aivo J, Hongell K, Soilu-Hanninen M, Surcel HM, Ascherio A. Vitamin D status during pregnancy and risk of multiple sclerosis in offspring of women in the Finnish maternity cohort. JAMA Neurol 2016;73:515–519.
31. Mokry LE, Ross S, Timpson NJ, et al. Obesity and multiple sclerosis: a mendelian randomization study. PLoS Med 2016;13:e1002053.
32. Gianfrancesco M, Glymour M, Walter S, et al. Genetic variants associated with body mass index demonstrate a causal effect on multiple sclerosis susceptibility. Am J Epidemiol Epub 2017 Jan 9.
33. Hedstrom AK, Lima Bomfim I, Barcellos L, et al. Interaction between adolescent obesity and HLA risk genes in the etiology of multiple sclerosis. Neurology 2014;82:865–872.
34. Aranow C. Vitamin D and the immune system. J Investig Med 2011;59:881–886.
35. Hart PH, Gorman S, Finlay-Jones JJ. Modulation of the immune system by UV radiation: more than just the effects of vitamin D? Nat Rev Immunol 2011;11:584–596.
36. Prietl B, Treiber G, Pieber TR, Amrein K. Vitamin D and immune function. Nutrients 2013;5:2502–2521.
37. Winer S, Paltser G, Chan Y, et al. Obesity predisposes to Th17 bias. Eur J Immunol 2009;39:2629–2635.
38. Brown K, DeCoffe D, Molcan E, Gibson DL. Diet-induced dysbiosis of the intestinal microbiota and the effects on immunity and disease. Nutrients 2012;4:1095–1119.
39. Manzel A, Muller DN, Hafler DA, Erdman SE, Linker RA, Kleinewietfeld M. Role of "Western diet" in inflammatory autoimmune diseases. Curr Allergy Asthma Rep 2014;14:404.
40. Graves J, Barcellos L, Belman A, et al. Vitamin D genetic risk score is strongly associated with vitamin D levels and relapse rate in pediatric MS patients. Presented at the ECTRIMS annual meeting; September 14–17, 2016; London.