Association between 25(OH) vitamin D and multiple sclerosis: cohort, shared genetics, and Causality

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Abstract

Background Multiple Sclerosis (MS), an autoimmune disorder causing demyelination and neurological damage, has been linked to 25-hydroxyvitamin D (25OHD) levels, suggesting its role in immune response and MS onset. This study used GWAS datasets to investigate genetic associations between 25OHD and MS.

Methods We utilized a large-scale prospective cohort to evaluate serum 25OHD levels and MS risk. Linkage Disequilibrium Score Regression (LDSC) assessed genetic correlations between 25OHD levels and MS. Cross-trait genome-wide pleiotropy analysis revealed shared genetic loci. MAGMA analysis identified pleiotropic genes, enriched tissues, and gene sets. Stratified LDSC estimated tissue-specific and cell-specific heritability enrichment, and multi-trait co-localization analysis identified shared immune cell subsets. Bidirectional Mendelian Randomization (MR) assessed the causal association between 25OHD and MS risk.

Results The observational study found a nonlinear relationship between 25OHD levels and MS risk, with the lowest quartile showing significant risk elevation. Our findings revealed shared genetic structure between 25OHD levels and MS, suggesting a common biological pathway involving immune function and CNS integrity. We found 24 independent loci shared between 25OHD levels and MS risk, enriched in brain tissues and involved in pathways like LDL, HDL, and TG metabolism. Four loci (6p24.3, 6p22.2, 12q14.1, and 19p13.2) had strong co-localization evidence, with mapped genes as potential drug targets. Bidirectional MR analysis supported a causal effect of 25OHD levels on MS risk, suggesting 25OHD supplementation could modulate MS risk.

Conclusion This study reveals the complex relationship between 25OHD levels and MS, indicating that higher levels are not always advantageous and recommending moderation in supplementation. We identified SMARCA4 as a potential therapeutic target and detailed key pathways influencing this interaction.

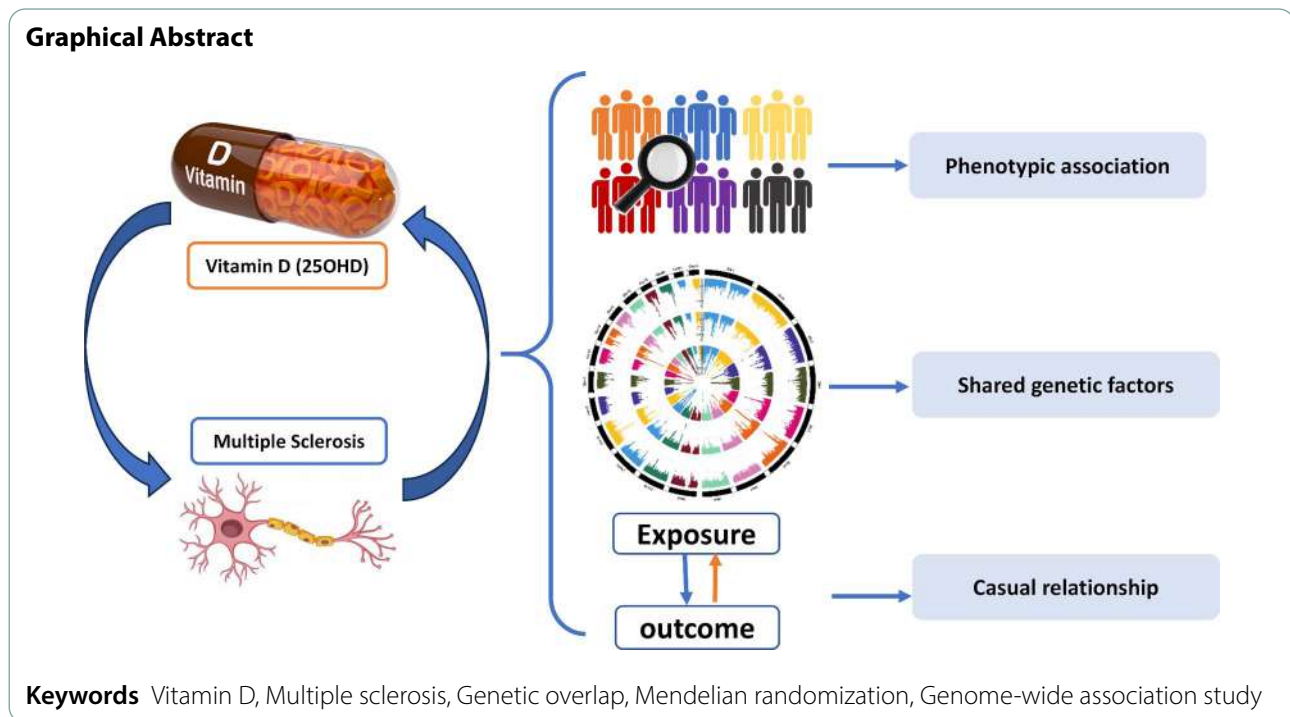†Xing-Hao Yu and Hui-Min Lu contributed equally to this work.

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Graphical Abstract

Keywords Vitamin D, Multiple sclerosis, Genetic overlap, Mendelian randomization, Genome-wide association study

Introduction

Multiple sclerosis (MS) is a multifaceted autoimmune disease characterized by the immune system's aberrant attack on the central nervous system (CNS), leading to demyelination and neuronal damage [1–3]. Epidemiological evidence suggests a geographical disparity in MS prevalence, with higher latitudes and reduced sunlight exposure correlating with increased incidence, resembling patterns observed in type 1 diabetes. (4–5) Observational studies report a statistically significant increase in MS incidence correlating with greater geographical distances from the equator [6]. Conversely, some studies report the absence of such a geographical gradient in MS prevalence. (7–8) This proposition of rising prevalence and incidence is rooted in the sunlight exposure hypothesis. Despite ongoing debates, it's noteworthy that this gradient is diminishing [9]. Previous studies have confirmed a significant association between MS risk and serum levels of 25OHD. For instance, several Mendelian randomization (MR) analyses have demonstrated that lower vitamin D levels increase MS risk [10–13]. The protective effect of higher vitamin D levels, as shown in studies such as the Nurses' Health Study and US Department of Defense study, strongly supports the role of vitamin D in reducing MS susceptibility. (14–15) An analysis of 35 studies revealed that 60% (21 studies) identified a statistically significant relationship between 25OHD levels and MS disease activity as detected by MRI, whereas 40% (14 studies) found no significant effect of 25OHD levels on MS [16].

Additionally, the therapeutic efficacy of vitamin D in MS remains a subject of ongoing investigation, with conflicting results arising from various studies [17]. While some studies suggest positive outcomes, such as improved relapse rates and magnetic resonance imaging (MRI) findings with increased vitamin D dosage, the optimal treatment regimen remains elusive [18]. Divergent dosages, ranging from high daily doses to prolonged supplementation, complicate the assessment of vitamin D's therapeutic potential in managing MS [19]. Numerous studies incorporating vitamin D supplementation have been conducted, yet they were often inadequately powered, lacking prolonged follow-up, and featuring diverse methodological biases, thereby limiting their ability to yield conclusive results [20]. Experts recommend that for a randomized controlled trial (RCT) on vitamin D, it may be important to ensure participants are vitamin D deficient at the start and minimize sunlight exposure during the study to control for external factors [21]. Given the complexity of these variables, further in-depth and well-controlled studies are essential to fully elucidate the role of vitamin D in MS risk, highlighting the importance of genetic-focused approach to provide deeper insights into this relationship.

Genome-wide association studies (GWAS) have identified approximately 238 genetic risk variants linked to MS, as reported by the International Multiple Sclerosis Genetics Consortium. (22–23) Therefore, leveraging large-scale GWAS datasets allows for a more comprehensive interpretation of the association between
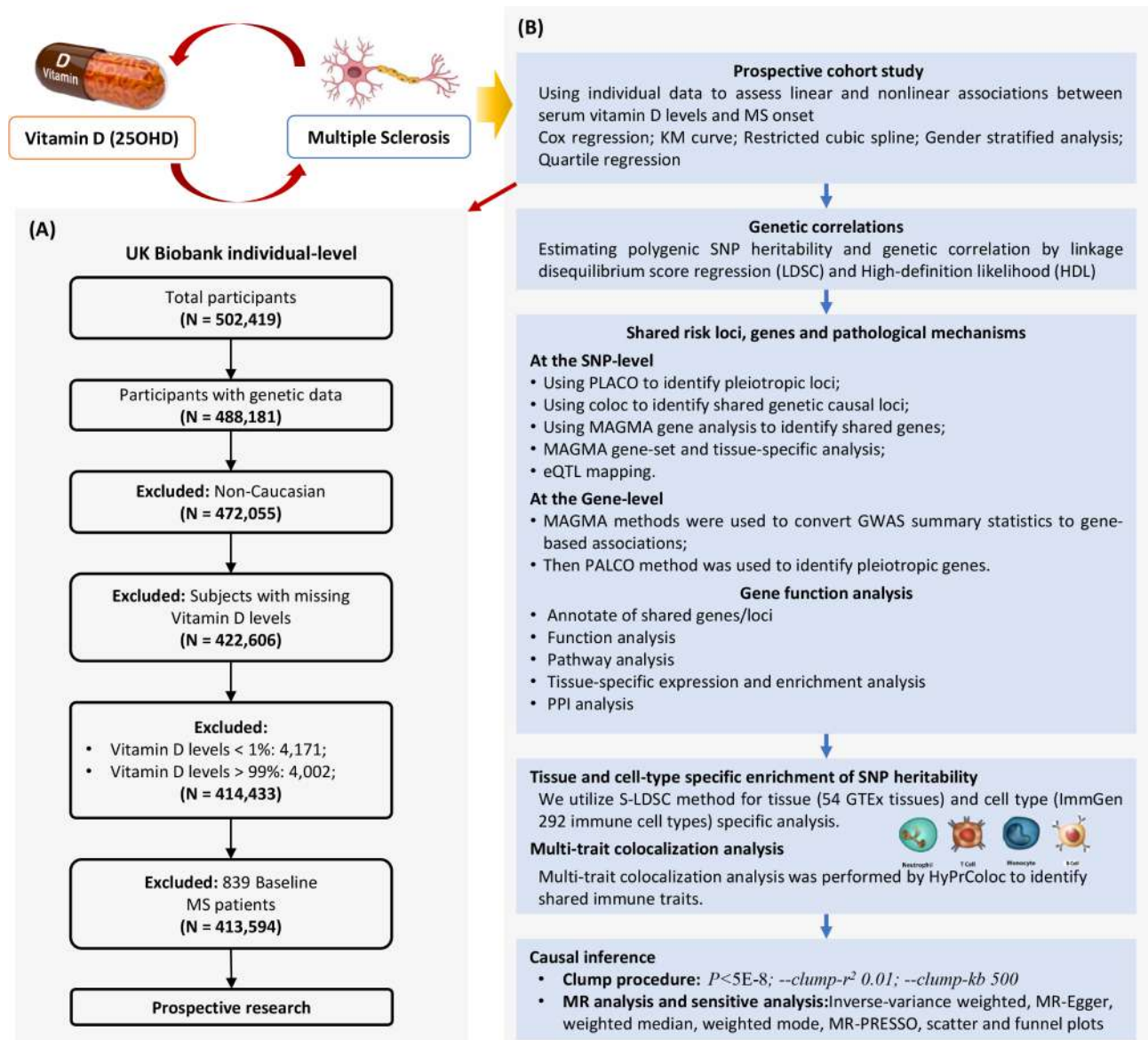
Fig. 1 Flowchart of overall study design. (**A**) Selection Process for UK Biobank Cohort in the study. (**B**) Overview of statistical analyses performed in the study. SNP: Single Nucleotide Polymorphism; LDSC: Linkage Disequilibrium Score Regression; HDL: high-definition likelihood; PLACO: Protein–Ligand Affinity by Comparable Co-Evolution; MAGMA: Multi-marker Analysis of GenoMic Annotation; GWAS: Genome-Wide Association Study; eQTL: Expression Quantitative Trait Locus; S-LDS: Stratified Linkage Disequilibrium Score

25OHD levels and MS. In this context, our study seeks to unravel the intricate genetic relationship between 25OHD and MS, employing sophisticated methodologies (Fig. 1B). First, we utilized the large-scale prospective cohort to evaluate serum 25OHD levels and MS risk. Then, Leveraging the Linkage Disequilibrium Score Regression (LDSC) method, we aim to evaluate the genetic correlation between 25OHD and MS, shedding light on shared genetic factors contributing to their association. Furthermore, a comprehensive exploration of pleiotropic loci and genes associated with both traits will

be undertaken, unraveling potential common pathways contributing to MS susceptibility and vitamin D metabolism. Crucially, our investigation extends beyond correlation, employing a two-sample two-way MR approach to assess causal associations between 25OHD and MS. By utilizing genetic variants as instrumental variables, we aspire to disentangle the complex web of causality, providing insights into the potential modifiability of MS risk through interventions targeting 25OHD status, and to discover potential intervention targets for the development of new drugs.

Materials and methods

Study design

This study used LDSC to explore the complex genetic relationship between 25OHD and MS, assessing their genetic correlation. We used cross-trait pleiotropy analysis to identify pleiotropic loci and genes and a series of functional analyzes to identify tissues and pathways involved in disease development. Furthermore, we applied a two-sample bidirectional Mendelian Randomization (MR) approach to determine the causal link between 25OHD and MS.

Observational study

The UK Biobank is a pivotal resource in biomedical research, offering an extensive database that includes genetic and health information from over half a million participants in the United Kingdom [24]. Recruitment began in 2006, targeting individuals aged 40–69 years from across 22 assessment centers. Each participant provided written informed consent and contributed to a wealth of data through baseline questionnaires, interviews, and physical measurements. Comprehensive blood biochemistry analyses and genotyping were also performed, paving the way for detailed investigations into the genetic and environmental underpinnings of various diseases.

In our observational study, we utilized the large-scale UK Biobank cohort (Application ID 76875) to evaluate the association between serum 25OHD levels and MS risk. These data items were collected during the initial assessment visit (2006–2010) and the first repeat assessment visit (2012-13). The concentration of 25OHD (Field ID=30890) was test for a total of 449,822 individuals, with value types continuous and expressed in nmol/L. Diagnostic for MS were categorized using the ICD-10-CM classification (G35). Participants were followed from the baseline assessment until the occurrence of the first diagnosis of MS, death, loss to follow-up, or until the end of the study period on December 31, 2021. Inclusion and exclusion of all samples are shown in Fig. 1A. Subjects with extreme 25OHD values (<1% or >99%) were eliminated from all analysis. In our analysis, we excluded participants diagnosed with MS within six months of baseline from all analyses to minimize the possibility of misclassification. A total of 413, 594 subjects with complete measurement of 25OHD levels were ultimately included in the study, of whom 861 developed MS during follow-up.

We used multivariable Cox proportional hazards models to evaluate the association between 25OHD levels and risk of MS, taking into account risk across quartiles of 25OHD levels. Restricted cubic splines (RCS) were used to evaluate the nonlinear relationship between 25OHD and MS. Our models were adjusted for potential confounders including age, sex, Townsend deprivation index (TDI), type 2 diabetes, cancer, osteoporosis, monocyte count, white blood cell leukocyte count, season of blood sampling and physical activity.

GWAS summary statistics

In order to avoid possible false positives caused by sample overlap, we selected 25OHD and MS summary data generated in completely different cohorts. 25OHD data originated from a GWAS that included 401,460 white British participants [25]. Utilizing a linear mixed model GWAS, 25OHD levels were measured in blood samples collected twice. The analysis incorporated standardized log-transformed 25OHD levels and included covariates such as age, sex, season, and 25OHD supplementation. A meta-analysis was conducted on GWAS results from 42,274 European samples, revealing 138 conditionally independent Single Nucleotide Polymorphisms (SNPs, including 63 novel ones). The estimated SNP heritability for 25OHD was determined to be 16.1%.The GWAS summary data for MS were derived from a two-stage investigation involving 76,755 people with MS and 243,649 control individuals [23]. This study identified 287 common variants associated with MS. In each individual cohort, the association tests were based on an additive logistic regression model using PLINK software, and the results were meta-analyzed by using an inverse variance-weighted fixed-effects model.

Genetic correlation

The LDSC method was employed to assess genetic correlations across phenotypes, facilitating the identification of shared genetic underpinnings across different traits [26]. This approach enabled the examination of the common polygenic structure among phenotypes, utilizing LD scores calculated from European ancestry samples derived from the 1000 Genomes Project and the Hapmap3 project as reference panels [27]. Rigorous quality control measures were applied to SNPs, including the exclusion of non-biallelic and SNPs with ambiguous strand information, removal of SNPs lacking rs tags, elimination of duplicate SNPs or those not present in the 1000 Genomes Project, and SNPs within the major histocompatibility complex (chr6: 28.5–33.5 Mb) were omitted due to their intricate LD structure. Moreover, we retained SNPs with a minor allele frequency (MAF) greater than 0.01. To further explore whether the SNP heritability of 25OHD and MS is concentrated in specific tissues, we utilized stratified LD score regression.

Tissue and cell-specific heritability estimation

We further utilized the S-LDSC method to investigate whether the genetic heritability of SNPs related to 25OHD and MS is enriched in specific tissues.

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Subsequently, LDSC was applied to diverse immune cell data to assess if specific cell types in these tissues exhibit significant genetic enrichment. We obtained data from 53 human tissues (including 13 brain tissues) [28] through GTEx and acquired information on 292 immune cell types (including B cells, gamma delta T cells, alpha beta T cells, innate lymphocytes, myeloid cells, stromal cells, and stem cells) from the ImmGen consortium [29]. After adjusting for baseline models and all gene sets, we assessed the significance of SNP heritability enrichment estimates in each tissue and cell type using p-values derived from the z-scores of regression coefficients.

Pleiotropic analysis under composite null hypothesis

SNP-Level PLACO is an innovative approach employed to investigate polygenic loci associated with complex traits using summary-level genotype-phenotype association statistics [30]. Specifically, we calculated the square of the Z-scores for each variant, excluding SNPs with excessively high Z [2] (>80). Considering the potential correlation between 25OHD and MS, we computed the correlation matrix of Z-scores. Subsequently, we applied a level-α intersection-union test (IUT) method to evaluate the hypothesis of no pleiotropy. The final *P* values for the IUT test was determined as the maximum p-value between H_0 and H_1.

Building on the PLACO findings, we further associated the identified loci with nearby genes to investigate shared biological mechanisms at these polygenic sites. Employing a Generalized Gene-Set Analysis of GWAS Data (MAGMA), we analyzed genes located at or overlapping with polygenic loci identified through PLACO output and single-trait GWAS, aiming to identify candidate pathways linked to polygenicity and tissue enrichment of polygenic genes [31]. For the MAGMA gene analysis, we applied a threshold of 2.68E-06, and for the gene set analysis, the threshold was 2.94E-06. To ascertain the biological functions of polygenic loci, we used Functional Mapping and Annotation (FUMA) with genome-wide association study data [32]. Pathway enrichment analysis was conducted across various pathways using the Molecular Signatures Database (MSigDB) to delineate the functions of mapped genes [33]. eQTL analysis incorporated SNP-gene association data derived from whole blood tissues.

Colocalization analysis

For multi-trait loci annotated with FUMA, we utilized the Bayesian colocalization analysis tool "coloc" [34] to explore potential shared causal variants between pairs of traits at each multi-trait locus. This analysis operates under the assumption of a single causal variant and provides posterior probabilities (PP) for five hypotheses at each multi-trait locus: H_0 - Neither of the two traits has a

genetic association in this region; H_1 - Only trait 1 exhibits a genetic association in this region; H2 - Only trait 2 shows a genetic association in this region; H_3 - Both traits are associated but have different causal variants; H_4 - Both traits are associated and share a common causal variant. Employing the "coloc.abf" function, we conducted colocalization analysis with significance thresholds set at $P_1 = P_2 = 1 \times 10^{-4}$ and $P_{12} = 1 \times 10^{-5}$.

Cell-type enrichment of pleiotropic signals

The cell-type MAGMA method is designed to map gene-level genetic associations to specific cell types using single-cell RNA sequencing (scRNA-seq) data [35]. First, gene-level association p-values are computed using MAGMA's gene-based analysis, which aggregates SNP-level associations for each gene. Then, the cell-type MAGMA method evaluates the overlap between these gene associations and the expression levels across various cell types. In our study, we used scRNA-seq data from large European single-cell datasets (Onek1K) [36] to perform cell-type MAGMA analysis, which allowed us to identify cellular subtypes that may be significantly associated with disease pathways. This method involved calculating specificity scores for each cell type by quantifying the relative expression of disease-associated genes in each cell subtype. By ranking cell types based on their enrichment scores, cell-type MAGMA enables the identification of cell populations that might play a role in the disease mechanism of interest, such as those relevant to MS risk.

Causal inference

In our analysis, we implemented the clumping procedure within the PLINK software to identify independently significant genetic loci serving as instrumental variables for two traits ($P < 5 \times 10 - 8$)[33]. The *r* [2] threshold for instrumental variables was set at 0.001, within a physical distance of a 10,000 kb window. Additionally, we computed the *r* [2] and *F*-statistic for each instrumental variable to ensure its strength [35] with the *F*-statistic calculated using the formula:

$$F = (\frac{n - 1 - k}{k})(\frac{r^2}{1 - r^2})$$

Here, *r* [2] represents the proportion of variance explained by the instrumental variable, *n* is the sample size, and *k* is the number of SNPs. For MR, we primarily employed the Inverse Variance Weighted (IVW) method, requiring instrumental variables (IVs) to satisfy three assumptions: (1) IV should be associated with the exposure; (2) IV should not be associated with confounding factors related to both the exposure and the outcome; (3) The effect of IV on the outcome is entirely mediated

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through the exposure. We searched the GWAS catalog (https://www.ebi.ac.uk/gwas) to exclude SNPs related to potentially relevant confounding indicators (such as BMI, metabolites, metabolic diseases, etc.); At the same time, during analysis, we used multiple testing correction to exclude instrumental variables related to outcomes ($P < 0.05/N$), where N is the number of IVs used for 25OHD and all IVs used are shown in Table S13. Various sensitivity analyses were conducted. Initially, the heterogeneity Q test of IVW and MR-Egger was employed to detect potential violations of assumptions through heterogeneity among individual IVs [36]. Subsequently, the MR-Egger intercept was applied to estimate horizontal pleiotropy, ensuring that genetic variation is independently associated with exposure and outcome [37]. Additional analyses using different modeling assumptions and robust MR methods (weighted median and weighted mode) were incorporated to enhance the stability and robustness of the results.

The multivariable MR analysis further minimizes potential horizontal pleiotropy. The MVMR analysis employs the IVW method to assess whether the association between multiple exposures and outcome risk is primarily influenced by other potential confounders. We incorporated the effects of other traits into a linear model using the following formula and retained instrumental variables present across the three datasets. We performed a multivariable MR framework (38–39) to investigate the potential mediating role of immune cells in the pathway from 25OHD to MS. First, we used the IVW method to estimate the causal effect of 25OHD on 280 immune cell phenotypes from European individuals. Next, we conducted an MVMR analysis to assess the causal impact of these immune cell on MS while adjusting for 25OHD. To test the indirect (mediated) effect, we employed a Bootstrap approach to determine if the pathway from 25OHD through immune cells to MS was significant.

Statistical analyses were conducted using R version 3.5.3 software, and for Mendelian randomization analyses, we employed the Mendelian Randomization package [40].

Results

Relationship between the 25OHD and the risk of developing MS

The relationship between serum levels of 25OHD and the risk of developing MS was examined using Cox regression and RCS analyses. Basic information was presented in Table S1. Cox regression analysis demonstrated a significant association between lower 25OHD levels and an increased risk of MS across three models: Model 1, accounting for batch effects, age, sex, and TDI; Model 2, additionally adjusted for potential confounders such as type 2 diabetes, cancer, osteoporosis, monocyte count,

and leukocyte count; and Model 3, additionally adjusted for season of blood sampling and regular physical activity (Table S2). Our RCS analysis identified a nonlinear trend between 25OHD levels and MS risk, with significant nonlinearity in Fig. 2A ($P = 0.024$) and Fig. 2C ($P = 0.044$), but not significant in Fig. 2B ($p = 0.3445$), which suggests different patterns of risk association by sex. In conclusion, A statistically significant P value for nonlinearity (P for nonlinear < 0.05) indicates that the risk relationship is not strictly linear across the range of 25OHD levels. During the follow-up period, our data suggest that participants with the lowest quartile (Q1) of 25OHD had the highest event rate of MS development, whereas those in the highest quartile (Q4) demonstrated the lowest event rate (Fig. 2D-F). This gradient effect reveals a distinct inverse relationship between 25OHD levels and the likelihood of developing MS, with deficiency marking a higher risk status. We also performed a sensitivity analysis and found that without excluding extreme values, the association between VD and MS risk was no longer significant in men but remained significant in women (Table S3). In additional sensitivity analysis, we further excluded participants with only a single MS diagnosis to reduce potential bias. While this adjustment slightly weakened the level of significance, the P value remained below 0.05, and the effect direction was consistent with that observed in the primary analysis(Table S4).

We conducted an interaction analysis to examine whether there is a significant interaction between serum 25OHD levels and sex in relation to MS risk. The interaction term's P-value was 0.052, which is just above the conventional threshold for statistical significance ($P < 0.05$). This suggests a trend towards a potential interaction between sex and 25OHD levels, but it does not reach statistical significance. Subgroup analyses stratified by gender revealed that this inverse association was consistent across male and female participants (Table S2). However, our analysis reveals an L-shaped relationship between 25OHD levels and the risk of MS. The inflection point of the L-shaped curve is identified at 47.9 nmol/L. Below this threshold, reductions in 25OHD are significantly correlated with an increased risk of MS. Conversely, above 47.9 nmol/L, serum 25OHD levels do not exhibit a significant correlation with MS risk, suggesting a threshold effect. Elevating 25OHD to this specific level can mitigate the risk of MS, but exceeding this threshold does not confer additional benefits. This finding underscores the importance of a balanced approach to vitamin D supplementation. The results from the quartile analysis further confirm the protective trend of higher 25OHD levels against the development of MS. Those in the lower quartiles of 25OHD, particularly Q1, demonstrated a significantly increased risk in both Model 1 and Model 2. The increased risk was consistent even after adjusting
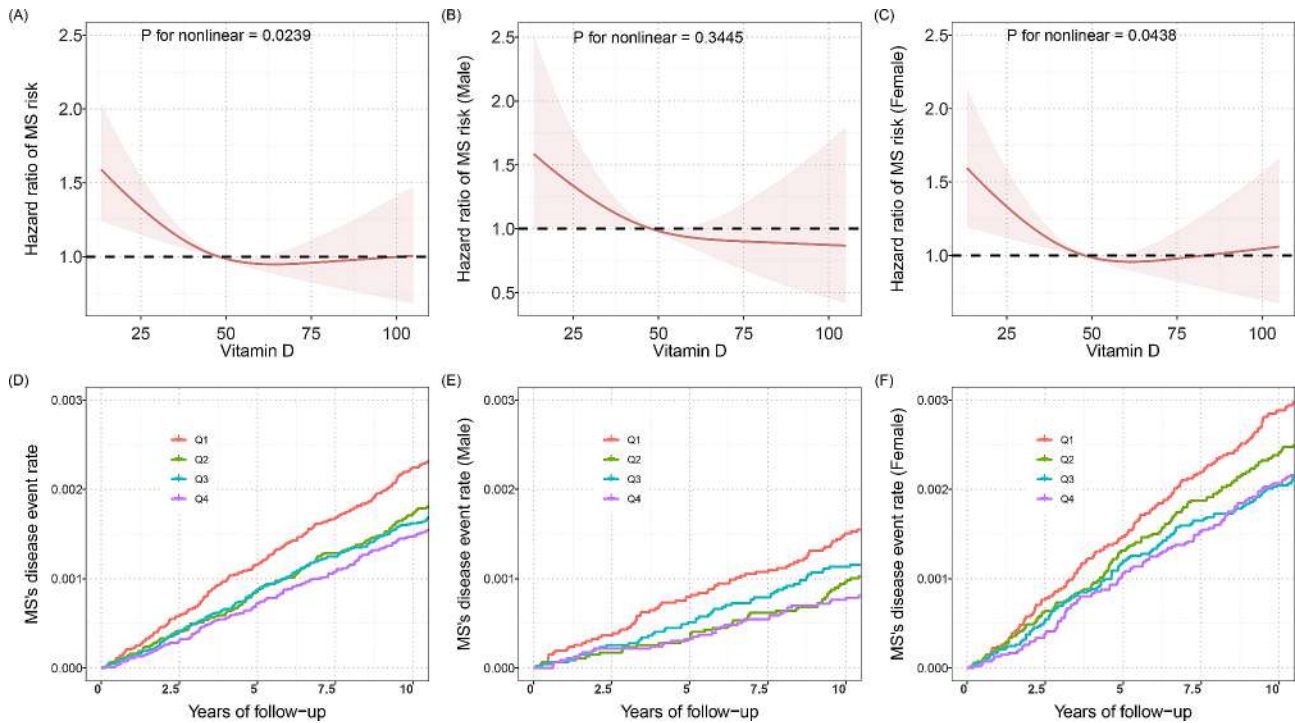
Fig. 2 (**A**) Non-linear association between serum 25OHD levels and Multiple Sclerosis risk in the UKB cohort. (**B**) Hazard ratios for MS by 25OHD levels in male participants of the UKB cohort. (**C**) Hazard ratios for MS by 25OHD levels in female participants of the UKB cohort. (**D**) Standardized rates of MS events in bottom quartile, quartiles 2, quartiles 3), and top quartile 25OHD groups in the UKB cohort. (**E**) Standardized rates of MS events in bottom quartile, quartiles 2, quartiles 3), and top quartile 25OHD groups in male participants of the UKB cohort. (**F**) Standardized rates of MS events in bottom quartile, quartiles 2, quartiles 3), and top quartile 25OHD groups in female participants of the UKB cohort. Adjustments were made for confounding variables including age, sex, genotyping batch, assessment center, Townsend Deprivation Index (TDI), and the first 10 principal components of ancestry. MS, Multiple Sclerosis; OR, odds ratio; HR, hazard ratio. The non-linearity of the associations was assessed and indicated by the p-value for nonlinearity in each graph

for a comprehensive set of confounders, underscoring the robustness of the association.

Genetic correlation between 25OHD and MS

Genetic correlation analysis revealed a significant genetic correlation between 25OHD and MS, estimated using the LDSC method without considering the intercept term (r_g = -0.096, $P=2.98\times10^{-8}$). Consistent findings were obtained when considering the intercept term in the LDSC analysis (r_g = -0.1379, $P=1.86\times10^{-8}$), with an intercept of 0.0134 (SE=0.0058). This intercept value helps mitigate potential interference from overlapping samples between the 25OHD and MS datasets, reinforcing the robustness of the results.

Pleiotropic gene loci identified for 25OHD and MS

Through PLACO analysis, we identified a total of 24 independent pleiotropic loci shared between 25OHD and MS. Figure 3A illustrates the circular Manhattan plot of the identified signals, while detailed information on these pleiotropic loci is provided in Table 1 and S5. Genomic inflation was not observed in the QQ plot (Figure S1 A), and Figure S1 B displays essential information for each genomic risk locus. The impact of pleiotropic SNPs

on gene function is depicted in Figure S1 C and Table S5. In our investigation of the genetic overlap between 25OHD levels and Multiple Sclerosis (MS), region-specific association plots revealed significant single nucleotide polymorphisms (SNPs). Risk loci with PP.H4>0.7 were symbolized as 6p24.3 (RREB1), 6p22.2 (SCGN), 12q14.1 (CDK4), and 19p13.2 (SMARCA4 and LDLR). The marginal signal distributions of these four strong co-localization evidences in 25OHD and MS, respectively, are shown in Fig. 2B-E, and the polytropic signal distributions are shown in Figures S1 D-G. Figure 3B presents a localized association plot on chromosome 6, where rs56329220 was identified as the most significantly associated SNP with 25OHD levels, and rs115740542 with MS. Figure 3C delineates a similar association pattern on chromosome 12, highlighting rs11533604 as a key SNP for 25OHD and rs701006 for MS, providing further evidence of shared genetic architecture between the two conditions. Figure 3D details another segment of chromosome 6, underscoring rs41302867's linkage to 25OHD and rs12211604 to MS, emphasizing the recurrent genetic signals in this chromosomal region. Figure 3E illustrates the findings on chromosome 19 with rs73015021 associated with 25OHD and rs12609500
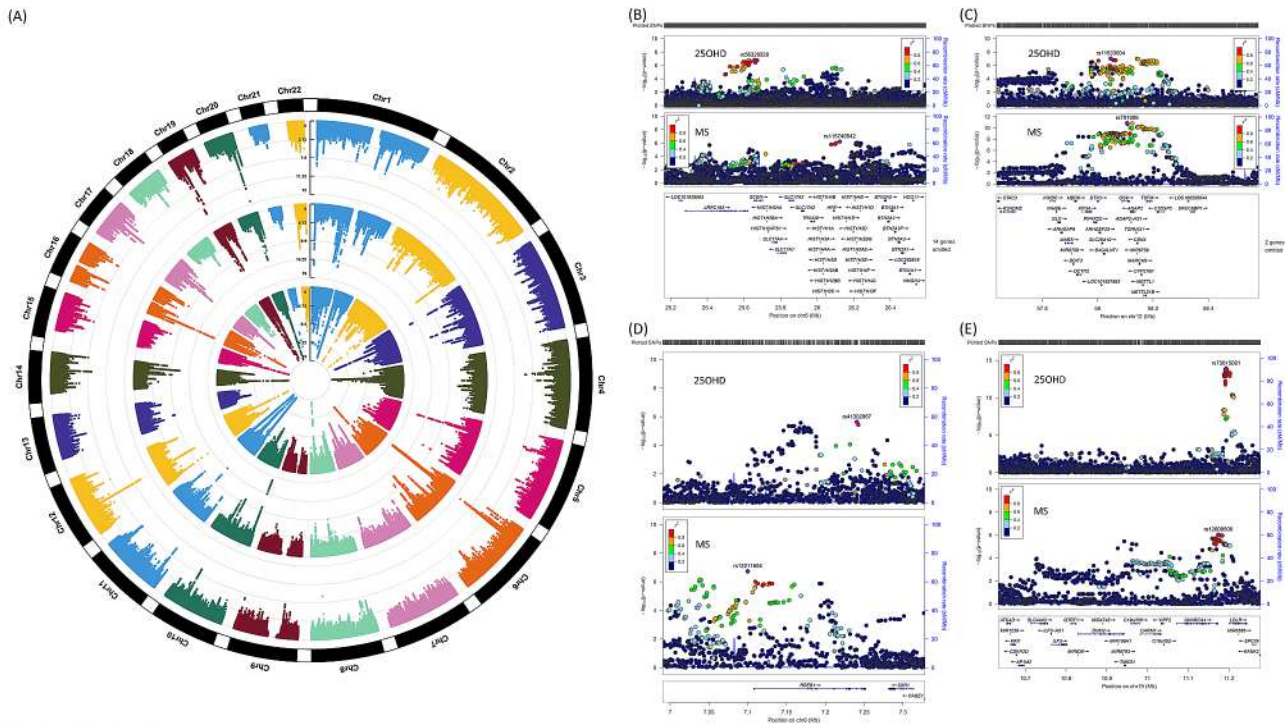
Fig. 3 (**A**) Circular Manhattan plot of pleiotropic signals between 25OHD and MS. (**B**) Regional Plot for shared genetic loci between 25OHD and MS (6p22.2). (**C**) Regional Plot for shared genetic loci between 25OHD and MS (6p24.3). (**D**) Regional Plot for shared genetic loci between 25OHD and MS (12q14.1). (**E**) Regional Plot for shared genetic loci between 25OHD and MS (19p13.2)

Table 1 Information on 24 pleiotropic loci identified between 25OHD and MS

Genomic Locus	Locus region	Lead SNP	*P*	symbol	PP.H4
1p36.32	1:2415191–2,756,397	rs4648390	7.83E-10	TTC34	0.045
1q21.3	1:150204405–151,246,241	rs11204708	1.45E-08	HORMAD1	0.357
1q25.2	1:178988598–179,807,727	rs3754133	7.93E-09	RP11-545A16.1	0.670
4q13.2	4:69509202–70,483,957	rs13123200	2.40E-08	UGT2B28, RP11-618I10.4	0.132
5p13.2	5:35649214–36,180,787	rs987106	4.19E-08	IL7R	0.185
6p24.3	6:6990747–7,328,165	rs4959426	4.26E-09	RREB1	0.967
6p22.2	6:25163549–26,594,542	rs1321247	1.40E-08	SCGN	0.954
6p22.1	6:28626101–29,614,419	rs3130248	1.86E-09	SUMO2P1	0.067
6p21.32	6:33179689–33,641,196	rs4484519	5.75E-13	ZBTB9, RN7SL26P	0.142
8q23.3	8:116464988–117,134,944	rs72681827	1.82E-09	LINC00536	0.212
10q23.33	10:93675891–94,791,270	rs7923837	3.48E-09	Y_RNA, EXOC6	0.479
11p15.2	11:13970901–15,126,262	rs3815984	7.61E-10	SPON1	0.335
11q23.3	11:116519358–117,144,194	rs5130	2.38E-08	APOC3	0.451
11q23.3	11:118459069–118,773,904	rs79504890	3.48E-08	RP11-158I9.1, SETP16	0.314
12q14.1	12:57634698–58,581,470	rs2069502	1.40E-13	CDK4	0.891
14q24.1	14:69138536–69,315,016	rs12435329	5.31E-09	RNU6-921P, ZFP36L1	0.286
14q32.33	14:103756160–104,537,680	rs2756119	3.78E-08	TRMT61A	0.663
16q23.2	16:79634279–79,656,673	rs6564681	4.62E-08	MAF, AC009159.1	0.575
17q12	17:34475471–35,008,350	rs3736166	4.48E-08	GGNBP2	0.533
17q21.32	17:45040389–46,646,427	rs11079784	1.37E-09	RP11-580I16.2	0.366
19p13.2	19:10633908–11,277,232	rs142158911	1.13E-13	SMARCA4, LDLR	0.956
19p13.11	19:18134596–18,409,068	rs62120396	1.22E-11	PDE4C	0.073
19q13.33	19:48322752–48,508,712	rs10405393	4.12E-08	BSPH1	0.308
20q13.2	20:52567414–52,820,687	rs6068816	3.98E-15	CYP24A1	0.000

with MS. Subsequently, we performed gene set enrichment analysis using MAGMA based on the results of pleiotropy. The analysis revealed the top 10 significantly ($P<2.94\times10^{-6}$) enriched gene sets (Fig. 4A, Table S6), with the top three being the metabolic pathway of LDL HDL and TG including diseases, statin inhibition of cholesterol production, and high-density lipoprotein particle remodeling. Additionally, our MAGMA tissue-specific analysis identified significant enrichments ($P<1\times10^{-3}$) in the top three tissues (Fig. 5A), namely cells EBV-transformed lymphocytes, spleen, and small intestine terminal ileum. It is noteworthy that this section of MAGMA gene set and tissue-specific analysis utilized the complete distribution of SNP for the analysis.

Identification of priority pleiotropic genes and functional enrichment

Given the intricate mechanisms through which SNPs influence gene regulation, we employed three distinct approaches to identify pleiotropic genes. All pleiotropic genes are shown in Table S7. Specifically, we first used the physical locations of lead SNPs to map pleiotropy risk sites to 31 nearby genes. The expression values of these nearby genes in various tissues are shown in Figure S2 and Table S8. Multiple genes (such as SCGN, RREB1, MAF, LDLR) were differentially expressed in brain, whole blood, and EBV-transformed lymphoid

tissues. Magma gene test identified 70 pleiotropic genes ($P<2.70E-6=0.05/18639$) (Figure S3 and Table S9). The QQ plot is shown in Figure S4; the expression of these genes in 54 different tissues is shown in (Figure S5 and Table S8), it was further found that genes such as AGAP2, KIF5A, and CTSS were differentially expressed in brain tissue, and genes such as DTX3 and APOE were differentially expressed in whole blood tissue. Finally, eQTL information in whole blood, EBV-transformed lymphocytes, and spleen tissue was used for gene mapping, and a total of 129 pleiotropic genes were identified, and a large number of genes were differentially expressed in brain tissue (Figure S6 and Table S8). The overlap of pleiotropic genes mapped in different ways is shown in Figure S7. HORMAD1, PDE4C, and TRMT61A were mapped by three methods at the same time. The nearby and magma methods simultaneously identified six genes: APOC3, CDK4, CYP24A1, RREB1, UGT2B28, ZFP36L1; Nearby genes and eQTL genes simultaneously identified three genes, SCGN, SMARCA4, and TTC34; Magma and eQTL simultaneously identified 21 shared genes (Table S7).

All identified pleiotropic genes (nearby genes, MAGMA genes, and eQTL genes) were further combined to annotate the gene functions. Tissue-specific enrichment analysis suggested that the mechanism shared by 25OHD and MS may be involved in pancreas,

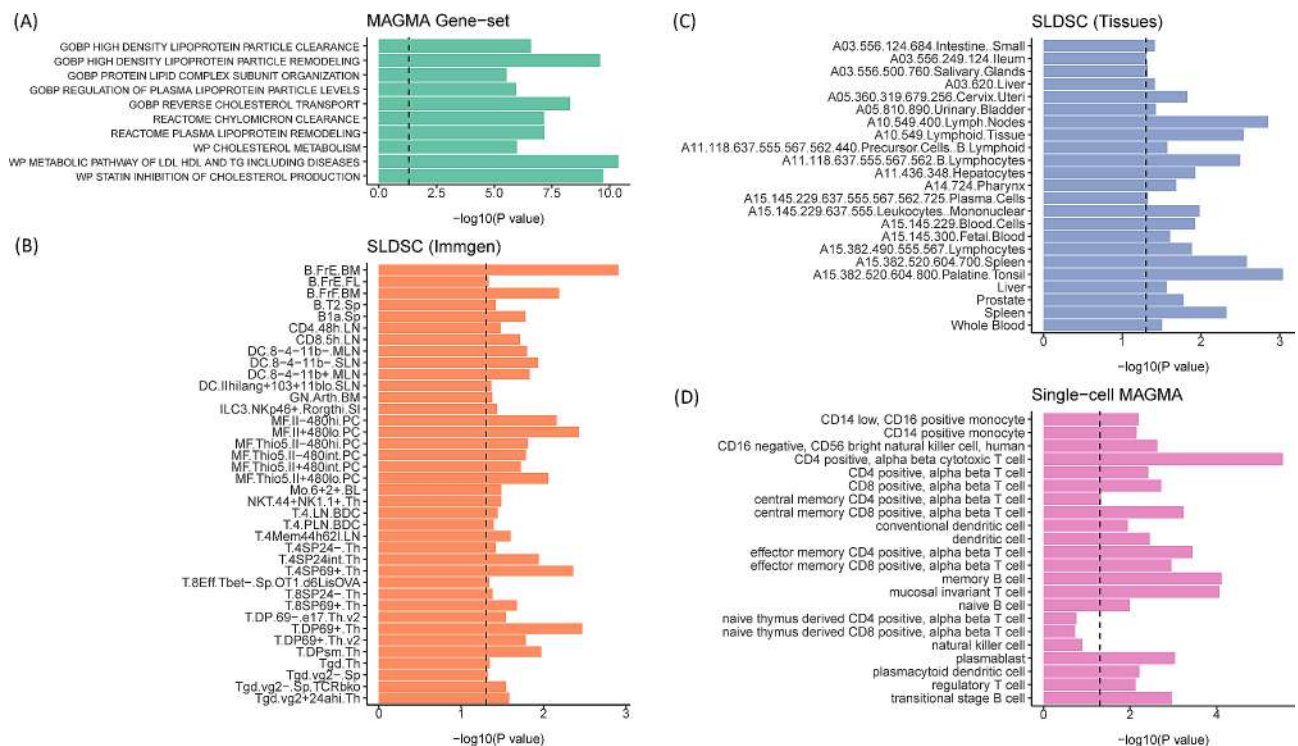**Fig. 4** (**A**) Results of the MAGMA gene-set analysis (top 10); (**B**) Enrichment of polygenic SNP heritability in ImmGen cells determined by stratified-LDSC analysis; (**C**) Enrichment of polygenic SNP heritability across various tissues determined by stratified-LDSC analysis. (**D**) Cell-type Enrichment of Polygenic SNP Heritability in Immune Cells Using Single-cell MAGMA Analysis
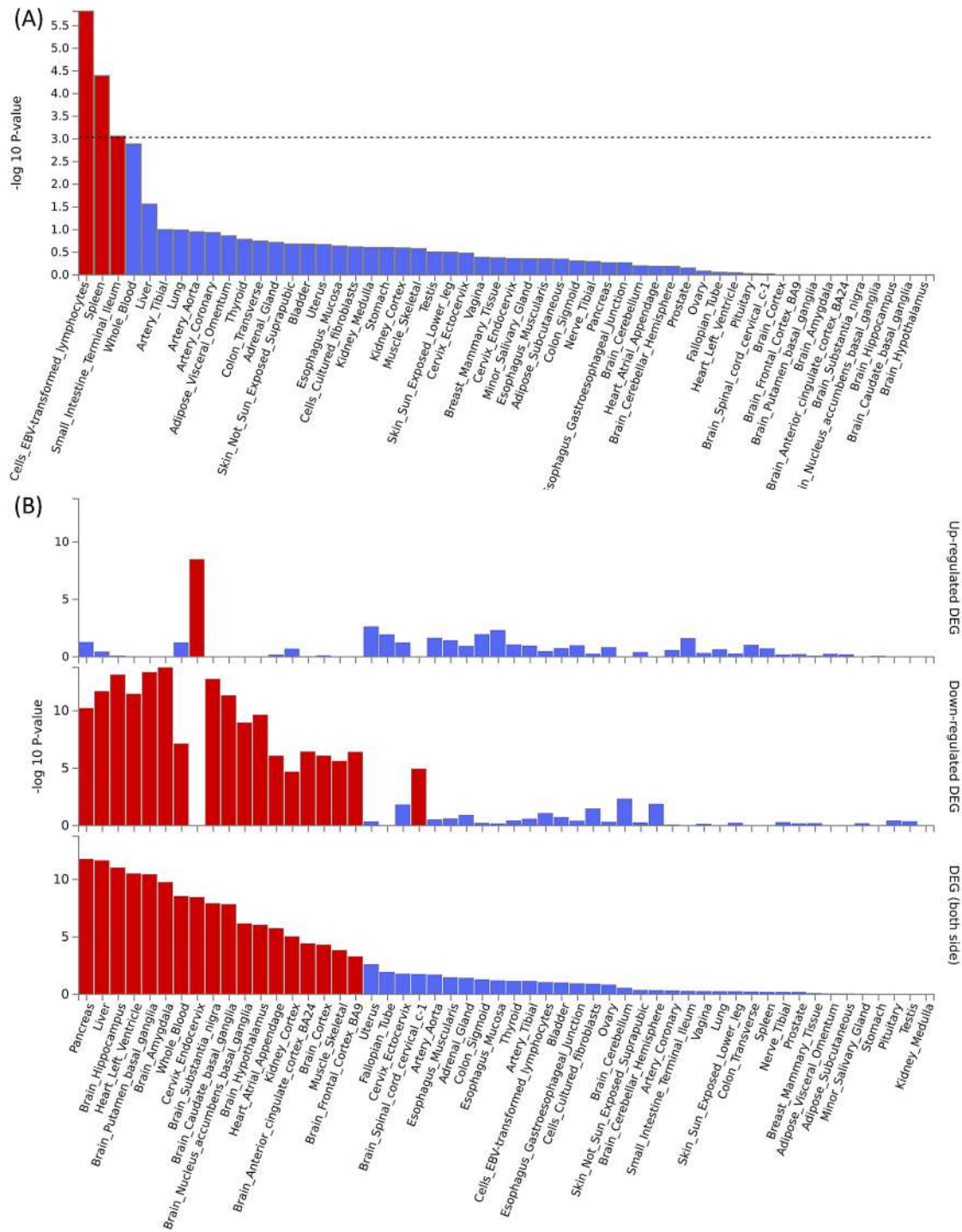
Fig. 5 (**A**) Tissue-specific analysis of genome-wide pleiotropy between 25OHD and MS; (**B**) Enrichment of pleiotropic genes between 25OHD and MS across different tissues

liver, and 11 brain tissues (Table S10, Fig. 5B). Pathway enrichment including KEGG, wiki, GO and other gene set information showed that pleiotropic genes were significantly enriched in pathways such as steroid metabolic process, statin inhibition of cholesterol production, and regulation of cellular catabolic process (Figure S8).

Cell-specific enrichment analysis showed that 18 types of cells were significantly enriched. The first three were FAN OVARY CL6 PUTATIVE EARLY ATRETIC FOLLICLE THECAL CELL 2, MANNO MIDBRAIN NEUROTYPES HMGL, ZHONG PFC MAJOR TYPES MICROGLIA (Figure S9), and the latter two Cells are closely related

to the central nervous system and brain diseases. Finally, PPI analysis was performed on the identified pleiotropic genes, and a total of 6 protein networks were identified (Figure S10).

Stratified-LDSC and single-cell MAGMA to Pinpoint critical Immune traits

We applied the S-LDSC method to the PLACO results of 25OHD and MS in an attempt to find potential heritability enrichment between two traits. As a result, significant enrichment of B.FrE. BM and B.FrF. BM in the B cell panel and T.DP69+. Th in the T cell panel was observed (Fig. 4B, Table S11). Macrophage steady-state MF.II+480lo. PC and MF.II-480hi. PC were also found to be significantly enriched. Tissue-specific S-LDSC enrichment also found that pleiotropy was significantly enriched in blood cells, B lymphocytes, lymphocytes and other tissues (Fig. 4C, Table S12). The Single-cell MAGMA results showed different degrees of enrichment in different subpopulations. Specifically, the pleiotropic signals of vitamin D and MS were most highly enriched in CD4_positive, alpha_beta_cytotoxic_T_cells, followed by memory_B_cells and mucosal_invariant_T_cells (Fig. 4D, Table S13).

Causal association between 25OHD and MS

Finally, the two-sample MR method was used to assess the causal relationship between 25OHD and MS, and IVs were presented in (Table S14). The heterogeneity test found heterogeneity ($P<0.05$), so random effects IVW was used as main results. IVW results supported that 25OHD could causally reduce the risk of MS, all other methods (i.e., MR-Egger, Weighted mode, Weighted median, DIVW, MR-RAPS and MR-PRESSO) obtained

consistent results (Table 2 and S15). The slope term of MR-Egger excluded possible horizontal pleiotropy ($P=0.508>0.05$). We further utilized scatter plots and funnel plots to assess the stability of the associations. Scatter plots exclude potential outliers and funnel plots exclude the possibility of potential bias (Fig. 6). In addition, the results of reverse MR did not support the causal effect of MS on 25OHD, and the results of multiple sensitivity analysis methods were consistent. After we used multivariate MR methods to further correct for metabolic indicators such as blood biochemical indicators, diabetes, and BMI, the association between 25OHD and MS is still statistically significant (Figure S11 and Table S16).

Table S17 shows that specific immune cells partially mediate the effect of 25OHD on MS, with mediation proportions between approximately 32% and 45%. Specifically, 25OHD was associated with increased levels of N_CD8bpos.panel1, X_CD8bpos_of_CD3pos.panel1, and N_HLADRpos_in_CD8bpos_EMRA.panel1. Conversely, 25OHD was associated with decreased ratio_ CD4_CD8.panel1 and X_CD4pos_of_CD3pos.panel1; these immune cells all showed negative indirect effects, consistent with main effects. These findings suggest that 25OHD may affect MS risk by modulating different immune cell populations in different ways.

Discussion

This study, we first utilized the large-scale UK Biobank cohort to evaluate the association between serum 25OHD levels and MS risk. Based on large-scale GWAS summary data, LDSC analysis revealed a significant genetic correlation between 25OHD and MS. Through cross-trait pleiotropy analysis, we found 24 pleiotropic

Table 2 Results of bidirectional MR analysis of 25OHD and MS

| Exposure	Outcome	Methods	Estimate	P	Heterogeneity test	
					Estimate	P
25OHD	MS	IVW (fixed)	0.733 (0.638, 0.843)	1.31E-05	153.087	0.005
		IVW (random)	0.733 (0.622, 0.864)	2.07E-04		
		MR-Egger (slope)	0.774 (0.614, 0.975)	0.030		
		MR-Egger (intercept)	-0.002 (-0.008, 0.004)	0.508		
		Weighted mode	0.739 (0.631, 0.865)	1.70E-04		
		Weighted median	0.785 (0.633, 0.973)	0.027		
		DIVW	0.732 (0.617, 0.867)	3.24E-04		
		MR-RAPS	0.738 (0.627, 0.869)	2.64E-04		
MS	25OHD	IVW (fixed)	0.999 (0.995, 1.003)	0.679	113.751	<0.001
		IVW (random)	0.999 (0.994, 1.005)	0.760		
		MR-Egger (slope)	1.012 (0.996, 1.028)	0.143		
		MR-Egger (intercept)	-0.002 (-0.004, 0)	0.094		
		Weighted mode	1.001 (0.987, 1.016)	0.859		
		Weighted median	0.998 (0.992, 1.005)	0.604		
		DIVW	0.999 (0.994, 1.005)	0.755		
		MR-RAPS	0.999 (0.993, 1.004)	0.658		
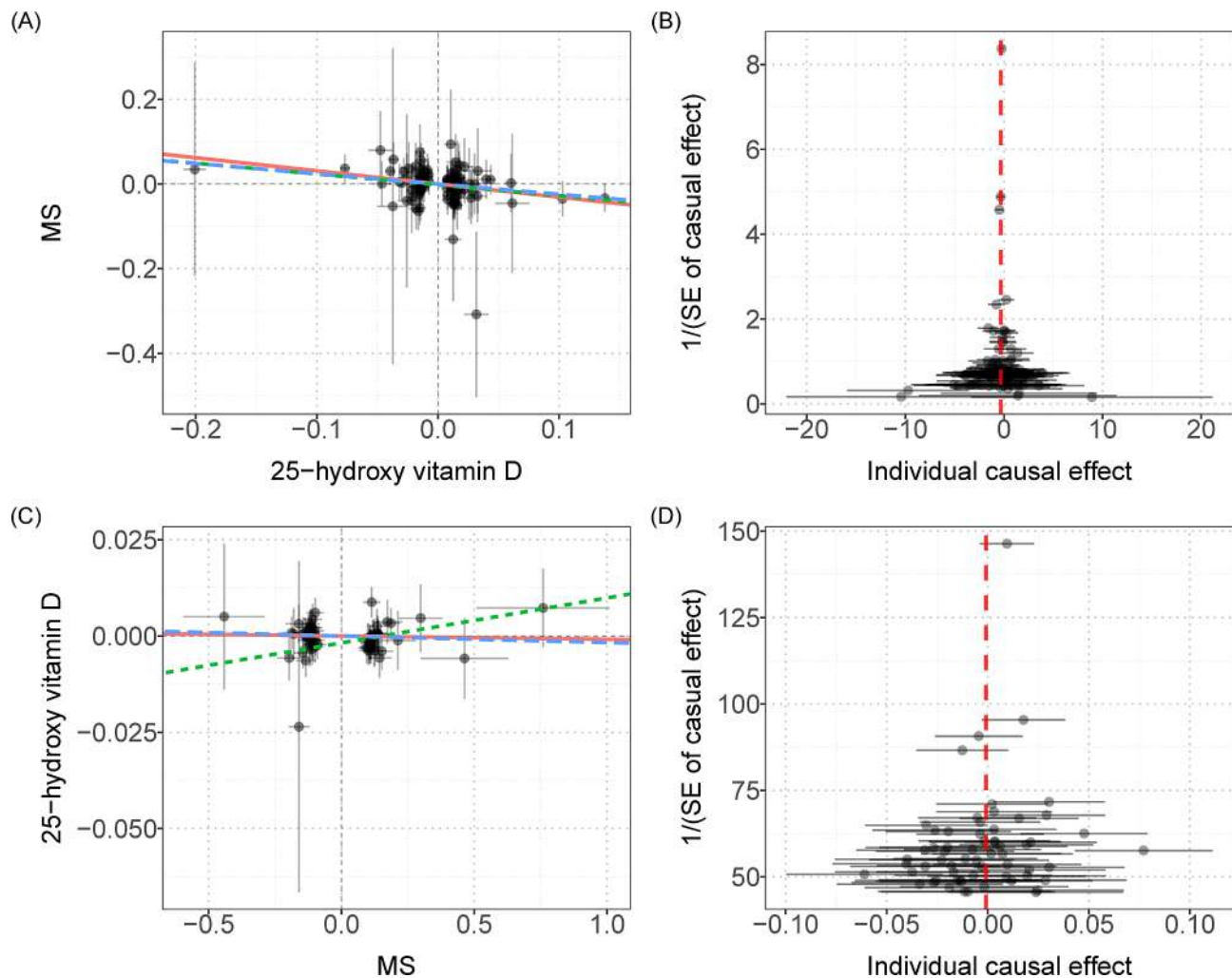
Fig. 6 Scatter and funnel plots from the Mendelian Randomization (MR) analysis. (**A**) Scatter plot for the causal effect of 25OHD on MS; (**B**) Funnel plot for the causal effect of 25OHD on MS; (**C**) Scatter plot for the causal effect of MS on 25OHD; (**D**) Funnel plot for the causal effect of MS on 25OHD

loci, four of which had high co-localization evidence: 6p24.3 (RREB1), 6p22.2 (SCGN), and 12q14.1 (CDK4), and 19p13.2 (SMARCA4 and LDLR). Subsequent investigations found the SNP heritability of 25OHD and MS is enriched in several tissues and immune cells (e.g., spleen, whole blood, lymphocytes). Gene set analysis identified multiple potential pathways that may be involved in the mechanisms of 25OHD deficiency and MS risk. Finally, two-sample MR analysis identified a potential causal effect of 25OHD deficiency on MS risk.

Both observational studies and genetic evidence in our analysis generally support a significant inverse association between 25OHD and MS risk. This association is consistent with studies by Mokry LE, Rhead B, Gianfrancesco MA, etc. (13, 41–42) Then there are also some studies that find vitamin D does not seem to have a therapeutic effect on EDSS scores or ARR in people with MS [43]. Previous inconsistent associations may be due to dosage factors. For example: A meta-study suggests that

vitamin D supplementation may have a therapeutic effect in the treatment of multiple sclerosis. However, there is uncertainty about the most appropriate dose, and higher doses may lead to worse outcomes [44]. Interestingly, this appears to be consistent with our finding in observational studies that there is an L-shaped association between 25OHD concentration and MS, that is, very high concentrations of 25OHD do not reduce the risk of MS and may even slightly increase. Expanding the scope of prior research, our bidirectional MR analysis enhances the evidence base with an enlarged cohort [13], substantially increasing the sample sizes to 417,580 for 25OHD and 76,755 for MS cases. However, MR studies only assume linear regression between VD and MS, and the developed nonlinear MR has also been proven to be unreasonable (45–46), so it is still impossible to infer the causal relationship between the two under nonlinear scenarios.

The Cox regression and RCS analyses point to a nonlinear association, suggesting an inverse correlation

where levels of 25OHD are linked to risk of MS, particularly pronounced in the lowest quartile of 25OHD. The implications of these results are multifaceted. 25OHD metabolism exhibits significant gender differences, most notably among people with MS [47]. The observed gender-based discrepancies in risk suggest potential biological or behavioral differences in 25OHD metabolism or MS pathogenesis. A study demonstrates that genetic variations, particularly those in 25OHD binding proteins and metabolism enzymes, can significantly influence serum 25OHD levels in response to high-dose vitamin D3 supplementation in relapsing-remitting people with MS [48]. In the male and combined gender subgroup analysis, the relationship between 25OHD levels and MS risk demonstrates an L-shaped curve, with a clear threshold at 47.5 nmol/L. Beyond this point, further increases in 25OHD do not provide additional benefits. However, in the female subgroup, once the 25OHD level exceeds a certain threshold, there is a slight increase in MS risk. These observations significantly diverge from traditional dose-response assumptions. Hormonal differences likely play a key role, as estrogen has been shown to enhance vitamin D receptor expression, which could potentiate the immune-regulatory properties of 25OHD in females [49]. The synergy between estrogen and vitamin D may provide insight into why women exhibit a stronger dose-response relationship to vitamin D, with more pronounced effects on immune modulation. This heightened sensitivity suggests that women might achieve optimal 25OHD levels more quickly with supplementation compared to men. However, this could also render women more susceptible to over-supplementation, where excessive 25OHD levels may paradoxically increase the risk of developing MS. Additionally, female sex has been identified as a significant risk factor for developing MS [50]. Our findings indicate that while vitamin D deficiency is strongly associated with an elevated risk of MS, surpassing a certain 25OHD threshold, particularly beyond the critical level of 47.5 nmol/L, may have adverse effects. This observation is reflected in the nonlinear risk patterns in females, which differ from the more straightforward L-shaped curve seen in males. These results emphasize the need for personalized vitamin D supplementation strategies that account for gender differences in vitamin D metabolism, ensuring that the benefits of supplementation are optimized while avoiding the potential risks of excessive vitamin D levels.

Among the five genes closest to the lead SNP in loci with high co-localization evidence, CDK4 and LDLR have been extensively reported to be associated with 25OHD and MS. The role of CDK4 in the pathogenesis of MS is elucidated through its engagement in various cellular and molecular pathways that intersect with the modulation of 25OHD [51–54], further confirming our research findings. An increasing amount of evidence suggests that LDL is associated with clinical risks and MRI outcomes in MS [55]. There are existing reports on the relationship between SMARCA4 and 25OHD, and SMARCA4 has been identified as potentially playing a role in regulating gender-specific immune responses and lipid metabolism in MS (56–57). Integrating our results, we consider SMARCA4 a potential therapeutic target between 25OHD and MS, deserving of further experimental investigation. RREB1 and SCGN have not been directly linked to 25OHD and MS, but there are indirect connections, which also warrant deeper future studies. We further discuss the functions of pleiotropic genes and possible mechanisms involved in the Supplementary.

Discussion

The findings revealed that the top 10 significantly enriched gene sets were all related to lipid metabolism pathways. The top three included the metabolic pathways of LDL, HDL, and TG, comprising diseases, statin inhibition of cholesterol production, and high-density lipoprotein particle remodeling. Further consolidation of nearby genes with matching positions, MAGMA genes, and eQTL genes led to tissue-specific enrichment analysis, indicating significant enrichment of pleiotropic genes associated with 25OHD and multiple sclerosis in the 'Steroid metabolic process' and 'Statin inhibition of cholesterol production' pathways. These enrichment results collectively suggest that lipid metabolism abnormalities may serve as risk factors for metabolic syndrome, providing additional insights into our discoveries concerning the pleiotropic loci of 25OHD and multiple sclerosis. The disruption of metabolism and fatty acid levels drives the occurrence and progression of central nervous system diseases, including MS [58]. The study reports the complex dynamics of microglial cell activation and their involvement in lipid metabolism, particularly the increased expression of key genes such as apolipoproteins, TREM2, and LPL [59]. This underscores the potential connection between microglial cell lipid metabolism and the pathogenesis of neurodegenerative diseases, including multiple sclerosis. The dysregulation of myelin health, due to the absence of microglia, is associated with the emergence of a myelinating oligodendrocyte state with altered lipid metabolism. Moreover, this mechanism is regulated through the disruption of the TGFβ1-TGFβR1 axis [60]. These insights open avenues for exploring targeted therapeutic interventions in MS, focusing on lipid metabolism as a significant factor in disease progression.

Cross-tissue eQTL analysis spanning whole blood, EBV-transformed lymphocytes, and spleen tissues revealed 129 multi-effect genes associated with 25OHD levels and MS. Through the integration of

position-matched nearby genes, MAGMA genes, and eQTL genes, tissue-specific enrichment analysis indicated that these genes are not only enriched in various brain tissues but also in organs such as the liver, pancreas, and endometrial tissue. This emphasizes the potential involvement of these gene loci in brain-related processes, highlighting their functional impact on lipid metabolism and hormone regulation. Existing research supports our findings, suggesting that alterations in lipid metabolism and hormone secretion may be risk factors for the onset and progression of MS [55, 61]. Hormone receptors are present on immune cells, and sex hormones (such as estrogen, progesterone, testosterone, and prolactin) can influence different aspects of the immune system, potentially affecting the risk, activity, and progression of MS [61]. Progesterone can increase the proliferation and maturation of oligodendrocyte precursor cells into mature oligodendrocytes, thereby attenuating the activation and proliferation of microglia and astrocytes [62–64]. A Mendelian randomization study found that high-density lipoprotein cholesterol has a certain impact on fatigue in MS [65]. Another study identified a direct anti-inflammatory effect of the secondary bile acid TUDCA on astrocytes and microglia in vitro, and supplementing TUDCA improved the disease condition in an MS mouse model through its action on GPBAR1, indicating abnormal changes in bile acid metabolism in MS [66]. APOE was found to be differentially expressed in whole blood, aligning with its known role in immune regulation. Notably, APOE is expressed in monocytes and macrophages, where it plays a critical role in modulating lipid metabolism and immune responses. Research has demonstrated that APOE4-expressing cells, such as microglia and macrophages, exhibit altered phagocytic activity and a heightened pro-inflammatory state, which may influence immune regulation. (67–68) Furthermore, studies on iPSC-derived cells have revealed that APOE4 impacts cholesterol trafficking and lipid droplet accumulation, contributing to increased inflammation [69]. These findings suggest that APOE's role in lipid metabolism could be linked to immune dysregulation in autoimmune diseases like MS. (69–70) These findings collectively underscore the intricate connections between hormone regulation, lipid metabolism, and immune system activities, suggesting that targeting these pathways could offer new therapeutic strategies for managing MS.

In addition to its well-established role in influencing lipid metabolism, 25OHD has emerged as a key player in immune regulation, exerting influence on both innate and adaptive immunity and effectively mitigating autoimmune responses. (71–72) A study on MS reveals heightened levels of 1,25-(OH)2D, increased EBV load, and a distinctive correlation between VDR expression and EBV load in people with MS, highlighting intricate

interactions and opposing effects of EBV and 25OHD on immune dysregulation [73]. The imbalance in Th17/Treg ratios has been implicated in the pathogenesis of various autoimmune and inflammatory diseases. (74–75) 1,25(OH)2D3 exhibits the capability to suppress the Th17 phenotype by inhibiting the transcription of key factors, including RORγt, IL-17, IL-23R, and IL-22. Simultaneously, it fosters the Treg subset by inducing the expression of immunoregulatory elements such as IL-10, Foxp3, and CTLA-4 [76]. Furthermore, 1,25(OH)2D3 suppresses the expression of IL-2 and IFN-γ, thereby modulating the differentiation of Th17 cells [77]. The intricate interaction between 25OHD and the immune system is substantiated in murine model experiments, where vitamin D treatment ameliorated symptoms in the experimental autoimmune encephalomyelitis (EAE, an MS model) mouse model, potentially through the direct action of 1,25OH2D3 on CD4+T cells [78]. Contingent upon the VDR recruitment site, 1,25(OH)2D3 has the potential to modulate the protein expression of specific vitamin D-sensitive genes, influencing a spectrum of cellular processes. This encompasses cellular growth, proliferation, differentiation, apoptosis, oxidative stress, and membrane transport [79]. In the EAE model, both the in vivo administration of vitamin D and the transfer of vitamin D-induced tolerogenic dendritic cells resulted in a notable elevation in the proportion of CD4(+) CD25(+) Foxp3(+) regulatory T cells and enhanced IL-10 production. Simultaneously, there was a reduction in the count of autoreactive T cells. These interventions demonstrated a considerable decrease in the incidence and severity of EAE. (80–81) Multi-trait colocalization analysis pinpointed 38 immune cell phenotypes with substantial genetic overlap. These molecular insights are pivotal for enhancing our genetic comprehension of MS, emphatically emphasizing the integral role of the immune system in the pathogenesis of the disease.

Future research should aim to delineate the mechanisms underlying these associations, explore the potential benefits of Vitamin D supplementation in at-risk populations, and establish the optimal ranges of serum 25OHD for MS prevention. Incorporating these insights, clinicians and policymakers could refine guidelines on Vitamin D intake, potentially incorporating genetic screening to identify individuals who might benefit most from preventive interventions.

Limitation

It is essential to acknowledge the limitations of our study. Despite the robust evidence from MR analysis, the observational nature of genetic associations cannot definitively prove causation and may overlook confounding factors. Our results, mainly derived from a European ancestry cohort, may not extend to all populations,

highlighting the need for validation across diverse ethnicities. Additionally, the potential existence of undetected pleiotropic loci or pathogenic variants could further complicate interpretations of our results. Future research must broaden the scope to include a more diverse genetic background and explore the multifactorial nature of MS and its association with 25OHD. Expanding the scope of investigation to diverse populations and employing complementary research methodologies will be crucial for confirming our findings and translating them into effective clinical interventions.While our interaction analysis suggests a trend towards a potential interaction between sex and 25OHD levels. This trend, despite not reaching statistical significance in our sample, may still hold biological or clinical relevance. Given the near-significant result, further studies with larger sample sizes are warranted to explore this potential interaction more definitively. Such studies could provide more robust evidence and help clarify whether sex modifies the effect of 25OHD levels on MS risk.

Conclusions

This study highlights a significant genetic correlation between 25OHD levels and MS, revealing shared genes and pathways that could contribute to disease etiology and progression. Causal inference analysis suggests a significant causal effect of 25OHD on MS, highlighting the potential of vitamin D-targeted interventions in modifying MS risk. Observational study revealed an "L"-shaped association between serum 25OHD levels and the risk of MS, indicating that increasing serum 25OHD to an optimal threshold of 47.5 nmol/L significantly reduces the risk of MS and higher levels do not necessarily provide additional benefits. Our findings provide a foundation for future research into the shared genetic mechanisms and interplay between 25OHD levels and MS.

Abbreviations

25OHD	25-hydroxyvitamin D
ASD	Autism Spectrum Disorder
BMI	Body Mass Index
CAD	Coronary Artery Disease
CDK4	Cyclin-Dependent Kinase 4
CNS	Central Nervous System
CRP	C-reactive Protein
DBP	Diastolic Blood Pressure
eQTL	Expression Quantitative Trait Loci
EDSS	Expanded Disability Status Scale
ERK1/2	Extracellular Signal-Regulated Kinase 1/2
FGF	Fibroblast Growth Factor
GWAS	Genome-Wide Association Study
HbA1c	Hemoglobin A1c
HDL	High-Density Lipoprotein
HOMA-IR	Homeostatic Model Assessment of Insulin Resistance
HOMA-β	Homeostatic Model Assessment of Beta-cell Function
LDL	Low-Density Lipoprotein
MAGMA	Multi-marker Analysis of GenoMic Annotation
MAPK	Mitogen-Activated Protein Kinase
MF	Macrophage
mTORC1	Mechanistic Target of Rapamycin Complex 1
MS	Multiple Sclerosis
MR	Mendelian Randomization
RREB1	Ras Responsive Element Binding Protein 1
SCGN	Secretagogin
SMARCA4	SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin, Subfamily A, Member 4
SNP	Single Nucleotide Polymorphism
SBP	Systolic Blood Pressure
TGFβ	Transforming Growth Factor Beta
TSC2	Tuberous Sclerosis Complex 2
VDR	Vitamin D Receptor
WBC	White Blood Cell Count
AC	Absolute Cell Counts
BRD4	Bromodomain Containing 4
CTSS	Cathepsin S
DTX3	Deltex E3 Ubiquitin Ligase 3
Esr1	Estrogen Receptor 1
FAN	Follicle-Associated Nucleus
GO	Gene Ontology
HDL-C	High-Density Lipoprotein Cholesterol
HORMAD1	HORMA Domain Containing 1
HyPrColoc	Hypothesis Prioritization for Multi-Trait Colocalization
IVs	Instrumental Variables
KEGG	Kyoto Encyclopedia of Genes and Genomes
KIF5A	Kinesin Family Member 5 A
LD	Linkage Disequilibrium
MAF	Minor Allele Frequency
MFI	Median Fluorescence Intensity
MRI	Magnetic Resonance Imaging
PPI	Protein-Protein Interaction
QQ plot	Quantile-Quantile plot
RC	Relative Cell Counts
RCS	Restricted Cubic Splines
TBNK	T, B, and Natural Killer cells
TTC34	Tetratricopeptide Repeat Domain 34
ZFP36L1	Zinc Finger Protein 36 Like 1
ARR	Annualized Relapse Rate
CTLs	Cytotoxic T Lymphocytes
EAE	Experimental Autoimmune Encephalomyelitis
GTEx	Genotype-Tissue Expression
ImmGen	Immunological Genome
IL	Interleukin
PLACO	Pleiotropic Analysis under Composite Null Hypothesis
Tregs	Regulatory T Cells
TUDCA	Tauroursodeoxycholic Acid
VDR	Vitamin D Receptor

Supplementary Information

Supplementary Material 1

Supplementary Material 2

Author contributions
Xing-Hao Yu and Hui-Min Lu: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data Curation, Writing - Original Draft, Visualization. Jun Li and Ming-Zhu Su: Validation, Formal analysis, Investigation, Writing - Original Draft. Xiaomin Li: Conceptualization, Methodology, Visualization, Supervision, Project administration. Yijin: Conceptualization, Methodology, Writing - Review & Editing, Visualization, Supervision, Project administration, Funding acquisition. All authors reviewed the manuscript.

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Data availability
Data is provided within the manuscript or supplementary information files.

Declarations

Ethical approval
This study did not involve any experiments with human or animal subjects directly; therefore, ethical approval was not required. The analysis was based on publicly available, anonymized datasets.

Consent for publication
All authors have approved the submission of this manuscript.

Competing interests
The authors declare no competing interests.

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References

1. Gandhi F, Jhaveri S, Avanthika C, Singh A, Jain N, Gulraiz A, Shah P, Nasir F. Impact of vitamin D supplementation on multiple sclerosis. Cureus 2021, 13 (10), e18487.
2. Compston A, Coles A. Multiple sclerosis. Lancet (London England). 2008;372(9648):1502–17.
3. Friese MA, Schattling B, Fugger L. Mechanisms of neurodegeneration and axonal dysfunction in multiple sclerosis. Nat Reviews Neurol. 2014;10(4):225–38.
4. Wessels I, Rink L. Micronutrients in autoimmune diseases: possible therapeutic benefits of zinc and vitamin D. J Nutr Biochem. 2020;77:108240.
5. Simpson S Jr., Blizzard L, Otahal P, Van der Mei I, Taylor B. Latitude is significantly associated with the prevalence of multiple sclerosis: a meta-analysis. J Neurol Neurosurg Psychiatry. 2011;82(10):1132–41.
6. Vukusic S, Van Bockstael V, Gosselin S, Confavreux C. Regional variations in the prevalence of multiple sclerosis in French farmers. J Neurol Neurosurg Psychiatry. 2007;78(7):707–9.
7. Melcon MO, Gold L, Carrá A, Cáceres F, Correale J, Cristiano E, Fernández Liguori N, Garcea O, Luetic G, Kremenchutzky M. Argentine Patagonia: prevalence and clinical features of multiple sclerosis. Mult Scler. 2008;14(5):656–62.
8. Poppe AY, Wolfson C, Zhu B. Prevalence of multiple sclerosis in Canada: a systematic review. Can J Neurol Sci. 2008;35(5):593–601.
9. Koch-Henriksen N, Sørensen PS. The changing demographic pattern of multiple sclerosis epidemiology. Lancet Neurol. 2010;9(5):520–32.
10. Jacobs BM, Noyce AJ, Giovannoni G, Dobson R. BMI and low vitamin D are causal factors for multiple sclerosis: a mendelian randomization study. Neurology(R) Neuroimmunol Neuroinflammation 2020, 7 (2).
11. Wang R. Mendelian randomization study updates the effect of 25-hydroxyvitamin D levels on the risk of multiple sclerosis. J Translational Med. 2022;20(1):3.
12. Fang A, Zhao Y, Yang P, Zhang X, Giovannucci EL. Vitamin D and human health: evidence from mendelian randomization studies. Eur J Epidemiol. 2024;39(5):467–90.
13. Mokry LE, Ross S, Ahmad OS, Forgetta V, Smith GD, Goltzman D, Leong A, Greenwood CM, Thanassoulis G, Richards JB. Vitamin D and risk of multiple sclerosis: a mendelian randomization study. PLoS Med 2015, 12 (8), e1001866.
14. Munger KL, Levin LI, Hollis BW, Howard NS, Ascherio A. Serum 25-hydroxyvitamin D levels and risk of multiple sclerosis. JAMA. 2006;296(23):2832–8.
15. Munger KL, Zhang SM, O'Reilly E, Hernán MA, Olek MJ, Willett WC, Ascherio A. Vitamin D intake and incidence of multiple sclerosis. Neurology. 2004;62(1):60–5.
16. Hajeer S, Nasr F, Nabha S, Saab MB, Harati H, Desoutter A, Al Ahmar E, Estephan E. Association between vitamin D deficiency and multiple sclerosis-MRI significance: a scoping review. Heliyon 2023, 9 (5), e15754.
17. Bouillon R, Manousaki D, Rosen C, Trajanoska K, Rivadeneira F, Richards JB. The health effects of vitamin D supplementation: evidence from human studies. Nat Rev Endocrinol. 2022;18(2):96–110.
18. Ramagopalan SV, Maugeri NJ, Handunnetthi L, Lincoln MR, Orton SM, Dyment DA, Deluca GC, Herrera BM, Chao MJ, Sadovnick AD, Ebers GC, Knight JC. Expression of the multiple sclerosis-associated MHC class II allele HLA-DRB1*1501 is regulated by vitamin D. PLoS Genet 2009, 5 (2), e1000369.
19. Sîrbe C, Rednic S, Grama A, Pop TL. An update on the effects of vitamin D on the Immune System and Autoimmune diseases. Int J Mol Sci. 2022;23:17.
20. Smolders J, Torkildsen Ø, Camu W, Holmøy T. An update on vitamin D and disease activity in multiple sclerosis. CNS Drugs. 2019;33(12):1187–99.
21. Heaney RP. Guidelines for optimizing design and analysis of clinical studies of nutrient effects. Nutr Rev. 2014;72(1):48–54.
22. Multiple sclerosis genomic. Map implicates peripheral immune cells and microglia in susceptibility. Sci (New York N Y). 2019;365:6460.
23. Low-Frequency. Rare-coding variation contributes to multiple sclerosis risk. Cell. 2018;175(6):1679–e16877.
24. Sudlow C, Gallacher J, Allen N, Beral V, Burton P, Danesh J, Downey P, Elliott P, Green J, Landray M, Liu B, Matthews P, Ong G, Pell J, Silman A, Young A, Sprosen T, Peakman T, Collins R. UK biobank: an open access resource for identifying the causes of a wide range of complex diseases of middle and old age. PLoS Med 2015, 12 (3), e1001779.
25. Manousaki D, Mitchell R, Dudding T, Haworth S, Harroud A, Forgetta V, Shah RL, Luan J, Langenberg C, Timpson NJ, Richards JB. Genome-wide Association Study for Vitamin D Levels Reveals 69 independent loci. Am J Hum Genet. 2020;106(3):327–37.
26. Bulik-Sullivan B, Finucane HK, Anttila V, Gusev A, Day FR, Loh PR, Duncan L, Perry JR, Patterson N, Robinson EB, Daly MJ, Price AL, Neale BM. An atlas of genetic correlations across human diseases and traits. Nat Genet. 2015;47(11):1236–41.
27. Auton A, Brooks LD, Durbin RM, Garrison EP, Kang HM, Korbel JO, Marchini JL, McCarthy S, McVean GA, Abecasis GR. A global reference for human genetic variation. Nature. 2015;526(7571):68–74.
28. The Genotype-Tissue. Expression (GTEx) project. Nat Genet. 2013;45(6):580–5.
29. Open-source ImmGen.: mononuclear phagocytes. Nat Immunol. 2016;17(7):741.
30. Ray D, Chatterjee N. A powerful method for pleiotropic analysis under composite null hypothesis identifies novel shared loci between type 2 diabetes and prostate Cancer. PLoS Genet 2020, 16 (12), e1009218.
31. de Leeuw CA, Mooij JM, Heskes T, Posthuma D. MAGMA: generalized gene-set analysis of GWAS data. PLoS Comput Biol 2015, 11 (4), e1004219.
32. Watanabe K, Taskesen E, van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. Nat Commun. 2017;8(1):1826.
33. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci USA. 2005;102(43):15545–50.
34. Hormozdiari F, van de Bunt M, Segrè AV, Li X, Joo JWJ, Bilow M, Sul JH, Sankararaman S, Pasaniuc B, Eskin E. Colocalization of GWAS and eQTL signals detects Target genes. Am J Hum Genet. 2016;99(6):1245–60.

35. Burgess S, Thompson SG. Avoiding bias from weak instruments in mendelian randomization studies. Int J Epidemiol. 2011;40(3):755–64.
36. Thompson SG, Sharp SJ. Explaining heterogeneity in meta-analysis: a comparison of methods. Stat Med. 1999;18(20):2693–708.
37. Burgess S, Thompson SG. Interpreting findings from mendelian randomization using the MR-Egger method. Eur J Epidemiol 2017, *32* (5), 377–89.
38. Yu X, Wang T, Huang S, Zeng P. Evaluation of the causal effects of blood lipid levels on gout with summary level GWAS data: two-sample mendelian randomization and mediation analysis. J Hum Genet. 2021;66(5):465–73.
39. Auwerx C, Sadler MC, Woh T, Reymond A, Kutalik Z, Porcu E. Exploiting the mediating role of the metabolome to unravel transcript-to-phenotype associations. *eLife* 2023, *12*.
40. Broadbent JR, Foley CN, Grant AJ, Mason AM, Staley JR, Burgess S. Mendelian-Randomization v0.5.0: updates to an R package for performing mendelian randomization analyses using summarized data. Wellcome open Res. 2020;5:252.
41. Rhead B, Bäärnhielm M, Gianfrancesco M, Mok A, Shao X, Quach H, Shen L, Schaefer C, Link J, Gyllenberg A, Hedström AK, Olsson T, Hillert J, Kockum I, Glymour MM, Alfredsson L, Barcellos LF. Mendelian randomization shows a causal effect of low vitamin D on multiple sclerosis risk. Neurol Genet 2016, 2 (5), e97.
42. Gianfrancesco MA, Stridh P, Rhead B, Shao X, Xu E, Graves JS, Chitnis T, Waldman A, Lotze T, Schreiner T, Belman A, Greenberg B, Weinstock-Guttman B, Aaen G, Tillema JM, Hart J, Caillier S, Ness J, Harris Y, Rubin J, Candee M, Krupp L, Gorman M, Benson L, Rodriguez M, Mar S, Kahn I, Rose J, Roalstad S, Casper TC, Shen L, Quach H, Quach D, Hillert J, Bäärnhielm M, Hedstrom A, Olsson T, Kockum I, Alfredsson L, Metayer C, Schaefer C, Barcellos LF, Waubant E. Evidence for a causal relationship between low vitamin D, high BMI, and pediatric-onset MS. Neurology. 2017;88(17):1623–9.
43. Zheng C, He L, Liu L, Zhu J, Jin T. The efficacy of vitamin D in multiple sclerosis: a meta-analysis. Multiple Scler Relat Disorders. 2018;23:56–61.
44. McLaughlin L, Clarke L, Khalilidehkordi E, Butzkueven H, Taylor B, Broadley SA. Vitamin D for the treatment of multiple sclerosis: a meta-analysis. J Neurol. 2018;265(12):2893–905.
45. Burgess S. Violation of the constant genetic Effect Assumption can result in biased estimates for non-linear mendelian randomization. Human Hered. 2023;88(1):79–90.
46. Wade KH, Hamilton FW, Carslake D, Sattar N, Davey Smith G, Timpson NJ. Challenges in undertaking nonlinear mendelian randomization. Obes (Silver Spring Md). 2023;31(12):2887–90.
47. Barnes MS, Bonham MP, Robson PJ, Strain JJ, Lowe-Strong AS, Eaton-Evans J, Ginty F, Wallace JM. Assessment of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D3 concentrations in male and female multiple sclerosis patients and control volunteers. Mult Scler. 2007;13(5):670–2.
48. Mimpen M, Rolf L, Poelmans G, van den Ouweland J, Hupperts R, Damoiseaux J, Smolders J. Vitamin D related genetic polymorphisms affect serological response to high-dose vitamin D supplementation in multiple sclerosis. PLoS ONE 2021, 16 (12), e0261097.
49. Wierzbicka A, Oczkowicz M. Sex differences in vitamin D metabolism, serum levels and action. Br J Nutr. 2022;128(11):2115–30.
50. Olsson T, Barcellos LF, Alfredsson L. Interactions between genetic, lifestyle and environmental risk factors for multiple sclerosis. Nat Reviews Neurol. 2017;13(1):25–36.
51. Romero-Pozuelo J, Figlia G, Kaya O, Martin-Villalba A, Teleman AA. Cdk4 and Cdk6 couple the cell-cycle Machinery to Cell Growth via mTORC1. Cell Rep. 2020;31(2):107504.
52. Manuel AM, Dai Y, Freeman LA, Jia P, Zhao Z. An integrative study of genetic variants with brain tissue expression identifies viral etiology and potential drug targets of multiple sclerosis. Mol Cell Neurosci. 2021;115:103656.
53. Yamamoto A, Saito T, Hosoya T, Kawahata K, Asano Y, Sato S, Mizoguchi F, Yasuda S, Kohsaka H. Therapeutic effect of cyclin-dependent kinase 4/6 inhibitor on dermal fibrosis in murine models of systemic sclerosis. Arthritis Rheumatol (Hoboken N J). 2022;74(5):860–70.
54. Irazoqui AP, Heim NB, Boland RL, Buitrago CG. 1α,25 dihydroxi-vitamin D3 modulates CDK4 and CDK6 expression and localization. Biochem Biophys Res Commun. 2015;459(1):137–42.
55. Lorincz B, Jury EC, Vrablik M, Ramanathan M, Uher T. The role of cholesterol metabolism in multiple sclerosis: from molecular pathophysiology to radiological and clinical disease activity. Autoimmun rev. 2022;21(6):103088.
56. McGill MM, Sabikunnahar B, Fang Q, Teuscher C, Krementsov DN. The sex-specific role of p38 MAP kinase in CNS autoimmunity is regulated by estrogen receptor alpha. J Neuroimmunol. 2020;342:577209.
57. Yap CX, Henders AK, Alvares GA, Giles C, Huynh K, Nguyen A, Wallace L, McLaren T, Yang Y, Hernandez LM, Gandal MJ, Hansell NK, Cleary D, Grove R, Hafekost C, Harun A, Holdsworth H, Jellett R, Khan F, Lawson LP, Leslie J, Levis Frenk M, Masi A, Mathew NE, Muniandy M, Nothard M, Miller JL, Nunn L, Strike LT, Cadby G, Moses EK; de Zubicaray, G. I.;, Thompson PM, McMahon KL, Wright MJ, Visscher PM, Dawson PA, Dissanayake C, Eapen V, Heussler HS, Whitehouse AJO, Meikle PJ, Wray NR, Gratten J. Interactions between the lipidome and genetic and environmental factors in autism. *Nature medicine* 2023, *29* (4), 936–949.
58. Bogie JFJ, Haidar M, Kooij G, Hendriks JJA. Fatty acid metabolism in the progression and resolution of CNS disorders. Adv Drug Deliv Rev. 2020;159:198–213.
59. Loving BA, Bruce KD. Lipid and lipoprotein metabolism in Microglia. Front Physiol. 2020;11:393.
60. McNamara NB, Munro DAD, Bestard-Cuche N, Uyeda A, Bogie JFJ, Hoffmann A, Holloway RK, Molina-Gonzalez I, Askew KE, Mitchell S, Mungall W, Dodds M, Dittmayer C, Moss J, Rose J, Szymkowiak S, Amann L, McColl BW, Prinz M, Spires-Jones TL, Stenzel W, Horsburgh K, Hendriks JJA, Pridans C, Muramatsu R, Williams A, Priller J, Miron VE. Microglia regulate central nervous system myelin growth and integrity. Nature. 2023;613(7942):120–9.
61. Ysrraelit MC, Correale J. Impact of sex hormones on immune function and multiple sclerosis development. Immunology. 2019;156(1):9–22.
62. Garcia-Ovejero D, González S, Paniagua-Torija B, Lima A, Molina-Holgado E, De Nicola AF, Labombarda F. Progesterone reduces secondary damage, preserves white matter, and improves locomotor outcome after spinal cord contusion. J Neurotrauma. 2014;31(9):857–71.
63. Guennoun R, Labombarda F, Gonzalez Deniselle MC, Liere P, De Nicola AF, Schumacher M. Progesterone and allopregnanolone in the central nervous system: response to injury and implication for neuroprotection. J Steroid Biochem Mol Biol. 2015;146:48–61.
64. Labombarda F, González S, Lima A, Roig P, Guennoun R, Schumacher M, De Nicola AF. Progesterone attenuates astro- and microgliosis and enhances oligodendrocyte differentiation following spinal cord injury. Exp Neurol. 2011;231(1):135–46.
65. Yuan S, Xiong Y, Larsson SC. An atlas on risk factors for multiple sclerosis: a mendelian randomization study. J Neurol. 2021;268(1):114–24.
66. Bhargava P, Smith MD, Mische L, Harrington E, Fitzgerald KC, Martin K, Kim S, Reyes AA, Gonzalez-Cardona J, Volsko C, Tripathi A, Singh S, Varanasi K, Lord HN, Meyers K, Taylor M, Gharagozloo M, Sotirchos ES, Nourbakhsh B, Dutta R, Mowry EM, Waubant E, Calabresi PA. Bile acid metabolism is altered in multiple sclerosis and supplementation ameliorates neuroinflammation. J Clin Investig. 2020;130(7):3467–82.
67. Jones L, Holmans PA, Hamshere ML, Harold D, Moskvina V, Ivanov D, Pocklington A, Abraham R, Hollingworth P, Sims R, Gerrish A, Pahwa JS, Jones N, Stretton A, Morgan AR, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Morgan K, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Love S, Kehoe PG, Mead S, Fox N, Rossor M, Collinge J, Maier W, Jessen F, Schürmann B, Heun R, Kölsch H, van den Bussche H, Heuser I, Peters O, Kornhuber J, Wiltfang J, Dichgans M, Frölich L, Hampel H, Hüll M, Rujescu D, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Singleton AB, Guerreiro R, Mühleisen TW, Nöthen MM, Moebus S, Jöckel KH, Klopp N, Wichmann HE, Rüther E, Carrasquillo MM, Pankratz VS, Younkin SG, Hardy J, O'Donovan MC, Owen. M. J.; Williams, J., Genetic evidence implicates the immune system and cholesterol metabolism in the aetiology of Alzheimer's disease. *PLoS one* 2010, *5* (11), e13950.
68. Lin Y-T, Seo J, Gao F, Feldman HM, Wen H-L, Penney J, Cam HP, Gjoneska E, Raja WK, Cheng J, Rueda R, Kritskiy O, Abdurrob F, Peng Z, Milo B, Yu CJ, Elmsaouri S, Dey D, Ko T, Yankner BA, Tsai L-H. APOE4 causes widespread Molecular and Cellular alterations Associated with Alzheimer's Disease Phenotypes in Human iPSC-Derived brain cell types. Neuron. 2018;98(6):1141–e11547.
69. Tcw J, Qian L, Pipalia NH, Chao MJ, Liang SA, Shi Y, Jain BR, Bertelsen SE, Kapoor M, Marcora E, Sikora E, Andrews EJ, Martini AC, Karch CM, Head E, Holtzman DM, Zhang B, Wang M, Maxfield FR, Poon WW, Goate AM. Cholesterol and matrisome pathways dysregulated in astrocytes and microglia. Cell. 2022;185(13):2213–e223325.
70. Machlovi SI, Neuner SM, Hemmer BM, Khan R, Liu Y, Huang M, Zhu JD, Castellano JM, Cai D, Marcora E, Goate AM. APOE4 confers transcriptomic and functional alterations to primary mouse microglia. Neurobiol Dis. 2022;164:105615.

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71. Pierrot-Deseilligny C, Souberbielle JC. Vitamin D and multiple sclerosis: an update. Multiple Scler Relat Disorders. 2017;14:35–45.
72. Zhang HL, Wu J. Role of vitamin D in immune responses and autoimmune diseases, with emphasis on its role in multiple sclerosis. Neurosci Bull. 2010;26(6):445–54.
73. Teymoori-Rad M, Sahraian MA, Mokhtariazad T, Nejati A, Mozdabadi RSK, Amiri MM, Shokri F, Marashi SM. Illuminating the in vitro effects of Epstein-Barr virus and vitamin D on immune response in multiple sclerosis patients. J Neurovirol. 2021;27(2):260–71.
74. Kleinewietfeld M, Hafler DA. The plasticity of human Treg and Th17 cells and its role in autoimmunity. Semin Immunol. 2013;25(4):305–12.
75. Pompura SL, Hafler DA, Dominguez-Villar M. Fatty acid metabolism and T cells in multiple sclerosis. Front Immunol. 2022;13:869197.
76. Urry Z, Chambers ES, Xystrakis E, Dimeloe S, Richards DF, Gabryšová L, Christensen J, Gupta A, Saglani S, Bush A, O'Garra A, Brown Z, Hawrylowicz CM. The role of 1α,25-dihydroxyvitamin D3 and cytokines in the promotion of distinct Foxp3 + and IL-10 + CD4 + T cells. Eur J Immunol. 2012;42(10):2697–708.
77. Joshi S, Pantalena LC, Liu XK, Gaffen SL, Liu H, Rohowsky-Kochan C, Ichiyama K, Yoshimura A, Steinman L, Christakos S, Youssef S. 1,25-dihydroxyvitamin D(3) ameliorates Th17 autoimmunity via transcriptional modulation of interleukin-17A. Mol Cell Biol. 2011;31(17):3653–69.
78. Mayne CG, Spanier JA, Relland LM, Williams CB, Hayes CE. 1,25-Dihydroxyvitamin D3 acts directly on the T lymphocyte vitamin D receptor to inhibit experimental autoimmune encephalomyelitis. Eur J Immunol. 2011;41(3):822–32.
79. Bartosik-Psujek H, Psujek M. Vitamin D as an immune modulator in multiple sclerosis. Neurol Neurochir Pol. 2019;53(2):113–22.
80. Chiuso-Minicucci F, Ishikawa LL, Mimura LA, Fraga-Silva TF, França TG, Zorzella-Pezavento SF, Marques C, Ikoma MR, Sartori A. Treatment with Vitamin D/MOG Association suppresses experimental autoimmune encephalomyelitis. PLoS ONE 2015, 10 (5), e0125836.
81. Mansilla MJ, Sellès-Moreno C, Fàbregas-Puig S, Amoedo J, Navarro-Barriuso J, Teniente-Serra A, Grau-López L, Ramo-Tello C, Martínez-Cáceres EM. Beneficial effect of tolerogenic dendritic cells pulsed with MOG autoantigen in experimental autoimmune encephalomyelitis. CNS Neurosci Ther. 2015;21(3):222–30.

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