UCSF
UC San Francisco Previously Published Works

Title
Vitamin D genes influence MS relapses in children.

Permalink
https://escholarship.org/uc/item/4m24z973

Journal
Multiple sclerosis (Houndmills, Basingstoke, England), 26(8)

ISSN
1352-4585

Authors
Graves, Jennifer S
Barcellos, Lisa F
Krupp, Lauren
et al.

Publication Date
2020-07-01

DOI
10.1177/1352458519845842

Peer reviewed

eScholarship.org Powered by the California Digital Library
University of California

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

HHS Public Access

Author manuscript

Mult Scler. Author manuscript; available in PMC 2021 July 01.

Published in final edited form as:

Mult Scler. 2020 July ; 26(8): 894–901. doi:10.1177/1352458519845842.

Vitamin D genes influence MS relapses in children

Jennifer S. Graves, MD, PhD, MAS[1], **Lisa F. Barcellos, PhD**[2], **Lauren Krupp, MD,PhD**[3], **Anita Belman, MD**[4], **Xiaorong Shao, MA**[2], **Hong Quach, BA**[2], **Janace Hart**[1], **Tanuja Chitnis, MD**[6], **Bianca Weinstock-Guttman, MD**[7], **Gregory Aaen, MD**[8], **Leslie Benson, MD**[9], **Mark Gorman, MD**[9], **Benjamin Greenberg, MD**[10], **Timothy Lotze, MD**[11], **Mar Soe, MD**[12], **Jayne Ness, MD, PhD**[13], **Moses Rodriguez, MD**[14], **John Rose, MD**[15], **Teri Schreiner, MD**[16], **Jan-Mendelt Tillema, MD**[14], **Amy Waldman, MD**[17], **T. Charles Casper, PhD**[5], **Emmanuelle Waubant, MD,PHD**[1] **U.S. Network of Pediatric MS Centers.**

[1]Neurology, University of California, San Francisco, San Francisco, United States;

[2]School of Public Health, University of California, Berkeley, Berkeley, United States

[3]Pediatric Multiple Sclerosis Center at New York University Langone Medical Center, Stony Brook, NY, United States.

[4]Stony Brook University, Stony Brook, NY, United States.

[5]Department of Pediatrics, University of Utah

[6]Partners Pediatric MS Center at the Massachusetts General Hospital for Children

[7]Jacobs Pediatric MS Center at SUNY University at Buffalo

[8]Pediatric Multiple Sclerosis Center at Loma Linda University Children's Hospital

[9]Pediatric Multiple Sclerosis and Related Disorders Program at Boston Children's Hospital

[10]University of Texas Southwestern

[11]The Blue Bird Circle Clinic for Multiple Sclerosis, Texas Children's Hospital

[12]Washington University Pediatric MS and other Demyelinating Disease Center, St. Louis

[13]Center for Pediatric-Onset Demyelinating Disease at the Children's of Alabama

[14]Mayo Clinic Pediatric MS Center

[15]Department of Neurology, University of Utah

[16]Rocky Mountain MS Center, University of Colorado at Denver

[17]The Children's Hospital of Philadelphia

Abstract

Objective: To determine whether a vitamin D genetic risk score is associated with 25-OH D level and MS relapses in children.

Corresponding author: Jennifer Graves MD, PhD, MAS, Address: 675 Nelson Rising Lane, Suite 221 Box 3206, San Francisco, CA, Telephone: (415) 297-2344, Fax: (415) 514-2170, Jennifer.Graves@ucsf.edu.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Methods: DNA samples were typed for single nucleotide polymorphisms (SNPs) from four genes previously identified to be associated with 25-OH D levels. SNPs with strong associations with 25-OH D after multiple comparisons correction were used to create a genetic risk score (vitDGRS). Cox regression models tested associations of vitDGRS with relapse hazard.

Results: Two independent SNPs within or near *GC* and *-NADSYN1/DHCR7,* genes were strongly associated with 25-OH D levels in the discovery cohort (n=182) after Bonferroni correction. The vitDGRS of these SNPs explained 4.5% of the variance of 25-OH D level after adjustment for genetic ancestry. Having the highest vs. lowest vitDGRS was associated with 1ng/ml lower 25-OH D level (95%CI=−17.5,−4.5.p=0.001) in the discovery cohort._Adjusting for ancestry, sex, disease-modifying therapy, and *HLA-DRB1*15* carrier status, the highest vs. lowest vitDGRS was associated with 2.6-fold (95%CI=1.37,5.03,p=0.00) and 2.0-fold (95%CI=0.75,5.20.p=0.16) higher relapse hazard in the discovery and replication cohorts, respectively.

Conclusions: The vitDGRS identifies children at greater risk of relapse. These findings support a causal role for vitamin D in MS course.

Keywords

genetics; epidemiology; vitamin D; pediatric multiple sclerosis

Introduction

Hypovitaminosis D has been associated with increased risk for multiple sclerosis (MS).[1,2] Low 25-OH vitamin D (25-OH D) level has also been associated with active disease in MS patients, including higher relapse rate, new T2 lesion formation, and increased disability.[3–6] However, concerns regarding unmeasured confounders and reverse causation still remain for this putative, modifiable risk factor. Genetic instrumental variables (IV) can directly address these concerns by serving as a proxy of the environmental or modifiable risk factor of interest upstream of any confounding effects.[7] A genetic IV facilitates a Mendelian randomization experiment for the risk factor of interest.[7] For example, such variables have been used to support a causal association between fasting glucose, type 2 diabetes, and coronary artery disease[8] and between obesity and MS.[9, 10] A number of prior studies including GWAS have examined the associations of individual SNPs in the vitamin D pathway with levels of vitamin D in healthy controls and subjects with various diseases and ancestries.[11–18]

Common genetic variants associated with the vitamin D pathway have emerged in genome wide association studies (GWAS) for MS[19, 20], further supporting a role for vitamin D in the risk of disease. However, it is not clear if these particular variants directly affect vitamin D levels in patients or if additional genetic interrogation of the vitamin D pathway will reveal more sensitive proxies for vitamin D level effects in MS. Prior work has shown that genetic IVs for low vitamin D level are also associated with risk to have MS[21–23], but it is not known if genetic factors driving vitamin D level are associated with the clinically pressing question of disease course.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

We sought to establish a genetic risk score for vitamin D pathway polymorphisms associated with serum vitamin D levels in pediatric MS patients and to determine whether that risk score could replicate the association of vitamin D level with disease activity, specifically relapse rate, in pediatric MS.

Materials and Methods

Subjects

Consecutive pediatric subjects presenting for care and meeting criteria for MS or clinically isolated syndrome (CIS) with high risk of MS were offered enrollment into the U.S. Network of Pediatric MS Centers registry. For the discovery cohort, subjects were enrolled from University of California, San Francisco and Stony Brook, New York from 2006–2011 and prospectively followed for relapses. For the replication cohort, subjects were recruited from 9 centers across the United States from 2011–2016 (none overlapping with the discovery set) as part of an investigation of risk factors in pediatric MS (R01NS071463, PI Waubant). Most centers have large catchment areas and provide care to families with and without private health insurance. Parents provided written consent and age appropriate children provided assent to participate in the study. Blood samples were collected upon enrollment. Parents reported race and ethnicity according to NIH standards. Disease-modifying therapy (DMT) use was recorded in both cohorts.

Vitamin D levels

Serum measures of 25-OH D for all subjects were determined by chemoluminescence assay as previously described (Heartlands, ARUP).[3] Assays were performed in batch within each cohort group.

Relapses

Clinical events had to meet standard criteria to be considered a clinical MS relapse: new or significantly worsened neurological symptoms lasting greater than 24 hours, no evidence of infection or fever, and no exposure to extreme heat (pseudo-exacerbation excluded).

Genotyping

DNA samples were prepared from whole blood using standard procedures. The Infinium 660K BeadChip or HumanOmniExpress BeadChip was used to genotype each study participant as previously described[19] for SNPs associated with four vitamin D pathway genes of interest (GC, *CYP2R1, DHCR7*, and *NADSYN1*), which have previously been shown in GWAS and other studies to be associated with vitamin D levels.[11, 14, 24–27] The Illumina GenomeStudio software was employed to perform standard quality control assessments including overall sample and SNP call rates (<90%), sex discrepancies, reproducibility of replicates and checks for Mendelian inheritance using CEPH control trios. Quality control measures and comparison of sample genotypes across the two Illumina platforms above were performed using PLINK v.1.07.[28]

We employed IMPUTE2 for genotype imputation using 1,000 Genomes Phase 3 reference haplotypes. To be used in the analyses, SNPs had to have high imputation quality (“info”

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

score > 0.3). SNPS were removed that were associated with being genotyped on a particular array, were imputed in fewer than 99% of individuals, or deviated from Hardy-Weinberg equilibrium in available controls. SNPs were also excluded if the minor allele frequency (MAF) was less than 0.05.

Ancestral estimates were generated as previously described using default parameters within STRUCTURE v2.3.1 and five ancestral populations, European, African, Central Asian, American, and East Asian.[29, 30]

Linkage disequilibrium (LD) analysis implemented in PLINK and using 1000 genomes data identified 32 haplotype blocks within genomic regions on chromosomes 4, 11, and 12 containing the genes of interest: *GC, CYP2R1, DHCR7*, and *NADSYN1*. Full variation within each gene, including the SNPs previously identified through GWAS,[1114, 24–27] were investigated for association with vitamin D level. SNPs associated with the genes *VDR* and *CYP24A1* were not included in these analyses, as they have been identified to be direct risk factors for MS in large GWAS, limiting their ability to be used in an IV for vitamin D level.

Carrier status for *HLA-DRB1*15:01/03* allele was determined by direct sequencing. Participants were noted as carriers vs. non-carriers of *HLA-DRB1*15*.

Statistical Analysis

Means, medians or frequencies were used to described patient characteristics as appropriate. Additive genotype models using linear regression were used to test association between each SNP and vitamin D level. To address concern for false positives resulting from multiple comparisons, a Bonferroni correction was applied for the 32 regions of DNA tested ($p<0.0016$). SNPs meeting these stringent criteria were then used to construct an unweighted risk score for low vitamin D level (vitDGRS). To assess whether vitDGRS and vitamin D levels approximated a normal distribution the quantiles of the risk score or vitamin D level were plotted against the quantiles of a normal distribution (qnorm function, STATA v12). Linear regression models compared vitDGRS to 25-OH vitamin D level. To determine variance of vitamin D level explained by the risk score we started first with a model only adjusting for genetic ancestry and then a second model with ancestry and the vitDGRS. We examine the additional variance of the outcome (vitamin D level) explained by the model with adding the vitDGRS variable.

Survival analysis (Cox proportional hazards regression) was employed to determine the association of vitDGRS with relapse hazard. The model was tested for proportional hazard assumption using Schoenfeld residuals. Potential confounding was assessed for genetic ancestry, sex, and *HLA-DRB1*15:01/03* carrier status (yes/no). While DMT use would not be expected to be a confounder, as it is not associated with genotype, treatment would be expected to have strong effects on the outcome and thus the standard errors of the models. Therefore we included DMT use as a time-varying covariate. Start and stop dates of DMT were used to determine amount of exposure. All analyses were performed using STATA 15 (Statacorp, TX).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Results:

Characteristics of the 182 pediatric subjects in the discovery cohort and 110 subjects in the replication cohort are described in Table 1. Briefly, in both groups roughly 65% of subjects were female, 65% were White and 30% were Hispanic. In the more recent replication cohort a higher percentage of patients had been exposed to DMT by the time of enrollment (81 vs. 72%). Mean 25-OH D levels in both patient cohorts were near lower limit of normal range (20 ng/ml) and the distribution of levels approximated a normal distribution (data not shown). There was no difference in vitamin D levels between girls and boys (p=0.44). Degree of European ancestry was associated with vitamin D level (explained 13% variance of 25-OH D level). For those with at least 50% European ancestry compared to non-European ancestry, vitamin D levels were 7.9 ng/ml higher on average (95% CI 5.02, 10.81, p<0.001).

Derivation of the Vitamin D Genetic Risk Score (vitDGRS)

A total of 32 independent genomic regions of DNA on chromosomes 4,11, and 12, containing the four genes of interest (GC, *CYP2R1, DHCR7*, and *NADSYN1*) from prior GWAS were evaluated for association with vitamin D levels in the discovery cohort of pediatric MS subjects (Figure 1). Of these, 2 independent regions were found to be associated with 25-OH vitamin D levels in the discovery cohort that survived correction for multiple comparisons (p<0.0016). SNPs in these regions with the lowest p-values were selected to represent these linkage disequilibrium (LD) blocks: rs7041_a (GC), and -rs12807827 *(NADSYN1/DHCR7)*. For the two loci there were additive effects for the risk allele. An unweighted genetic risk score comprised of the alleles at these loci was normally distributed in the discovery cohort (data not shown).

For the discovery cohort univariate analysis, one unit of the vitDGRS was associated with 3.94 ng/ml lower 25-OH vitamin D level (95% CI −5.21, −2.61, p=2.95×10−9). Having the highest vitDGRS vs. carrying no risk alleles was associated with 15.6 ng/ml lower 25-OH D level on average (95%)CI=−20.8, −10.4, p=2.95×10−9) in the discovery cohort. Adjusting for genetic ancestry, the highest vs. lowest vitDGRS was associated with 11 ng/ml lower 25-OH D level (95%CI=−17.5−4.5, p=0.001). The vitDGRS explained 4.5%of the variance of 25-OH D level after adjustment for genetic ancestry. If the analysis was restricted only to those of European descent, similar results were observed (n=107 participants): the highest vitDGRS vs. the lowest was associated with 13.5 ng/ml lower vitamin D level on average (95%CI=−22.1, −5.1, p=0.0022). Adjustments for sex, *HLA-DRB1*15:01/03* status (yes/no)or DMT use did not change the results (data not shown).

The vitDGRS was associated with serum 25-OH vitamin D levels in the replication cohort. Each unit of the score was associated with 3.2 ng/ml lower 25-OH D level and the highest vs. lowest vitDGRS with 12.8 ng/ml lower level in this independent dataset (Table 2). After adjusting for genetic ancestry, the highest vs. lowest vitDGRS was associated with 5.7 ng/ml lower 25-OH D level (95%CI=−16.3, 4.8, p=0.28). When the analysis was limited to those of European descent, similar point estimates to unadjusted were observed, though given limited sample size (n=70) the results did not reach nominal statistical significance: the

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

highest vitDGRS vs. the lowest was associated with 11.6 ng/ml lower vitamin D levels (95%CI=−23.9, 0.74, p=0.065).

Association between vitDGRS and relapses

Of important potential clinical significance and to support a causal role for vitamin D in disease course, we examined whether the vitDGRS was associated with relapses in children. In a multivariable Cox regression model adjusting for ancestry, sex, DMT use and *HLA-DRB1*15:01/03* status, one unit of the vitDGRS was associated with 27% higher hazard to relapse in the discovery cohort (Table 3). Comparing the highest to the lowest vitDGRS score, there was a 2.6 fold (260%) higher hazard to relapse (Table 3). Similar results were observed in the replication cohort, but in this smaller sample of participants with greater DMT exposure and fever overall relapses this result did not reach nominal statistical significance (p=0.16). For each unit of vitDGRS there was 19% higher hazard to relapse and for highest vs. lowest vitDGRS score, a 2-fold (200%) higher hazard to relapse (Table 3). Furthermore even though the primary multivariable analysis for the replication cohort did not reach nominal statistical significance there was a clear dosage effect of increasing vitDGRS score in this cohort (e.g. having 2 risk alleles = 11% increased relapse hazard, 3 risk alleles = 34% increase, 4 risk alleles=44% increased relapse hazard).

Restricting the analyses to those of European descent, there was 38% higher relapse hazard per unit of vitDGRS (HR 1.38, 95%CI 1.12, 1.67, p=0.003) in discovery set and 18% higher hazard (HR 1.18, 95%CI 0.87, 1.59, p=0.29) in the replication dataset. Comparing highest to lowest vitDGRS, there was 3.6 fold (360%) higher relapse hazard (95%CI 1.53, 8.55 p=0.003) in the discovery set and 1.9 fold (190%) higher hazard (95%CI 0.58, 6.42, p=0.29) in the replication dataset.

Discussion:

We show for the first time that polymorphisms in the vitamin D pathway are associated with 25-OH D levels and relapse rate in pediatric MS patients. A vitamin D genetic risk score captures lower levels of 25-OH D in children with MS. Importantly, we demonstrate that a high-risk score was associated with a clinically relevant increased relapse rate, supporting a causal role of vitamin D in disease course. It is of future interest to determine how the risk score may affect subgroup responses in treatment trials of vitamin D supplementation.

The two polymorphisms in the risk score have strong rationale for driving vitamin D levels and relapses. The rs7041 SNP is within the protein encoded by *GC*. This protein binds vitamin D and transports it to various targets.[31] The second SNP (rs12807827) in the risk score is annotated as tagging *NADSYN1*, the gene product of which is glutamine-dependent NAD(+) synthetase. It is an enzyme that catalyzes the final step in the synthesis of nicotinamide adenine dinucleotide. However, according to HaploReg (http://archive.broadinstitute.org/mammals/haploreg/haploreg.php) and as illustrated in the figure, rs12807827 also tags some variants in *DHCR7*. This latter gene encodes an enzyme which acts on 7-dehydrocholesterol, precursor to 25-OH vitamin D. The enzyme is thought to play a critical role as a switch between cholesterol and vitamin D production.[32]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Conclusive evidence that vitamin D supplementation improves clinical outcomes in MS is still pending results of large ongoing clinical trials[33]; in light of this evidence gap, our results further support a causal role for vitamin D level and the related pathway and disease course in MS. Our data are consistent with some of the completed vitamin D pilot trials. One of the largest trials to date SOLAR, with over 200 participants randomized demonstrated a lower annualized relapse rate in those receiving add-on high dose vitamin D3 compared to those only receiving interferon (ARR 0.28 vs 0.41), though this was a secondary aim of the study and did not reach statistical significance.[34] In the CHOLINE study of 63 patients, high dose vitamin D3 was associated with decreased relapses in those who completed the trial, but not in- the intention to treat analysis.[35] EVIDIMS, a small pilot trial of 53 MS patients with primary outcome reported in 2018, examined high dose vs low dose vitamin D supplementation on new T2 lesion development and did not find a significant difference between groups.[36] However, the sample size was small and even the control low dose arm had average increase of 8ng/ml in vitamin D level from below to above the normal cut-off value for vitamin D according to the US FDA (20 ng/ml).

Previous IVs for vitamin D and obesity (high BMI) have demonstrated association with risk to have MS, but were not compared to measured serum 25-OH D levels or BMI in pediatric MS patients.[21] The use of genetic IVs, which drive the levels of environmental risk factors, reduces concerns about confounding and reverse causation. In addition, it has thus far not been extensively explored what the heterogeneity may be in the role of vitamin D in MS across patients who carry different genotypes and how polymorphisms may affect vitamin D supplementation response. Further study of vitamin D genetic polymorphisms may help clarify these clinically relevant questions.

Strengths of this study include 1) the use of very well-phenotyped cohorts of pediatric MS patients with prospective relapse capture; 2) replication with similar effect sizes of the association of the vitDGRS with vitamin D levels and relapses in an independent cohort of children: and 3) rigorous analytical methods. We demonstrate that the genetic IV is associated with disease course and the magnitude of this association is clinically relevant. Limitations include the possibility of violations of the assumptions underlying use of genetic IVs, that the genes studied have (unknown) direct links to relapses independent of vitamin D level or pathway, and possible exclusion of the other SNPs that may contribute, but due to sample size did not survive the conservative Bonferroni correction for multiple comparisons. The results in the replication dataset did not all reach nominal statistical significance, but the effect sizes were reassuringly similar to the discovery set and there was a linear dosage effect on relapse hazard. The replication dataset had decreased statistical power due to smaller N and fewer relapses with greater use of and more potent disease modifying therapies. Lastly studies of Mendelian randomization in which the IV may be associated with both disease incidence and course have the possibility of collider bias in which unmeasured factors associated with incidence and disease course could drive an association between the IV and disease course variable.[37]

In summary, a genetic IV captures risk for lower 25-OH vitamin D serum levels and is associated with relapses in pediatric MS patients. Future work will determine whether these

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

particular SNPs have an impact on vitamin D supplementation success with respect to increasing levels, decreasing MS activity or affecting immunological function.

ACKNOWLEDGEMENTS:

We acknowledge with gratitude our families and staff members of the pediatric MS centers participating in this study. Without them these investigations would not be possible. We thank Dr. Michaela George for assistance in this project. We also acknowledge the support for this study from the NIH (R01NS071463, PI Waubant), NMSS (HC 0165 PI Casper), and the Race to Erase MS (PI Graves).

DISCLOSURES

Dr. Graves has no disclosures for the content of this manuscript. She has received speaking honoraria from Novartis.

Dr. Barcellos has no disclosures.

Dr. Waubant is section editor for ACTN and co-editor in chief for MSARD.

Drs. Krupp and Belman have no disclosures for the content of this manuscript.

Xiaorong Shao, Hong Quach and Janace, Hart have no disclosures.

Dr. Chitnis has no disclosures.

Dr Weinstock-Guttman has no disclosures for the content of this manuscript. She has grant supports from Biogen, TEVA, Novartis, EMD Serono and Genentech. She has also received compensation as board advisor and speaker from Biogen, TEVA and Genentech.

Drs. Aaen, Benson, Gorman, Greenberg, Lotze, Mar, Ness, Rodriguez, Rose, Schreiner, and Tillema have no disclosures for the content of this manuscript.

Dr. Waldman has no disclosures for the content of this manuscript. She has received grant support from the NIH (K23NS069806) and from Biogen and IONIS for investigation-initiated studies.

Drs. Casper and Waubant have no disclosures.

Appendix

Appendix 1:

Authors

Name	Location	Role	Contribution
Dr. Lisa F. Barcellos	UC Berkeley	Author	Designed the study and revised the manuscript
Dr. Lauren Krupp	NYU	Author	Collected data and revised the manuscript
Dr. Anita Belman	Stony Brook	Author	Collected data and revised the manuscript
Xiaorong Shao	UC Berkeley	Author	Contributed to genetic analyses and revised the manuscript
Hong Quach	UC Berkely	Author	Contributed to genetic analyses and revised the manuscript
Janace, Hart	UCSF	Author	Collected data and revised the manuscript
Dr. Tanuja Chitnis	Partner's Pediatric MS Center, Boston	Author	Collected data and revised the manuscript
Dr. Bianca Weinstock-Guttman	Jacob's Pediatric MS Center, Buffalo	Author	Collected data and revised the manuscript
Dr. Gregory Aaen	Loma Linda	Author	Collected data and revised the manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Name	Location	Role	Contribution
Dr. Leslie Benson	Boston Children's Hospital	Author	Collected data and revised the manuscript
Dr. Mark Gorman	Boston Children's Hospital	Author	Collected data and revised the manuscript
Dr. Benjamin Greenberg	UTSW, Dallas	Author	Collected data and revised the manuscript
Dr. Timothy Lotze	Texas Children's	Author	Collected data and revised the manuscript
Dr. Mar Soe	Washington University, St. Louis	Author	Collected data and revised the manuscript
Dr. Jayne Ness	University of Alabama, Birmingham	Author	Collected data and revised the manuscript
Dr. Moses Rodriguez	Mayo Clinic	Author	Collected data and revised the manuscript
Dr. John Rose	University of Utah	Author	Collected data and revised the manuscript
Dr. Teri Schreiner	Denver Children's	Author	Collected data and revised the manuscript
Dr. Jan-Mendelt Tillema	Mayo Clinic	Author	Collected data and revised the manuscript
Dr. Amy Waldman	Children's Hospital of Philadephia	Author	Collected data and revised the manuscript
Dr. T. Charles Casper	University of Utah	Author	Contributed to data preparation and analysis plan and revised the manuscript
Dr. Emmanuelle Waubant	UCSF	Author	Designed the study, collected data and revised the manuscript

REFERENCES:

1. Nielsen NM, Munger KL, Koch-Henriksen N, et al. Neonatal vitamin D status and risk of multiple sclerosis: A population-based case-control study. Neurology. 2016.
2. Munger KL, Levin LI, Hollis BW, Howard NS and Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. Jama. 2006; 296: 2832–8. [PubMed: 17179460]
3. Mowry EM, Krupp LB, Milazzo M, et al. Vitamin D status is associated with relapse rate in pediatric-onset multiple sclerosis. Annals of neurology. 2010; 67: 618–24. [PubMed: 20437559]
4. Simpson S Jr., Taylor B, Blizzard L, et al. Higher 25-hydroxyvitamin D is associated with lower relapse risk in multiple sclerosis. Annals of neurology. 2010; 68: 193–203. [PubMed: 20695012]
5. Mowry EM, Pelletier D, Gao Z, Howell MD, Zamvil SS and Waubant E. Vitamin D in clinically isolated syndrome: evidence for possible neuroprotection. European journal of neurology. 2016; 23: 327–32. [PubMed: 26518224]
6. Fitzgerald KC, Munger KL, Kochert K, et al. Association of Vitamin D Levels With Multiple Sclerosis Activity and Progression in Patients Receiving Interferon Beta-1b. JAMA neurology. 2015; 72: 1458–65. [PubMed: 26458124]
7. Davey Smith G and Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Human molecular genetics. 2014; 23: R89–98. [PubMed: 25064373]
8. Ahmad OS, Morris JA, Mujammami M, et al. A Mendelian randomization study of the effect of type-2 diabetes on coronary heart disease. Nature communications. 2015; 6: 7060.
9. Mokry LE, Ross S, Timpson NJ, Sawcer S, Davey Smith G and Richards JB. Obesity and Multiple Sclerosis: A Mendelian Randomization Study. PLoS medicine. 2016; 13: e1002053. [PubMed: 27351487]
10. Gianfrancesco M, et al. Increased body mass index is causally associated with pediatric MS onset: A Mendelian randomization study. Neurology. 2016; 86: Supplement P1375. [PubMed: 26718566]
11. Ahn J, Yu K, Stolzenberg-Solomon R, et al. Genome-wide association study of circulating vitamin D levels. Human molecular genetics. 2010; 19: 2739–45. [PubMed: 20418485]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

12. Nissen J, Rasmussen LB, Ravn-Haren G, et al. Common variants in CYP2R1 and GC genes predict vitamin D concentrations in healthy Danish children and adults. PloS one. 2014; 9: e89907. [PubMed: 24587115]
13. Bu FX, Armas L, Lappe J, et al. Comprehensive association analysis of nine candidate genes with serum 25-hydroxy vitamin D levels among healthy Caucasian subjects. Human genetics. 2010; 128: 549–56. [PubMed: 20809279]
14. Wang TJ, Zhang F, Richards JB, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet. 2010; 376: 180–8. [PubMed: 20541252]
15. Agliardi C, Guerini FR, Saresella M, et al. Vitamin D receptor (VDR) gene SNPs influence VDR expression and modulate protection from multiple sclerosis in HLA-DRB1*15-positive individuals. Brain, behavior, and immunity. 2011; 25: 1460–7.
16. Simon KC, Munger KL, Kraft P, Hunter DJ, De Jager PL and Ascherio A. Genetic predictors of 25-hydroxyvitamin D levels and risk of multiple sclerosis. Journal of neurology. 2011; 258: 1676–82. [PubMed: 21431378]
17. Shui IM, Mucci LA, Kraft P, et al. Vitamin D-related genetic variation, plasma vitamin D, and risk of lethal prostate cancer: a prospective nested case-control study. Journal of the National Cancer Institute. 2012; 104: 690–9. [PubMed: 22499501]
18. Signorello LB, Shi J, Cai Q, et al. Common variation in vitamin D pathway genes predicts circulating 25-hydroxyvitamin D Levels among African Americans. PloS one. 2011; 6: e28623. [PubMed: 22205958]
19. International Multiple Sclerosis Genetics C, Wellcome Trust Case Control C, Sawcer S, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. Nature. 2011; 476: 214–9. [PubMed: 21833088]
20. International Multiple Sclerosis Genetics C. Network-based multiple sclerosis pathway analysis with GWAS data from 15,000 cases and 30,000 controls. American journal of human genetics. 2013; 92: 854–65. [PubMed: 23731539]
21. Gianfrancesco MA, Stridh P, Rhead B, et al. Evidence for a causal relationship between low vitamin D, high BMI, and pediatric-onset MS. Neurology. 2017; 88: 1623–9. [PubMed: 28356466]
22. Rhead B, Baarnhielm M, Gianfrancesco M, et al. Mendelian randomization shows a causal effect of low vitamin D on multiple sclerosis risk. Neurology Genetics. 2016; 2: e97. [PubMed: 27652346]
23. Mokry LE, Ross S, Ahmad OS, et al. Vitamin D and Risk of Multiple Sclerosis: A Mendelian Randomization Study. PLoS medicine. 2015; 12: e1001866. [PubMed: 26305103]
24. Anderson D, Holt BJ, Pennell CE, Holt PG, Hart PH and Blackwell JM. Genome-wide association study of vitamin D levels in children: replication in the Western Australian Pregnancy Cohort (Raine) study. Genes and immunity. 2014; 15: 578–83. [PubMed: 25208829]
25. Gallicchio L, Moore LE, Stevens VL, et al. Circulating 25-hydroxyvitamin D and risk of kidney cancer: Cohort Consortium Vitamin D Pooling Project of Rarer Cancers. American journal of epidemiology. 2010; 172: 47–57. [PubMed: 20562187]
26. Coskun S, Simsek S, Camkurt MA, Cim A and Celik SB. Association of polymorphisms in the vitamin D receptor gene and serum 25-hydroxyvitamin D levels in children with autism spectrum disorder. Gene. 2016; 588: 109–14. [PubMed: 27155524]
27. Kitanaka S, Isojima T, Takaki M, Numakura C, Hayasaka K and Igarashi T. Association of vitamin D-related gene polymorphisms with manifestation of vitamin D deficiency in children. Endocrine journal. 2012; 59: 1007–14. [PubMed: 22785457]
28. 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: 559–75. [PubMed: 17701901]
29. Graves JS, Barcellos LF, Shao X, et al. Genetic predictors of relapse rate in pediatric MS. Multiple sclerosis. 2016; 22: 1528–35. [PubMed: 26769066]
30. Pritchard JK, Stephens M and Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000; 155: 945–59. [PubMed: 10835412]
31. Speeckaert M, Huang G, Delanghe JR and Taes YE. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clinica chimica acta; international journal of clinical chemistry. 2006; 372: 33–42. [PubMed: 16697362]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

32. Prabhu AV, Luu W, Li D, Sharpe LJ and Brown AJ. DHCR7: A vital enzyme switch between cholesterol and vitamin D production. Progress in lipid research. 2016; 64: 138–51. [PubMed: 27697512]

33. Shoemaker TJ and Mowry EM. A review of vitamin D supplementation as disease-modifying therapy. Mult Scler. 2018; 24: 6–11.

34. Smolders J, Peelen E, Thewissen M, et al. Safety and T cell modulating effects of high dose vitamin D3 supplementation in multiple sclerosis. PLoS One. 2010; 5: e15235. [PubMed: 21179201]

35. Camu W. Cholecalciferol supplementation in relapsing multiple sclerosis patients treated with subcutaneous interferon beta-1a: A randomized controlled trial. Neurology. 2017; 88: S44.004.

36. AUB P. Koduah, Piper SK, Bellmann-Strobl J, Wuerfel J, Paul F, Dörr J-M. Vitamin D supplementation in multiple sclerosis: primary efficacy endpoint and safety of a randomized, controlled, double-blind phase II trial (EVIDIMS). ECTRIMS. Berlin2018.

37. Paternoster L, Tilling K and Davey Smith G. Genetic epidemiology and Mendelian randomization for informing disease therapeutics: Conceptual and methodological challenges. PLoS genetics. 2017; 13: e1006944. [PubMed: 28981501]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

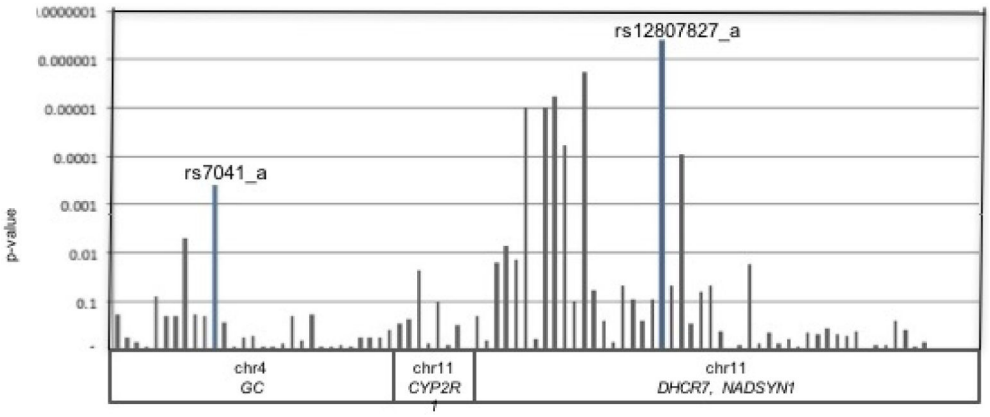

Figure 1: Association of SNPs related to genes *GC, CYP2R1, NADSYN1, and DHCR7,* and 25-OH vitamin D level in children with MS

Manhattan plot of the p-value (y-axis) of the association between vitamin D pathway SNPs (x-axis) and 25-OH vitamin D level. Three final SNPs selected from independent linkage disequilibrium blocks are indicated by the blue lines (rs7041_a (*GC*), and -rs12807827 (*NADSYN1*).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1:

Participant Characteristics

	Discovery Set (n=182)	Replication Set (n=110)
Age at baseline, mean yrs (± SD)	13.1 (± 4.2)	13.7 (± 3.5)
Median follow-up, months (range)	34 (2–174)	32 (1–69)
Patient-years of follow-up	611	221
Median relapses over follow-up period (range)	1 (0–12)	0 (0–7)
Total relapses in cohort	408	130
White (%)	120 (65.1)	70 (65.4)
Hispanic (%)	55 (30.3)	29 (26.4)
Female (%)	124 (65.8)	71 (64.5)
Median 25(OH)D ng/ml (range)	23(2–63)	25(4–66)
*HLA-DRB1*15:01/03 positive* (%)	74 (41.4)	39 (35.4)
DMT exposure (%)	131 (72)	90 (81)

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 2:

Vitamin D genetic risk score association with 25-OH vitamin D level

	Discovery (n=182)		Replication (n=110)	
	β (95% CI)	**p-value**	**β (95% CI)**	**p-value**
Unadjusted				
Per Unit vitDGRS *	−3.90 ng/ml (−5.21, −2.61)	2.95×10^{-9}	−3.2 ng/ml (−5.44, −0.95)	6.0×10^{-3}
4 Units vitDGRS	−15.6 ng/ml (−20.8, −10.4)	2.95×10^{-9}	−12.8 ng/ml (−21.8, −3.78)	6.0×10^{-3}
Adjusted for genetic ancestry				
Per Unit vitDGRS *	−2.74 ng/ml (−4.38, −1.11)	1.0×10^{-3}	−1.43 ng/ml (−4.07, 1.21)	2.8×10^{-1}
4 Units vitDGRS	−11.0 ng/ml (−17.5, −4.50)	1.0×10^{-3}	−5.7ng/ml (−16.3, 4.8)	2.8×10^{-1}

* vitDGRS – Unweighted 25-OH vitamin D genetic risk score derived from number of risk genotypes from rs7041 (*GC*), and rs12807827 *(DHCR7/NADSYN1).*

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Table 3:

Vitamin D genetic risk score association with relapse rate

	Discovery (n=182)		Replication (n=110)	
	HR (95% CI)	**p-value**	**HR (95% CI)****	**p-value**
Univariate models				
Per Unit vitDGRS*	1.27 (1.08, 1.50)	0.004	1.17 (0.98, 1.41)	0.079
4 Units vitDGRS	2.62 (1.37, 5.03)	0.004	1.91 (0.93, 3.92)	0.079
Multivariable models**				
Per Unit vitDGRS*	1.27 (1.08, 1.50)	0.004	1.19 (0.93, 1.51)	0.16
4 Units vitDGRS	2.62 (1.37 5.03)	0.004	1.98 (0.75, 5.20)	0.16

* vitDGRS – Unweighted 25-OH vitamin D genetic risk score derived from number of risk genotypes from rs7041 (*GC*) and, rs12807827 *(DHCR7/NADSYN1*).

** Adjusted for ancestry, sex, disease-modifying therapy use, and *HLA-DRB1*15* carrier allele status