Effects of Vitamin D and Body Mass Index on Disease Risk and Relapse Hazard in Multiple Sclerosis

A Mendelian Randomization Study

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Abstract

Background and Objectives

Decreased vitamin D levels and obesity are associated with an increased risk for multiple sclerosis (MS). However, whether they also affect the disease course after onset remains unclear. With larger data sets now available, we used Mendelian randomization (MR) to determine whether serum 25-hydroxyvitamin D (25OHD) and body mass index (BMI) are causally associated with MS risk and, moving beyond susceptibility toward heterogeneity, with relapse hazard.

Methods

We used genetic variants from 4 distinct genome-wide association studies (GWASs) for serum 25OHD in up to 416,247 individuals and for BMI from a GWAS in 681,275 individuals. Applying 2-sample MR, we examined associations of 25OHD and BMI with the risk of MS, with summary statistics from the International Multiple Sclerosis Genetics Consortium GWAS in 14,802 MS cases and 26,703 controls. In addition, we examined associations with relapse hazard, with data from our GWAS in 506 MS cases.

Results

A 1-SD increase in genetically predicted natural-log transformed 25OHD levels decreased odds of MS up to 28% (95% CI: 12%–40%, $p = 0.001$) and decreased hazard for a relapse occurring up to 43% (95% CI: 15%–61%, $p = 0.006$). A 1-SD increase in genetically predicted BMI, corresponding to roughly 5 kg/m^2, increased risk for MS with 30% (95% CI: 15%–47%, $p = 3.76 \times 10^{-5}$). On the contrary, we did not find evidence for a causal role of higher BMI with an increased hazard for occurrence of a relapse.

Discussion

This study supports causal effects of genetically predicted serum 25OHD concentrations and BMI on risk of MS. In contrast, serum 25OHD but not BMI is significantly associated with relapse hazard after onset. These findings might offer clinical implications for both prevention and treatment.

Glossary

BMI = body mass index; **GWAS** = genome-wide association study; **HR** = hazard ratio; **IV** = instrumental variable; **IVW** = inverse-variance weighted; **LD** = linkage disequilibrium; **MHC** = major histocompatibility complex; **MR** = Mendelian randomization; **MR-PRESSO** = MR Pleiotropy RESidual Sum and Outlier; **MS** = multiple sclerosis; **RCT** = randomized controlled trial; **SNV** = single nucleotide variation; **25OHD** = 25-hydroxyvitamin D.

Low levels of serum 25-hydroxyvitamin D (25OHD) and higher body mass index (BMI) are causally associated with increased risk for multiple sclerosis (MS).[1-12] Notwithstanding 25OHD and BMI being established risk factors for MS, it remains to be determined whether intervening on these risk factors after disease onset has an effect on disease severity measures, such as relapse rate. Relapses are a core feature of relapsing remitting MS and a common primary outcome of clinical trials. Observational studies show that lower 25OHD levels are associated with a higher relapse rate in MS,[13-16] whereas there are conflicting results for BMI.[17,18] However, observational studies are prone to reverse causation and recall bias and thereby preclude drawing conclusions regarding causality.

Progress in availability of genome-wide association studies (GWASs) for heterogeneity measures, such as relapse hazard in MS,[19] enables us to apply the Mendelian randomization framework (MR) for causal inference assessments with heterogeneity measures as outcome. In this way, MR is an elegant tool to inform intervention strategies, also after disease onset, when the patient is followed up by the neurologist.[20] In MR analysis, genetic variants that are a proxy for environmentally modifiable exposures are used to assess the presence of a causal relationship between these exposures and an outcome.[21]

We first explore whether genetically predicted serum 25OHD levels, BMI, and risk of MS are causally associated by using the latest GWAS summary statistics to date and by comparing different sources for 25OHD-associated genetic variants. Subsequently, we move beyond susceptibility toward heterogeneity and assess whether 25OHD and BMI are causally associated with relapse hazard in MS.

Methods

Data Sources
Data sources for 25OHD, adult BMI, MS risk, and relapse hazard are summarized in eTable 1, links.lww.com/NXI/A709. Instrumental variables (IVs) associated with 25OHD levels were derived from 4 GWASs from 2 populations (SUNLIGHT and UK Biobank).[22-25] The SUNLIGHT consortium identified 6 common single nucleotide variations (SNVs [formerly SNPs]) in 79,366 participants of European ancestry.[22] A GWAS for low-frequency variants in 42,274 European-descent individuals added 1 variant.[23] Finally, a total of 138 and 143 genetic variants (with 61 SNVs in LD with $r^2 > 0.5$ across the 2 data sets) were discovered for serum 25OHD levels, in respectively, 401,460 and 417,580 UK Biobank participants of European ancestry.[24,25] For the latter, primary analyses were conducted with results

expressed per 1-SD change in natural-log transformed 25OHD levels from GWAS in 416,247 individuals but with BMI adjustment. Secondary analyses were conducted with results expressed per unit change in rank-based inverse normal transformed 25OHD levels, without adjustment for BMI (eTable 1, links.lww.com/NXI/A709).

For BMI, we included 656 primary genome-wide significant associations listed in the GIANT consortium GWAS in 681,275 individuals.[9,26] Corresponding effects of 25OHD- and BMI-associated SNVs on MS susceptibility and on relapse hazard in MS were derived from the discovery cohorts of the International Multiple Sclerosis Genetics Consortium meta-analysis, including up to 41,505 participants (14,802 MS and 26,703 controls),[27] and our GWAS for relapse hazard performed in 506 individuals of European descent,[19] respectively.

Selection of Instrumental Variables
Clumping and data harmonization were implemented in R v3.6.1 using the TwoSampleMR package (v0.5.5).[28] For each genetic variant, alleles were aligned and matched so that their effects correspond to an increase in the corresponding exposure based on marginal effect estimates. To prevent result bias by strongly correlated SNVs, for SNVs in linkage disequilibrium (LD) with $r^2 > 0.05$ in the European samples of 1000 Genomes, only the SNV with the lowest p value for exposure is retained. To prevent strand ambiguity issues, palindromic SNVs were replaced by nonpalindromic proxy SNVs in high LD ($r^2 \geq 0.9$) identified with LDlinkR package v1.1.2 in R v4.0.2.

An overview of IVs included in each MR analysis is provided in eTables 2–12, links.lww.com/NXI/A709. The proportion of variance in exposure explained by each IV set and mean F-statistic are shown in eTable 13 and eTables 15–17, links.lww.com/NXI/A709.

Statistical Analyses
MR analyses were implemented in R v3.6.1 with the TwoSampleMR package (v0.5.5).[28] As primary analysis, the multiplicative random-effects inverse-variance weighted (IVW) analysis was used to estimate the effects of IVs on outcomes.[28,29] Cochran Q test and I^2 statistic[30] were calculated to measure the degree of heterogeneity across individual effect estimates derived from each genetic variant.[31]

For an IVW analysis to be valid, 3 key MR assumptions need to be satisfied. These assumptions are as follows: (1) the genetic variants are associated with the exposure of interest; (2) the genetic variants are independent of confounding factors for the association of

exposure and outcome; and (3) the genetic variants must not be associated with the outcome through other pathways other than the exposure of interest (horizontal pleiotropy).[21]

To relax the assumptions regarding pleiotropic variants, additional sensitivity tests were performed, including MR Egger,[32] weighted median regression,[33] and weighted mode-based estimator.[34] Horizontal pleiotropy was evaluated based on the intercept obtained from the MR Egger analysis being significantly different from 0[32,35] and by visual inspection of the funnel plot, where asymmetry is indicative of horizontal pleiotropy.[28] Furthermore, we used the MR Pleiotropy RESidual Sum and Outlier (MR-PRESSO) method for detection of horizontal pleiotropy (MR-PRESSO global test) and correction of horizontal pleiotropy, if detected, via outlier removal (MR-PRESSO outlier test).[36] In addition, sensitivity tests were applied in which we excluded variants within the major histocompatibility complex (MHC) region, if present, as it is strongly associated with MS risk and susceptible to bias from pleiotropy due to its complex LD patterns. As multiple exposure data sets for the same exposure variable and the multiple MR tests performed are correlated, we considered as primary analysis the IVW MR in the largest IV data set and as further indicative of causal effects those that were concordant in direction across multiple IV data sets and MR approaches.

For the secondary analyses with the 25OHD summary statistics[25] unadjusted for BMI, results are expressed per unit rank-based inverse normal transformed 25OHD levels. For all other 2-sample MR analyses, results are expressed per 1-SD increase in natural-log transformed 25OHD levels or in BMI. One SD equals a mean of 4.70 BMI units (kg/m^2) among cohorts in the GIANT consortium[26] and 0.33–0.46 units of natural log-transformed 25OHD.[25,37]

Standard Protocol Approvals, Registrations, and Patient Consents
All data sources used in this study received approval from institutional review boards and obtained informed consent from all participants.[19,22-25]

Data Availability
Summary-level data for the genetic associations with 25OHD, adult BMI and MS are publicly available and can be obtained through the sources provided in eTable 1, links.lww.com/NXI/A709. The relapse hazard GWAS summary statistics are available from the corresponding author on request. Please see supplementary data.

Results

Vitamin D and BMI Are Associated With the Risk of MS

25OHD SNVs Derived From the SUNLIGHT Consortium GWAS
Including five 25OHD SNVs (eTable 2, links.lww.com/NXI/A709) from the SUNLIGHT Consortium GWAS,[22] the odds

for MS decreased with 17% per 1-SD increase in genetically predicted natural log-transformed 25OHD levels with the random-effects IVW method (Figure 1). Findings from sensitivity tests were consistent (eFigure 1A, eFigure 2A, and eTable 13, links.lww.com/NXI/A709). The Cochran Q test and I^2 statistic did not provide evidence for substantial heterogeneity among the individual SNV effect estimates in the IVW analysis, and there was no evidence for horizontal pleiotropy from the MR Egger regression intercept (eTable 14, links.lww.com/NXI/A709). However, the funnel plot was found to be asymmetric (eFigure 3A, links.lww.com/NXI/A709).
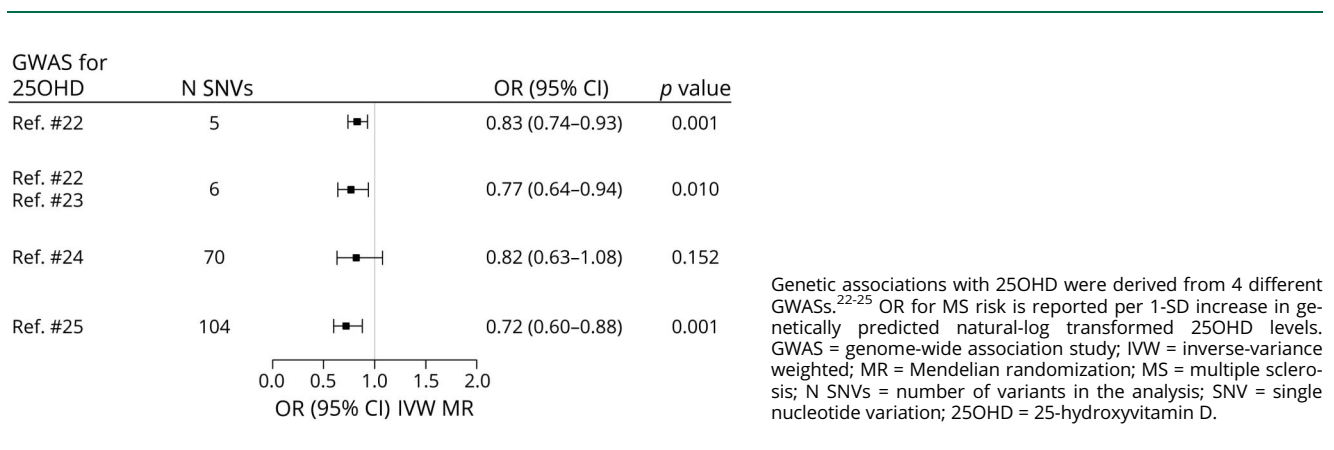
Adding the low-frequency SNV rs117913124 from Manousaki et al.[23] (eTable 3, links.lww.com/NXI/A709), the odds for MS decreased with 23% per 1-SD increase in genetically predicted natural log-transformed 25OHD levels (Figure 1), and findings from sensitivity tests were consistent (eFigure 1B, eFigure 2B, and eTable 13, links.lww.com/NXI/A709). There was no evidence for horizontal pleiotropy (eTable 14, links.lww.com/NXI/A709). However, the funnel plot was found to be asymmetric (eFigure 3B, links.lww.com/NXI/A709), and there was evidence for substantial heterogeneity among the individual SNV effect estimates (eTable 14, links.lww.com/NXI/A709).

25OHD SNVs Derived From UK Biobank GWASs
First, we selected a total of 70 independent SNVs for MR[24] (eTable 4, links.lww.com/NXI/A709). Results from the IVW method did not show clear evidence for an effect of 25OHD on risk of MS due to the large CI (Figure 1), although causal effect estimates were concordant in direction across multiple MR approaches (eFigure 1C, eFigure 2C, and eTable 13, links.lww.com/NXI/A709). Heterogeneity was present among the individual SNV effect estimates, and the MR-PRESSO global test indicated overall pleiotropy (eTable 14, links.lww.com/NXI/A709). SNVs rs2762942 and rs73015021 were excluded in the outlier-corrected MR-PRESSO test. These outliers are indicated on the scatter plot (eFigure 1C, links.lww.com/NXI/A709). MR-PRESSO outlier-corrected estimates were consistent with the main IVW analysis (eTable 13, links.lww.com/NXI/A709). Both the MR Egger regression intercept (eTable 14, links.lww.com/NXI/A709) and visual inspection of funnel did not show evidence for horizontal pleiotropy (eFigure 3C, links.lww.com/NXI/A709).

Second, we included a total of 104 independent SNVs in MR[25] (eTable 5, links.lww.com/NXI/A709). The odds for MS decreased with 28% per 1-SD increase in genetically predicted natural log-transformed 25OHD levels (OR 0.72, 95% CI: 0.60–0.88, $p = 0.001$) (Figure 1), with consistent findings across sensitivity tests (eFigure 1D, eFigure 2D, and eTable 13, links.lww.com/NXI/A709). However, heterogeneity among individual SNV effect estimates was present (eTable 14, links.lww.com/NXI/A709). Excluding MHC SNV rs28374650 slightly improved precision (eTable 13, links.lww.com/NXI/A709). The MR-PRESSO

Figure 1 Inverse-Variance Weighted MR Estimates of the Association Between 25OHD and Risk of MS

GWAS for 25OHD	N SNVs	OR (95% CI)	p value
Ref. #22	5	0.83 (0.74–0.93)	0.001
Ref. #22 Ref. #23	6	0.77 (0.64–0.94)	0.010
Ref. #24	70	0.82 (0.63–1.08)	0.152
Ref. #25	104	0.72 (0.60–0.88)	0.001

OR (95% CI) IVW MR
0.0 0.5 1.0 1.5 2.0

Genetic associations with 25OHD were derived from 4 different GWASs.[22-25] OR for MS risk is reported per 1-SD increase in genetically predicted natural-log transformed 25OHD levels. GWAS = genome-wide association study; IVW = inverse-variance weighted; MR = Mendelian randomization; MS = multiple sclerosis; N SNVs = number of variants in the analysis; SNV = single nucleotide variation; 25OHD = 25-hydroxyvitamin D.

global test p was <0.0001. SNVs rs116970203, rs142158911, rs2762943, and rs28374650 were identified as outliers, as also visible on the scatter plot (eFigure 1D, links.lww.com/NXI/A709). The distortion test p value of 0.01 indicated a significant difference between raw- and outlier-corrected MR-PRESSO estimates, although findings from outlier-corrected MR-PRESSO estimates were in line with the main IVW analysis (eTable 13, links.lww.com/NXI/A709). Horizontal pleiotropy was not evident from the MR Egger regression intercept (eTable 14, links.lww.com/NXI/A709) nor from visual inspection of the funnel plot (eFigure 3D, links.lww.com/NXI/A709). The association between 25OHD and risk of MS remains when summary statistics unadjusted for BMI are used, with effect estimates within the same range (eTable 13, links.lww.com/NXI/A709).

BMI

Genetic predisposition to an increased BMI was associated with an increased risk for MS (OR 1.30, 95% CI: 1.15–1.47, $p = 3.76 \times 10^{-5}$) (Figure 2), with a total of 588 SNVs included (eTable 6 and eTable 7, links.lww.com/NXI/A709). Findings were consistent across sensitivity analyses (Figure 2 and eTable 15, links.lww.com/NXI/A709). The Cochran Q test and I^2 statistic did show heterogeneity among individual SNV effect estimates (Q = 1,026, I^2 = 43%, $p = 2.21 \times 10^{-26}$). Excluding the MHC SNV rs498240 did not alter our results (eTable 15, links.lww.com/NXI/A709). The MR-PRESSO global test p was <0.0001, with 11 SNVs identified as outliers (rs1048932, rs1106908, rs2010281, rs273504, rs3803286, rs3810291, rs3814883, rs419261, rs498240, rs7535528, and rs7941030). The outliers are indicated on the scatter plot (eFigure 4, links.lww.com/NXI/A709). MR-PRESSO outlier-corrected estimates were consistent with the main IVW (eTable 15, links.lww.com/NXI/A709). Finally, there was no evidence for directional pleiotropy from the MR Egger regression intercept (Egger intercept −0.002, 95% CI: −0.008 to 0.003, $p = 0.42$) nor from visual inspection of the funnel plot (eFigure 5, links.lww.com/NXI/A709).

Vitamin D but Not BMI Is Associated With Relapse Hazard in MS

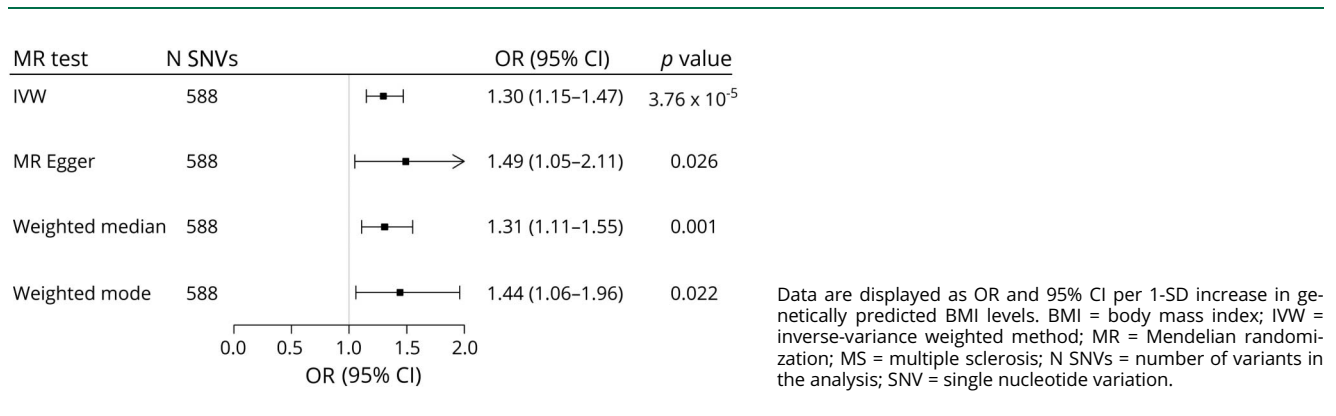### 25OHD SNVs Derived From the SUNLIGHT Consortium GWAS

Using the 6 SUNLIGHT Consortium 25OHD GWAS SNVs (eTable 8, links.lww.com/NXI/A709), a 1-SD increase in genetically predicted natural-log transformed 25OHD levels is associated with a 41% decreased hazard for a relapse occurring in IVW analysis (hazard ratio [HR] 0.59, 95% CI: 0.38–0.94, $p = 0.025$) (Figure 3). Findings from sensitivity tests were consistent (eFigure 6A, eFigure 7A, and eTable 16, links.lww.com/NXI/A709). The Cochran Q test and I^2 statistic did not provide evidence for substantial heterogeneity among the individual SNV effect estimates (Q = 8, $p = 0.18$; I^2 = 34%). There was no evidence for horizontal pleiotropy from the MR Egger regression intercept (intercept 0.021, 95% CI: −0.102 to 0.144, $p = 0.75$) and MR-PRESSO global test ($p = 0.39$) nor from visual inspection of funnel plot (eFigure 8A, links.lww.com/NXI/A709).

Adding the low-frequency SNV rs117913124 (eTable 9, links.lww.com/NXI/A709) led to a slightly improved precision in causal effect estimation (HR 0.59, 95% CI: 0.41–0.86, $p = 0.007$) (Figure 3), with consistent findings across sensitivity tests (eFigure 6B, eFigure 7B, and eTable 16, links.lww.com/NXI/A709). There was no evidence for pleiotropy nor heterogeneity (intercept 0.019, 95% CI: −0.084 to 0.121, $p = 0.74$; MR-PRESSO global test $p = 0.43$; Q = 8, $p = 0.27$, I^2 = 21%) (eFigure 8B, links.lww.com/NXI/A709).

25OHD SNVs Derived From UK Biobank GWASs

Using genetic estimates for 25OHD for 71 SNVs (eTable 10, links.lww.com/NXI/A709),[24] no clear evidence for an effect was observed in IVW analysis (HR 0.77, 95% CI: 0.41–1.44, $p = 0.414$) (Figure 3), although causal effect estimates were concordant in direction, i.e., negative, across MR sensitivity analyses (eFigure 6C, eFigure 7C, and eTable 16, links.lww.com/NXI/A709). There was no evidence for heterogeneity among the individual SNV effect

Figure 2 Forest Plot of MR Estimates of BMI With Risk of MS

MR test	N SNVs		OR (95% CI)	p value
IVW	588		1.30 (1.15–1.47)	3.76×10^{-5}
MR Egger	588		1.49 (1.05–2.11)	0.026
Weighted median	588		1.31 (1.11–1.55)	0.001
Weighted mode	588		1.44 (1.06–1.96)	0.022

OR (95% CI)

Data are displayed as OR and 95% CI per 1-SD increase in genetically predicted BMI levels. BMI = body mass index; IVW = inverse-variance weighted method; MR = Mendelian randomization; MS = multiple sclerosis; N SNVs = number of variants in the analysis; SNV = single nucleotide variation.

estimates in the IVW analysis (Q = 70, p = 0.48; I^2 = 0.01%), and the MR-PRESSO global test did not indicate pleiotropy (p = 0.45). In contrast, there was evidence for horizontal pleiotropy from the MR Egger regression intercept (intercept 0.028, 95% CI: 0.001–0.056, p = 0.05). The funnel plot is depicted in eFigure 8C, links.lww.com/NXI/A709.

Finally, when we include a total of 103 SNVs[25] (eTable 11, links.lww.com/NXI/A709), genetic predisposition to increased levels of natural-log transformed serum 25OHD levels was associated with a decreased hazard for a relapse occurring (HR: 0.57, 95% CI: 0.39–0.85, p = 0.006) (Figure 3, eFigure 6D, and eFigure 7D, links.lww.com/NXI/A709), with no evidence for pleiotropy nor heterogeneity (intercept 0.015, 95% CI: −0.002 to 0.032, p = 0.09; MR-PRESSO global test p = 0.92; Q = 81, p = 0.93, I^2 = 25%) (eFigure 8D, links.lww.com/NXI/A709). Exclusion of the MHC SNV rs28374650 had no influence on the results (eTable 16, links.lww.com/NXI/A709). The association between 25OHD and relapse hazard remains when summary statistics unadjusted for BMI are used, with effect estimates within the same range (eTable 16, links.lww.com/NXI/A709).

BMI

Genetic predisposition to an increased BMI was not associated with relapse hazard under IVW method (HR 0.88, 95% CI: 0.63–1.23, p = 0.453), with concordant direction across weighted median and weighted mode. The MR Egger estimate was in the opposite direction due to the large CI (Figure 4, eFigure 9, and eTable 17, links.lww.com/NXI/A709).

The Cochran Q test and I^2 statistic did not provide evidence for heterogeneity among the individual SNV effect estimates in the IVW analysis (Q = 663, p = 0.05; I^2 = 9%). There was no evidence for directional pleiotropy from the MR Egger regression intercept (intercept −0.005, 95% CI: −0.020 to 0.010, p = 0.54) or MR-PRESSO global test (p = 0.05) and no evidence for asymmetry in the funnel plot (eFigure 10, links.lww.com/NXI/A709). Excluding the MHC SNV rs498240 led to very similar findings (eTable 17, links.lww.com/NXI/A709).

Discussion

In keeping with previous studies[1-12] and validating our IV sets, we identified genetically predicted increased BMI to be a risk factor and increased levels of serum 25OHD to be protective

Figure 3 Inverse-Variance Weighted MR Estimates of the Association Between 25OHD and Relapse Hazard
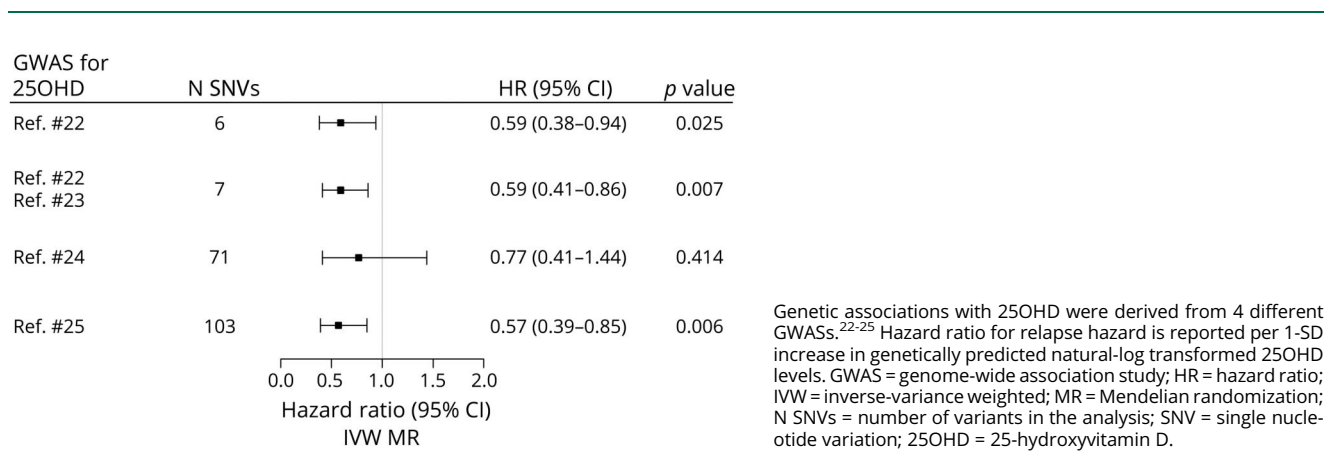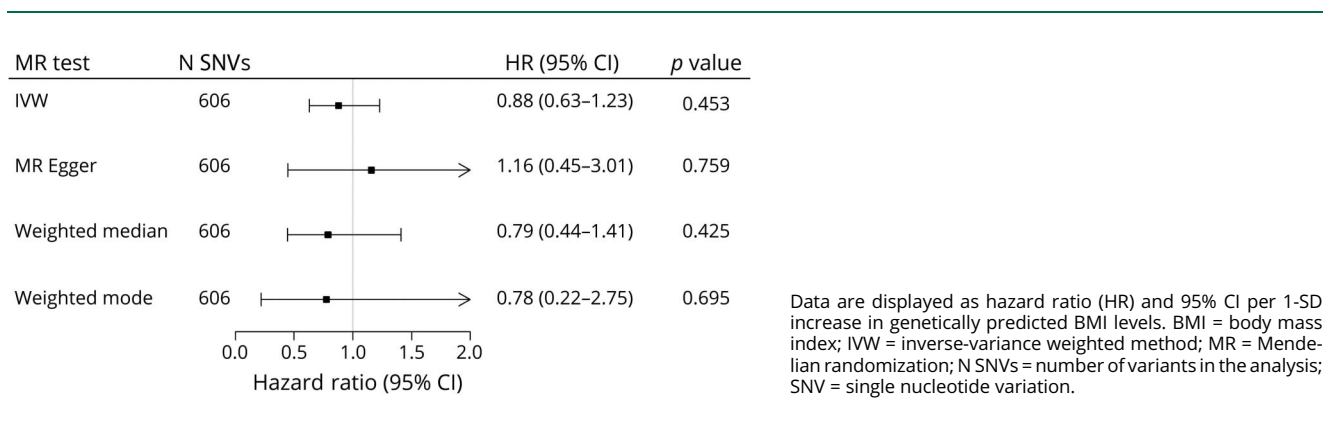
GWAS for 25OHD	N SNVs		HR (95% CI)	p value
Ref. #22	6		0.59 (0.38–0.94)	0.025
Ref. #22 Ref. #23	7		0.59 (0.41–0.86)	0.007
Ref. #24	71		0.77 (0.41–1.44)	0.414
Ref. #25	103		0.57 (0.39–0.85)	0.006

Hazard ratio (95% CI)
IVW MR

Genetic associations with 25OHD were derived from 4 different GWASs.[22-25] Hazard ratio for relapse hazard is reported per 1-SD increase in genetically predicted natural-log transformed 25OHD levels. GWAS = genome-wide association study; HR = hazard ratio; IVW = inverse-variance weighted; MR = Mendelian randomization; N SNVs = number of variants in the analysis; SNV = single nucleotide variation; 25OHD = 25-hydroxyvitamin D.

Figure 4 Forest Plot of MR Estimates of BMI With Relapse Hazard

MR test	N SNVs		HR (95% CI)	p value
IVW	606		0.88 (0.63–1.23)	0.453
MR Egger	606		1.16 (0.45–3.01)	0.759
Weighted median	606		0.79 (0.44–1.41)	0.425
Weighted mode	606		0.78 (0.22–2.75)	0.695

Hazard ratio (95% CI)

Data are displayed as hazard ratio (HR) and 95% CI per 1-SD increase in genetically predicted BMI levels. BMI = body mass index; IVW = inverse-variance weighted method; MR = Mendelian randomization; N SNVs = number of variants in the analysis; SNV = single nucleotide variation.

for MS risk. Each SD increase in BMI, corresponding to a shift from normal weight to overweight, confers a 30% increase in MS risk. Per SD increase in genetically predicted natural log-transformed 25OHD levels, corresponding to $\sim$37–75 nmol/L[1], the odds for MS decrease with approximately 20%.

With risk factors for MS now being well established, we moved beyond susceptibility and investigated whether intervening on these factors after onset of disease does have a beneficial effect on disease course,[20] building on our previous GWAS for relapse hazard in MS in a cohort of 506 untreated patients with MS.[19] Across the 4 25OHD data sets as exposures, we identified genetically predicted increased levels of serum 25OHD to be protective for a relapse occurring. In the primary analysis, each SD increase in genetically predicted natural-log transformed 25OHD levels decreases the hazard for a relapse occurring with approximately 40%. In contrast to 25OHD, our MR study does not provide support for a substantial causal role of BMI in relapse hazard, although larger studies will be required to exclude smaller effects.

In our previous GWAS, we have demonstrated that genetic associations with relapse hazard are enriched for genes in the response to vitamin D gene ontology set by performing a competitive gene-set enrichment analysis.[19] In addition, studies applying a polygenic score approach have shown an association between increased 25OHD levels and a decreased relapse hazard in MS.[38,39] These studies differ from our 2-sample MR design by combining multiple genetic variants into a single variable, i.e., polygenic score, and/or by not limiting to genome-wide significant SNVs. Moreover, tools for pleiotropy and heterogeneity assessment are limited in the polygenic score setting.[21]

Strengths of our study include the reduction of concerns about confounding and reverse causation by applying an MR approach, as genetic variants are fixed at conception and less associated with confounders than directly measured environmental exposures.[40] To fulfill the first and second assumption, i.e., the genetic variants are associated with the

exposure of interest, independent of confounding factors for the association of exposure and outcome, we only selected as IVs genetic variants that are robustly associated with the exposure of interest ($p < 5 \times 10^{-8}$) from large GWASs including roughly 40,000–700,000 individuals. However, we cannot entirely rule out confounding as it is currently not feasible to test for associations with each potential (un)known confounding factor. To overcome population stratification, we only included SNVs that were found in GWASs in individuals of European ancestry, although this inherently limits generalization of our findings to other populations.[41] When collider bias is present, unmeasured factors associated with both disease incidence and disease course drive an association between the IVs and disease course variable.[42] To illustrate, our MR study of 25OHD and relapse hazard among cases with MS would be subject to collider bias if there are variants associated with both risk of MS and relapse hazard, but not 25OHD, potentially inducing spurious association between 25OHD and relapse hazard.[43] However, if collider bias were present, we would also expect to see a spurious association between BMI and relapse hazard, which is not the case. Furthermore, we have previously shown that a genetic burden for risk of MS is not associated with relapse hazard,[19] in line with other studies.[44] Therefore, we believe that the effect of 25OHD on relapse hazard cannot be explained by the genetic burden for risk of MS. Collider bias may also arise from the fact that the estimates from the SUNLIGHT consortium GWAS[22] and the UK Biobank GWAS data[25] used in this study were adjusted for another risk factor, BMI. A simulation study[45] demonstrated that using covariable-adjusted summary associations may bias MR analyses. Nonetheless, additional analyses using summary statistics from rank-based inverse normal transformed 25OHD values without adjustment for BMI do not change the conclusions regarding the causal effect of 25OHD on risk of MS and relapse hazard. The third MR assumption entails that the genetic variants must not be associated with the outcome through pathways other than the exposure of interest (horizontal pleiotropy). BMI-associated genes are enriched for genes involved in neurogenesis.[26] The IV sets for 25OHD range from the inclusion of

SNVs predominantly in biologically plausible genes to larger sets of SNVs also involved outside the vitamin D canonical metabolic pathway, such as in lipid metabolism and dermal tissue properties and even brain-related and behavioral phenotypes.[24,25] We assessed the potential limitation of pleiotropy by performing additional sensitivity tests. Consistent estimates across different IV sets and across additional pleiotropy robust MR methods support the validity of our findings. Nevertheless, pleiotropy can only be assessed indirectly and therefore not completely excluded. MR approaches are dependent on the instrument strength of the IVs. The proportion of variance explained by the selected SNVs is 2%–5% for 25OHD, with the weakest IV data set[24] corresponding to the largest CIs, and 6% for BMI. Importantly, our MR study agrees with observational studies in pointing to an overall protective effect of higher baseline 25OHD levels on subsequent relapse occurrence in cohorts of pediatric or adult MS cases (between 73 and 1,482 participants per study)[13,15,16,46] or meta-analyses of up to 3,130 patients.[14]

On the contrary, randomized controlled trials (RCTs), of which the largest to date include up to 412 individuals,[47-49] provide inconclusive evidence regarding the benefits of vitamin D supplement intake after disease onset.[16] In the CHOLINE study, add-on supplementation with high-dose vitamin D3 (cholecalciferol) was associated with decreased relapses in the post hoc analysis of completers after 2 years of follow-up.[48] In addition, in an RCT testing the effect of the vitamin D analog Alfacalcidol (1-hydroxycholecalciferol), the treated group had a reduced number of relapses and a higher proportion of patients remained relapse-free.[47] Finally, the SOLAR RCT with high-dose vitamin D3 add-on supplementation could not identify a significant effect on relapse.[49] Main limitations of the completed RCTs include small sample size and short follow-up (from 6 months up to 2 years).[50] In addition, the add-on design may mask small effects of vitamin D3, disease-modifying treatment may affect vitamin D levels,[51] patients enrolled in RCTs may already have an adequate vitamin D status,[52] and timing of intervention may be important, i.e., early in disease course or even before onset of disease.

Cholecalciferol and 25OHD need to be metabolized and are subject to complex regulatory mechanisms. The correlation between 25OHD and the active form of vitamin D (1,25-dihydroxyvitamin D or calcitriol) is highly significant but limited in magnitude.[53] The small sample size of the available 1,25-dihydroxyvitamin D GWAS limits the power for characterization of 1,25-dihydroxyvitamin D loci.[24] Yet, overlapping genetic associations in the canonical vitamin D metabolic pathway indicate that genetically determined interindividual natural metabolic variation may affect the vitamin D supplementation response as well.[24] In addition, gene-environment interactions, including interactions with season, may need to be considered.[25]

Ongoing trials such as VIDAMS,[54] PrevANZ (ACTRN1261 2001160820), and D-Lay-MS (NCT01817166), including up to 316 individuals with maximum follow-up of 2 years, might further clarify the efficacy of vitamin D supplementation on MS disease activity.[50] The D-Lay MS RCT will, in addition to its primary end point, investigate the influence of genetic variation in metabolism of vitamin D on vitamin D levels after supplementation.

In conclusion, our study supports a causal effect of serum 25OHD concentrations and BMI on risk of MS. Furthermore, we moved beyond susceptibility toward heterogeneity and found that 25OHD levels, but not BMI, are associated with relapse hazard after onset.

Disclosure
The authors declare no disclosures relevant to the manuscript. Go to Neurology.org/NN for full disclosures.

Appendix Authors

Name	Location	Contribution
Marijne Vandebergh, MSc	KU Leuven, Belgium	Designed and conceptualized the study; analyzed the data; interpreted data; and drafted the manuscript for intellectual content
Bénédicte Dubois, MD, PhD	KU Leuven University Hospitals Leuven, Belgium	Major role in the acquisition of the data and revised the manuscript for intellectual content
An Goris, PhD	KU Leuven, Belgium	Designed and conceptualized the study; interpreted the data; and revised the manuscript for intellectual content

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