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Revealing the Causal Relationship Between Vitamin D and Autism Spectrum Disorder Through Bidirectional Mendelian Randomization

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Abstract
Objective: *Current research indicates a potential association between autism spectrum disorder (ASD) and vitamin D (Vitamin D, VitD), though the causal relationship remains unclear. This study employed Mendelian randomization (MR) to investigate the underlying correlation between vitamin D and ASD.*
Methods: *Five MR analysis methods were applied to examine the bidirectional association between vitamin D data from the International Equivalence Unit (IEU) database and ASD GWAS data in the International Equivalence Unit (IEU) database. These included inverse variance weighting (IVW), weighted median method (WME), MR-Egger regression test, weighted number method (WM), and simple number method (SM). Statistical power was evaluated using the Power value, while sensitivity analyses were conducted through Cochran's Q test, MR-Egger regression, and leave-one-out method.*
Results: *Vitamin D levels showed no association with genetic susceptibility to autism spectrum disorder [OR = 0.978 (95% CI: 0.868-1.103), P = 0.719, no significant causal effect; F-statistic = 198.3, sufficient instrument strength]. However, ASD was identified as a causal risk factor for reduced vitamin D levels [OR = 0.976 (95% CI: 0.956-0.997), P = 0.0256, significant causal effect; ASD increased vitamin D levels by approximately 2.4%, F-statistic = 27.14, sufficient instrument strength].*
Conclusion: *Special attention should be given to children with ASD, with regular monitoring of vitamin D levels and timely interventions through supplements or lifestyle adjustments to prevent potential health issues caused by vitamin D deficiency, such as skeletal health and immune dysfunction.*

Keywords: *Mendelian randomization, autism spectrum disorder, vitamin D, Genome-wide association study.*

Abbreviation: Autism spectrum disorder (ASD), Vitamin D (Vit D), Mendelian randomization (MR), Simple number method (SM), Genome-wide association study (GWAS), single nucleotide polymorphisms (SNPs), instrumental variables (IVs)

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by social interaction difficulties, repetitive behaviors, and restricted interests. Its prevalence has been rising steadily in recent years [1].

Vitamin D (Vit D), a steroid hormone obtained through dietary intake, plays a vital role in numerous physiological processes, including cartilage formation [2], Stimulate liver regeneration [3], Stimulate insulin secretion[4], Neuroprotection, etc.

Research indicates that Vit D may be associated with ASD in cognitive function, synaptic formation, anti-inflammatory activity, and neuroprotective effects [5]. Magdalena Yvonne Kohet al. [6] indicates that 36.5% of children with ASD suffer from Vit D deficiency. Pu Tianet al. [7] has shown that Vit D deficiency in children with ASD affects brain structure and development, as well as the severity of clinical symptoms. This is consistent with the findings of Liu Jia et al. [8].

Therefore, this study employs bidirectional Mendelian randomization to investigate the causal relationship between ASD and Vit D. By identifying genetic variants associated with both, we can better understand their interaction.

Mendelian randomization (MR) is a statistical method that uses genetic variants as instrumental variables to investigate causal relationships between exposures and outcomes[9], it can reduce the influence of confounding factors and reverse causation.

Materials and Methods

Research design

The MR design for ASD and Vit D is based on three fundamental assumptions: association, independence, and exclusivity[10]. The association hypothesis requires that single nucleotide polymorphisms (SNPs) are closely linked to exposure factors. The independence hypothesis requires that SNPs are independent of confounding variables. The exclusivity hypothesis requires that SNPs influence outcomes exclusively through exposure factors, not through other pathways.
Sources of date

This study utilized publicly available genome-wide association study (GWAS) summary data from the MRC IEU Open GWAS database (https://gwas.mrcieu.ac.uk).

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The ASD identifier was ieu-a-1185. The dataset was sourced from a study conducted by the Integrated Psychiatric Research-Psychiatric Genomics Consortium (iPSYCH-PGC), where ASD cases were diagnosed using Danish ICD-8, ICD-9, and ICD-10 criteria in psychiatric hospitals or clinics. The iPSYCH-PGC study included 46,351 ASD patients (18,382 cases and 27,969 controls). Vit D data were obtained from the EBI database (identifier ebi-a-GCST90000618), encompassing 496,946 individuals from the UK Biobank and other major biobanks. The analysis underwent rigorous quality control filtering, population stratification adjustments, and seasonal variation control in Vit D measurements. All ASD patients and control subjects were of European ancestry to minimize population heterogeneity.

Statistical treatment

Instrument Variable Selection: We selected SNPs significantly associated with exposure factors from GWAS exposure studies as instrumental variables, with a significance threshold of P <5×10^-8. To ensure instrument independence, we performed SNP clustering (cluster parameters: r^2 <0.001, clump distance> 10,000 kb). If the number of valid instruments was insufficient, we relaxed the significance threshold to P <1×10^-6. Bidirectional Mendelian Randomization Analysis: We conducted two-way MR analyses: (1) Forward MR: evaluating the causal effect of Vit D on ASD risk; (2) Reverse MR: assessing ASD's causal effect on Vit D. For each analysis, we primarily used IVW as the main method, supplemented by MR-Egger regression, WME, WM and SM.

Sensitivity Analysis: To evaluate the robustness of MR analysis results, we conducted the following sensitivity analyses: **Heterogeneity Test:** We used the Cochran's Q statistic to assess heterogeneity among instrumental variables. A P-value <0.05 indicates heterogeneity.

Horizontal Multifunctionality Test: The MR-Egger intercept test was employed to evaluate horizontal multifunctionality. A P-value <0.05 suggests significant horizontal multifunctionality.

Leave-One Analysis: By sequentially removing each SNP and repeating the MR analysis, we examined whether individual SNPs exerted excessive influence on the results.

Instrumental variable strength assessment: The F-statistic is calculated to evaluate the strength of the instrumental variable. A value of F> 10 indicates sufficient strength to effectively avoid weak instrument bias.

Results were presented as odds ratios (OR) with 95% confidence intervals (CI). Statistical analysis was performed using R software (version 4.1.0) and the Two Sample MR package (version 0.5.6). A P <0.05 was considered statistically significant.

Results

We conducted forward Mendelian randomization with Vit D as the exposure and ASD as the outcome, as shown in Table 1.

Table 1: IVs screening statistics

exposure	outcome	nsnp	f_statistic	beta	Se	pval	or	or_lci95	or_uci95
Vit D	ASD	115	198.3	-0.022	0.061	0.719	0.978	0.868	1.102
ASD	Vit D	13	27.14	-0.024	0.011	0.026	0.976	0.956	0.997

A total of 115 SNPs was identified with F=198.3, demonstrating sufficient instrument strength. Causal effects were evaluated using multiple methods including IVW, MR Egger regression, WME, WM and SM. Results from Figures 1 and Table 2 indicate that the causal estimates from all five methods showed inconsistent directions and lacked statistical significance (P>0.05).

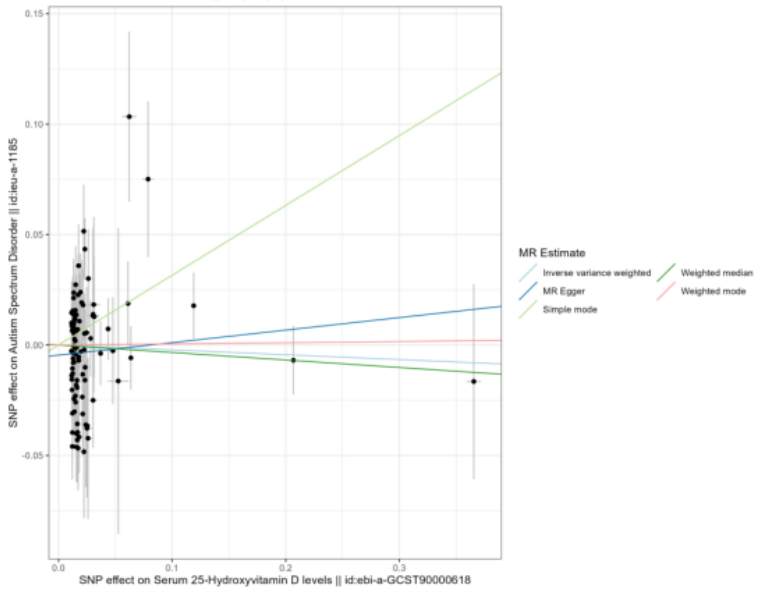

Figure 1: Scatter Plot of Causal Effect of Vitamin D on ASD.

Table 2: Five MR analysis methods of Causal Effect of Vitamin D on ASD.

method	nsnp	b	se	pval
IVW	100	-0.022	0.061	0.719
MR Egger	100	0.057	0.077	0.463
WME	100	-0.034	0.068	0.620
WM	100	0.005	0.052	0.919
SM	100	0.316	0.220	0.154

Sensitivity analysis (Table 3) revealed significant heterogeneity (Q_pval <0.05), while Egger's intercept test showed no evidence of horizontal pleiotropy (Egger_pval = 0.101). Comprehensive analysis of multiple methods and sensitivity results suggests no definitive causal link between Vit D and ASD. As illustrated in Figure 2, the leave-one-out analysis demonstrated fluctuating effect estimates near zero, supporting the conclusion of no causal effect.

Table 3: Sensibility analysis for Causal Effect of Vitamin D on ASD.

exposure	outcome	Q_pval	Egger_pval
Vit D	ASD	1.437e-05	0.101

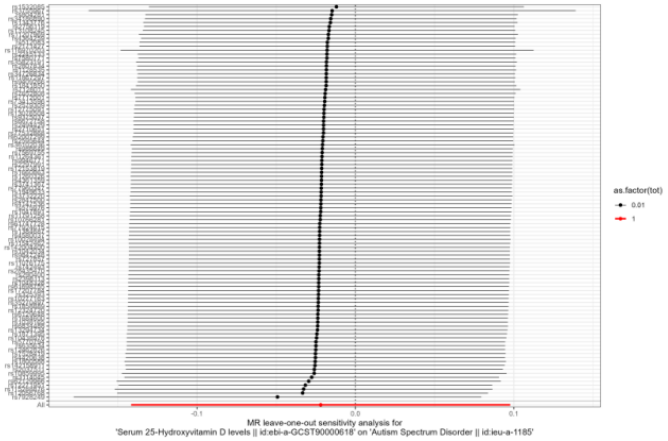

Figure 2: Leave-One-Out Sensitivity Analysis for Causal Effect of Vitamin D on ASD.

We conducted reverse Mendelian randomization using ASD as an exposure and Vit D as an outcome, as shown in Table 1. A total of 13 SNPs was identified with F=27.14, indicating sufficient strength of instrumental variables. Multiple methods including IVW, MR Egger regression, WME, WM and SM were employed to evaluate causal effects. Results in Table 4 demonstrate that the causal relationship between ASD and Vit D levels calculated by inverse variance weighting was statistically significant [OR = 0.976 (95% CI: 0.956-0.997), P=0.0256]. ASD increased Vit D levels by approximately 2.4%, while the other four methods showed inconsistent and non-significant causal estimates (P>0.05).

Table 4: Five MR analysis methods of Causal Effect of ASD on Vitamin D.

method	nsnp	b	se	pval
IVW	12	-0.024	0.011	0.026
MR Egger	12	-0.037	0.044	0.425
WME	12	-0.021	0.012	0.095
WM	12	-0.024	0.020	0.242
SM	12	-0.025	0.022	0.265

Sensitivity analysis in Table 5 indicated no significant heterogeneity (Q_pval> 0.05), and the Egger intercept test revealed no substantial horizontal pleiotropy (Egger_pval = 0.773).

Table 5: Sensibility analysis for Causal Effect of ASD on Vitamin D.

exposure	outcome	Q_pval	Egger_pval
ASD	Vit D	0.074	0.773

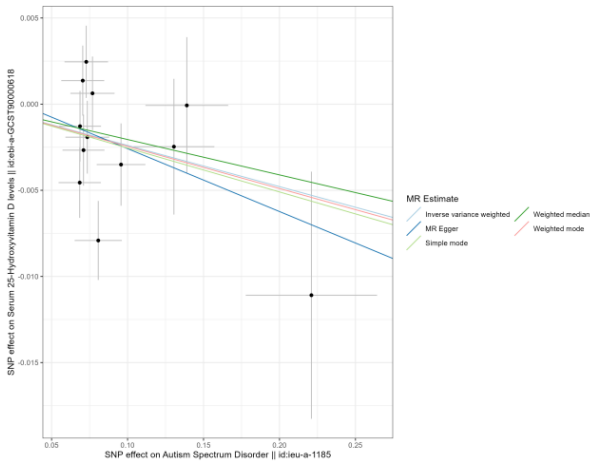

Figure 3: Scatter Plot of Causal Effect of ASD on Vitamin D.

Our conclusions remain consistent with the primary analysis method (IVW), confirming ASD's causal influence on Vit D levels and the association between ASD elevation and decreased Vit D. As shown in Figure 4, leave-one-out analysis revealed no single SNP driving the results, indicating robust causal effect estimation in reverse MR and consistent negative associations between ASD and Vit D levels.

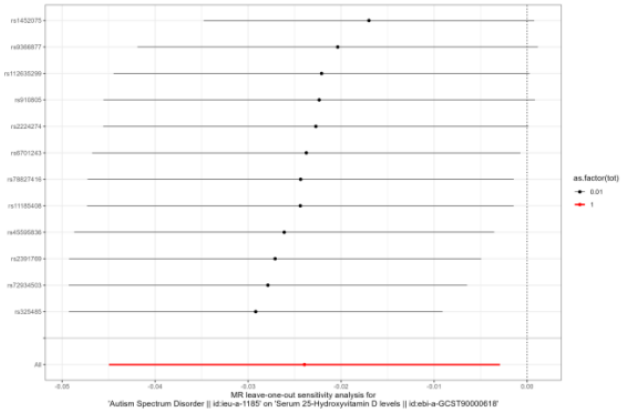

Figure 4: Leave-One-Out Sensitivity Analysis for Causal Effect of ASD on Vitamin D.

Discussion

Multiple meta-analyses have demonstrated a significant correlation between elevated maternal 25(OH)D levels and reduced incidence of autism-related traits in off spring.W L Zhaoet al. [11] It is found that sufficient Vit D intake during pregnancy can reduce the risk of ASD, which may be related to the neuroprotective effect of Vit D. Bing Wanget al. [12] By establishing a valproate-induced ASD rat model, we demonstrated that 1,25(OH)2D3 enhances Tph1/2 mRNA and 5-HT levels through increased VitD receptor activity, thereby alleviating ASD symptoms in male rats. However, this study found no causal relationship between Vit D levels and ASD genetic risk. Future research should aim to expand the sample size for more accurate conclusions.

Reversed MR analysis indicates that ASD may reduce serum Vit D levels. The exact mechanism remains unclear, though current research suggests it involves complex interactions. Dietary patterns may play a role, as individuals with ASD often exhibit restricted food preferences and heightened sensory sensitivity, which increases risks of nutritional deficiencies—including Vit D deficiency and subsequent hypocalcemia [13] on the other hand ,Ling Shanet al. [14]. The study found that Vit D levels in children with ASD showed a negative correlation with age and screen time, while positively correlated with daylight exposure. in addition, The association between ASD and Vit D levels may also involve inflammation [15, 16], immune [17], and metabolic factors, but more data are needed for further investigation.

Conclusion

Research indicates that children with ASD exhibit reduced serum Vit D levels, which may be linked to dietary patterns, gastrointestinal dysfunction, limited outdoor activity, or the complex pathophysiological mechanisms of ASD itself. These children require special attention, with regular monitoring of Vit D levels and supplementation to improve their status.

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