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Null association between serum 25-hydroxyvitamin D levels with allergic rhinitis, allergic sensitization and non-allergic rhinitis: A Mendelian randomization study

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Abstract

Background: Previous observational studies have not found a conclusive association between serum 25-hydroxyvitamin D (25(OH)D) levels and allergic rhinitis (AR) or allergic sensitization (AS).

Objective: To investigate a causal association between 25(OH)D levels with risk of AR and AS, using a two-sample Mendelian randomization (MR) approach.

Methods: Seven single nucleotide polymorphisms (SNPs), previously shown to be associated with serum 25(OH)D levels, were identified as instrumental variables. The primary outcome was AR, and the secondary outcomes were AS and non-allergic rhinitis (NAR). The genome-wide association (GWA) summary statistics of the outcomes were obtained from two cohort studies (EAGLE Consortium and UK Biobank). An MR analysis with random-effects inverse-variance weighted method was performed as the primary analysis to estimate overall effect size (odds ratio [OR] and 95% confidence interval [CI]). Sensitivity analysis using weighted median method and MR-Egger regression method was conducted. A subgroup analysis based on 25(OH)D synthesis-related SNPs was further applied.

Results: Serum 25(OH)D levels were not causally associated with risk of AR (OR: 0.960; 95% CI: 0.779-1.184), AS (OR: 1.059; 95% CI: 0.686 to 1.634) or NAR (OR: 0.937; 95% CI: 0.588-1.491). Subgroup analysis also showed null association between 25(OH)D synthesis-related SNPs and the outcomes. Sensitivity analyses yielded similar results.

Conclusions and Clinical Relevance: This MR study found no evidence supporting a causal association between serum 25(OH)D levels and risk of AR, AS and NAR in European-ancestry population. This argues against the previous postulation that vitamin D supplementation is effective in prevention of allergic diseases.

KEYWORDS

25-hydroxyvitamin D, allergic rhinitis, allergic sensitization, causation, Mendelian randomization, vitamin D

1 | INTRODUCTION

Allergic rhinitis (AR) and allergic sensitization (AS) are the most common allergic disorders and highly prevalent world-wide.¹⁻³ Serum 25-hydroxyvitamin D (25(OH)D) has been shown to have a role in immunomodulation and has been suggested to have an effect on the development of AR and AS.^{4,5} The underlying mechanisms are thought to involve paracrine feedback loops that reduce inflammation, differentiation of activated CD4+ T cells and enhancement of suppressor regulatory T-cell count and functions.⁶ Previous studies have examined a vitamin D-based strategy for AR and AS prevention, assuming the presence of causality between vitamin D deficiency with allergic disorders.⁶⁻¹¹ If a true causality exists, given the high prevalence of vitamin D deficiency world-wide,¹² vitamin D supplementation would be a promising strategy for AR and AS prevention. However, this causality has not been fully confirmed.

An 11-year follow-up study in a European population found that serum 25(OH)D levels were negatively associated with risk of AR, and each 25 ng/mL reduction in serum 25(OH)D concentration was associated with 84% higher risk.¹³ Similar findings have also been shown in other populations.^{14,15} However, a Japanese study found no such association.¹⁶ Systematic reviews and meta-analyses found that lower 25(OH)D level was related to higher AR and AS prevalence, but not with incidence, and that the effect might be modified by gender and age.¹⁷⁻¹⁹ However, this association is suspected to be distorted by confounding factors, such as geographic latitude and dietary habits.⁵

The effect of vitamin D supplements on prevention of AR also remains inconsistent. Previous systematic reviews have found that vitamin D supplements during pregnancy,^{8,18} breastfeeding,⁸ infancy,^{8,18} childhood or adolescence²⁰ did not significantly reduce AR risk, but the majority of eligible studies were observational. It has been suggested that sufficient serum vitamin D levels or vitamin D supplement in AR patients with nutrient deficiency could enhance the efficacy of allergy treatment, such as subcutaneous immunotherapy²¹ and antihistamines,²² but these studies had small sample size and high risk of bias.

Observational studies are likely to suffer from reverse causality and residual confounding. Although there are a few randomized trials on vitamin D supplementation and AR prevention, they are flawed because of small sample size and use of surrogate outcomes.^{23,24} Mendelian randomization (MR) has the potential to overcome the limitations of conventional observational studies and small randomized trials.²⁵ It uses genetic variants (usually single nucleotide polymorphisms [SNPs]) as instrumental variables for an exposure to estimate the causal effect of the exposure on outcomes. It ascertains causation based on the theory that if genetic variants produce phenotypic difference that alters disease risk, they should be associated with disease risk to the extent predicted by their influence on the exposure.²⁶ MR is conceptually similar to randomized controlled trial in the way that genes are randomly assorted at conception prior to the occurrence of any behavioural or environmental confounders.²⁵ The principles, methodological details and limitations of MR analysis have been discussed previously.^{27,28}

Therefore, we conducted a two-sample MR study to investigate the causality between serum 25(OH)D with AR. Secondly, we performed MR analyses of (a) the mechanistic substrate of allergy (AS), which might be more powerful if vitamin D specifically had effect on sensitization, and (b) non-allergic rhinitis (NAR) in order to address any potential effect on non-allergic epithelial mechanisms.

2 | METHODS

This two-sample MR study used summary-level data from published genome-wide association (GWA) studies on 25(OH)D,^{29,30} AR,^{31,32} AS³² and NAR,³² which have obtained appropriate patient consents and ethical approval.

2.1 | SNPs selection

Seven SNPs for serum 25(OH)D levels were selected based on genome-wide significant association ($P < 5 \times 10^{-8}$) from two large GWA studies.^{29,30} These SNPs have also been used in other published MR studies.³³⁻³⁵ Six SNPs (rs8018720, rs3755967, rs17216707, rs12785878, rs10745742 and rs10741657) were extracted from a GWA study of 79 366 unrelated European-ancestry individuals recruited from 31 cohorts in Europe, Canada and the United States,²⁹ while the other SNP (rs117913124) was extracted from a GWA study of 41 336 unrelated European-ancestry individuals.³⁰ Both GWA studies were adjusted for age, sex, body mass index, dietary vitamin D intake, month or season of measurement and principal components of ancestry. Rs117913124 was not directly available in an outcome GWA study, so we selected rs116970203 as its proxy ($r^2 = 1.00$). The effect of this proxy was measured in the GWA study of 41,138 individuals, adjusted for the same covariates.³⁰ The SNP rs8018720 was not available in the UK Biobank GWA study, and no suitable proxy was identified. The seven SNPs were not in linkage disequilibrium. The seven SNPs explained 5.3% of the variance in serum 25(OH)D levels.³³ Three SNPs (rs12785878, rs116970203 and rs10745742) were located within or near CYP2R1 and NADSYN1/DHCR7 genes, modulating 25(OH)D synthesis, while the other four SNPs (rs8018720, rs3755967, rs17216707 and rs10745742) were located within or near genes modulating its transport (SEC23A and GC) and catabolism (CYP24A1 and AMDHD1).

2.2 | Outcome data

The primary outcome was AR, while the secondary outcomes were AS and NAR. Summary-level data for the association of the 25(OH)D-influencing SNPs with AR risk were extracted from two independent GWA studies: the Early Genetics and Lifecourse Epidemiology (EAGLE) Rhinitis Consortium (excluding 23andMe)³² and UK Biobank.³¹

The EAGLE GWA study³² included 38 838 unrelated European-ancestry individuals (about 10 941 AR cases and 27 897 controls) recruited from 17 cohorts and examined 16 531 985 genetic variants. AR cases were defined as individuals who had ever received a diagnosis or experienced AR symptoms. The association analysis was adjusted for age, sex and ancestry principal components.

The UK Biobank GWA study³¹ included 258 688 unrelated European-ancestry individuals (18 915 AR cases and 239 773 controls) and examined 15 688 218 genetic variants. AR cases were defined as individuals who had physician-diagnosed AR via interview. The association was adjusted for deprivation index, sex, age, smoking and ancestry principal components and genotype batch.

For AS and NAR, we used the summary-level data from the EAGLE GWA study only,³² because they have not been examined in UK Biobank GWA study. AS cases were defined as individuals with measured high levels of specific immunoglobulin E or positive skin prick test, and controls were defined as those who were not sensitized against any tested allergens. NAR cases were defined as individuals with current AR symptoms but without AS, while controls were defined as individuals who had never experienced AR symptoms and had no AS. The GWA analysis of AS and NAR was conducted in 24 481 (8040 cases and 16 441 controls) and 11 634 (2028 cases and 9606 controls) unrelated European individuals, respectively, and were adjusted for age, sex and ancestry principal components.

2.3 | Data analysis

All summary-level data were harmonized according to the methods recommended by Hartwig et al,³⁶ to ensure that the effects of each SNP on exposure and outcomes were shown for the same effect allele. Power calculation for MR analysis was based on the method of Brion et al³⁷

We first conducted a meta-analysis combining the effects of 25(OH)D-influencing SNPs on AR from the EAGLE Consortium and the UK Biobank, using random-effects inverse-variance weighted method. We did not choose a fixed-effect model for primary analysis, because the purpose of this meta-analysis was not to discover novel significant genetic variants, but to estimate average effects with higher statistical power.³⁸ However, meta-analysis using fixed-effect model was conducted as sensitivity analysis. The pooled results were used in MR analysis.

In MR analysis, the random-effects inverse-variance weighted method was used in the primary analysis. The weighted median method and the MR-Egger regression method were performed as sensitivity analysis. A subgroup analysis was also conducted using only synthesis-related SNPs (rs12785878, rs116970203, rs10745742), to investigate the association between 25(OH)D synthesis and risk of the outcomes. A statistical test for the intercept in MR-Egger method was used to examine potential pleiotropy, with a *P* value <.05 suggesting the presence of pleiotropy.³⁹ Pleiotropy occurs when SNPs affect multiple phenotypes via independent

biological pathways, which may violate the underlying assumptions of MR analysis and introduce bias.²⁵ The PhenoScanner v2 database (<http://www.phenoscanner.medschl.cam.ac.uk/>) was also searched to investigate whether the selected SNPs are associated with other related traits at a significance level of 5×10^{-8} .

For the outcome of AR, we additionally performed sensitivity analyses by using different outcome data sources in the MR analysis, which were EAGLE GWA study only, UK Biobank GWA study only and meta-analysis of the two GWA studies using a fixed-effect model. We also performed sensitivity analysis by changing the order of data analysis, that is, conducting MR analysis in EAGLE consortium and UK Biobank separately first and combining the effect estimates using meta-analysis later. More details on data analysis are shown in File S1.

The effect measure was odds ratio (OR) and its 95% confidence interval (CI), which should be interpreted as relative change of the outcome risk for each standard deviation increment in serum 25(OH)D level. The statistical analysis was performed with *meta* package (version 4.9-9) and *TwoSampleMR* package (version 0.4.25)⁴⁰ in R environment.⁴¹

3 | RESULTS

The characteristics of the seven SNPs and their effects on serum 25(OH)D are shown in Table 1. With a total sample size of 297 526 for AR, we had 80% and more statistical power to detect an OR <0.923 or >1.075, given alpha of 0.05, case proportion of 10% and proportion of explained exposure variation of 5.3%. For AS, with a sample size of 24 482 and case proportion of 33%, we had at least 80% power to identify an OR <0.843 or >1.175. For NAR, with a sample size of 11 634 and a case proportion of 17%, we were able to detect an OR <0.715 or >1.315 with sufficient power (Figure S1).

Meta-analysis was performed for six matched SNPs (rs12785878 was not available in EAGLE), as shown in Table 2. The SNP rs10745742 and rs12785878 seemed to have the largest effect on AR risk, with beta (standard error) of -0.0215 (0.0094) and 0.0213 (0.0202), respectively. Results were consistent in the meta-analysis with fixed-effect model and random-effects model.

Overall, there was no evidence for a causal association between serum 25(OH)D levels and AR risk regardless of the MR method employed. The OR (95% CI) in inverse-variance weighted method, weighted median method and MR-Egger regression method were 0.960 (0.779-1.184), 0.979 (0.803-1.194) and 1.038 (0.823-1.308), respectively (Figure 1A). Similar results of null association were also observed when using outcome data source from EAGLE GWA study (OR: 0.962 [0.647-1.432]), UK Biobank GWA study (OR: 1.020 [0.827-1.258]) and meta-analysis with fixed-effect model (OR: 1.005 [0.861-1.175]) (Table 3). When limiting instrument variables to the three synthesis-related SNPs, there was still no effect of 25(OH)D on AR (OR: 0.966 [0.789-1.183]).

The intercept test of MR-Egger regression method showed no evidence of pleiotropy (*P* = .32). A manual search in PhenoScanner

TABLE 1 The SNP characteristics and their associations with serum 25(OH)D levels

SNP	Chromosome:Position ^b	Nearby gene	Effect allele	Other alleles	EAF	Function	Beta	SE	Sample size	P value
rs8018720	14:39086981	SEC23A	G	C	0.18	Transport	0.017	0.003	79 366	1.11×10^{-11}
rs3755967	4:71743681	GC	C	T	0.72	Transport	0.089	0.002	79 366	4.74×10^{-343}
rs17216707	20:54115823	CYP24A1	T	C	0.79	Catabolism	0.026	0.003	79 366	8.14×10^{-23}
rs12785878	11:71456403	NADSYN1/DHCR7	T	G	0.75	Synthesis	0.036	0.002	79 366	3.80×10^{-62}
rs116970203 ^a	11:14855172	CYP2R1	G	A	0.98	Synthesis	0.430	0.020	42 274	2.20×10^{-90}
rs10745742	12:95964751	AMDHD1	T	C	0.40	Catabolism	0.017	0.002	79 366	2.10×10^{-20}
rs10741657	11:14893332	CYP2R1	A	G	0.40	Synthesis	0.031	0.002	79 366	2.05×10^{-46}

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; EAF, effect allele frequency; SNP, single nucleotide polymorphism.

^aProxy for rs117913124, $r^2 = 1.00$.^bChromosome:position is based on GRCh38.

database (Table S1) revealed that rs3755967 was associated with blood white cell count, neutrophil count and eosinophil count, which may be related to immune response and AR risk; removing rs3755967 from MR analysis yielded an OR of 0.961 (0.804-1.148), which was in line with the primary analysis. Sensitivity analysis by changing the order of data analysis also showed non-significant associations. (Table S2).

Null association was also observed between 25(OH)D and AS (OR: 1.059 [0.686-1.634]) or NAR (0.937 [0.588-1.491]; Table 3), which remained consistent when using other MR methods (Figure 1B,C). Using only synthesis-related SNPs showed null association for AS (OR: 1.075 [0.777-1.487]) and NAR (0.866 [0.500-1.500]). The intercept test of MR-Egger regression method revealed no evidence for pleiotropy ($P = .13$ and $.55$ for AS and NAR, respectively).

4 | DISCUSSION

This MR study included a large sample of nearly 300 000 European descents and revealed no evidence supporting a causal link between serum 25(OH)D levels and AR risk, which remained consistent in sensitivity analyses. This suggests that there is little potential for vitamin D supplementation in prevention of AR. This study also showed null effects of 25(OH)D on AS and NAR.

Use of SNPs as instrumental variable in MR relies on three assumptions⁴²: (1) the SNPs are strongly associated with exposure, (2) the SNPs affect the outcome only through their effect on the exposure, and (3) the SNPs are independent of confounders of the association between the exposure and the outcome. For assumption 1, the seven SNPs were significantly associated with serum 25(OH)D levels as demonstrated in GWA studies,^{29,30} which explain 5.3% of the total variance in serum 25(OH)D and form a strong composite instrumental variable with an F statistic of 634.⁴³ For assumption 2, statistical tests for pleiotropy³⁹ yielded P values of 0.32, 0.13 and 0.55 for AR, AS and NAR, suggesting no pleiotropy. This was further supported by the results of sensitivity analyses removing rs3755967, which might be associated with immune response and allergic disorders according to the PhenoScanner database. For assumption 3, we found obesity was associated with vitamin D deficiency (presumably due to vitamin D being fat-soluble resulting in lower circulating levels)¹² and AR⁴⁴ simultaneously, which could be a potential confounder. However, we have not found evidence that any of the seven SNPs are significantly associated with obesity-related traits according to the PhenoScanner database; body mass index has already been adjusted for in the GWA studies of 25(OH)D.^{29,30} Therefore, the three assumptions are plausible in this study, which enhances the reliability of our results.

The reliability of MR results also depends on other conditions, such as no population stratification, no segregation distortion and no parent-of-origin effect.^{26,45} The GWA studies included only European-ancestry individuals and have adjusted for principal ancestry components to control for potential population stratification effect. For the seven SNPs included in this study, neither segregation

TABLE 2 The effects of the seven 25(OH)D-related SNPs on allergic rhinitis

SNP	GWA study	Sample size	Beta	SE	P value	I ²
rs10741657	EAGLE	38 838	-0.0306	0.0175	.0804	
	UK Biobank	258 688	0.0218	0.0109	.0453	
	Meta-analysis ^a	297 526	-0.0026	0.0261	.92	0.8449
rs10745742	EAGLE	38 838	-0.0323	0.0176	.0669	
	UK Biobank	258 688	-0.0171	0.0111	.1242	
	Meta-analysis ^a	297 526	-0.0215	0.0094	.0225	0.0000
rs116970203	EAGLE	38 838	-0.086	0.0616	.1632	
	UK Biobank	258 688	0.0094	0.0329	.7743	
	Meta-analysis ^a	297 526	-0.024	0.0455	.5977	0.4633
rs12785878	EAGLE	38 838	0.0213	0.0202	.2921	
	UK Biobank	NA	NA	NA	NA	
	Meta-analysis ^a	38 838	0.0213	0.0202	.2921	NA
rs17216707	EAGLE	38 838	-0.0361	0.0221	.1027	
	UK Biobank	258 688	0.0009	0.0142	.9515	
	Meta-analysis ^a	297 526	-0.0137	0.0181	.4489	0.4963
rs3755967	EAGLE	38 838	0.0459	0.0196	.0192	
	UK Biobank	258 688	0.0014	0.0118	.9067	
	Meta-analysis ^a	297 526	0.0209	0.0221	.3439	0.7358
rs8018720	EAGLE	38 838	0.0315	0.0232	.1746	
	UK Biobank	258 688	-0.0391	0.0142	.0059	
	Meta-analysis ^a	297 526	-0.0062	0.0352	.8611	0.8513

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; GWA, genome-wide association; SE, standard error; SNP, single nucleotide polymorphism.

^aMeta-analysis with random-effects model as the primary analysis.

distortion effect nor parent-of-origin effect has been previously reported; even if there were any, their influences should be minor on MR results, as suggested by Davey Smith G and Ebrahim S.⁴⁶

The evidence from epidemiological studies is inconclusive regarding vitamin D levels and AR risk. Cross-sectional studies have observed that AR patients had lower vitamin D level,¹⁵ that vitamin D deficiency was associated with higher probability of AR⁷ and that the severity of nasal symptoms was negatively associated with serum vitamin D levels in AR patients.⁴⁷ Findings from cohort studies were controversial. Hennessy et al⁴⁸ found no association between antenatal vitamin D levels measured in maternal serum and umbilical cord blood with the offspring's AR risk at age of 2 and 5 years. Chawes et al⁴⁹ revealed similarly null effect between cord blood 25(OH)D and childhood risk of AR and AS until age 7 years. A US birth cohort (n = 219)⁵⁰ observed a U-shaped dose-response relationship between cord vitamin D level with childhood AS, but not with AR. Mai et al¹³ observed an effect modification by gender in a Norwegian population, where an association was only found in males but not in females.

Similar to the limited observational evidence of the association between vitamin D and AR risk, quantitative and qualitative reviews^{8,18-20,51} of observational studies on the association between vitamin D supplementation and AR treatment generally concluded that evidence was limited and of low quality, which may provide

hints for not supporting the effect of vitamin D on AR risk. For example, Joudi et al²¹ found that the vitamin D level was a predictive factor for efficacy of subcutaneous allergen immunotherapy, but the study included only 55 AR patients. Agarwal et al⁷ found that vitamin D supplementation relieved nasal symptoms, but the study had a small sample size (n = 80). More importantly, residual confounding (such as sunlight exposure, diet and latitude) should always be borne in mind when interpreting observational results.

Randomized controlled trials also generated limited evidence on disease causation. Goldring et al²³ found vitamin D supplementation in late pregnancy did not decrease childhood AR risk in a small sample of 180 pregnant women. Chawes et al found that prenatal vitamin D supplementation did not decrease the risk of AS before the age of 3 years⁵² nor AS or AR at the age of 6 years.⁵³ Grant et al²⁴ found that vitamin D supplementation during both pregnancy and infancy reduced incidence of aeroallergen sensitization and primary care visits due to asthma at 18 months, but not AR; this trial had a high attrition rate of 29%, which may introduce bias.

In vivo and in vitro studies have found that vitamin D has multiple effects in the immune system, affecting a wide range of immune cells and cytokines. A number of reviews^{4,6,54,55} have summarized potential physiological mechanisms linking vitamin D with AR and found conflicting effects. On the one hand, vitamin

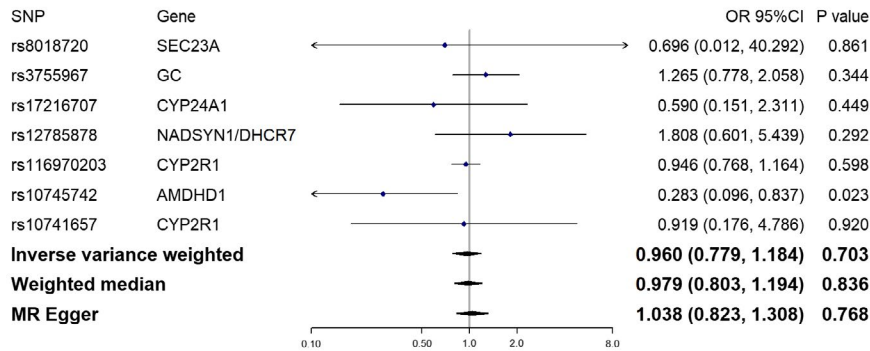
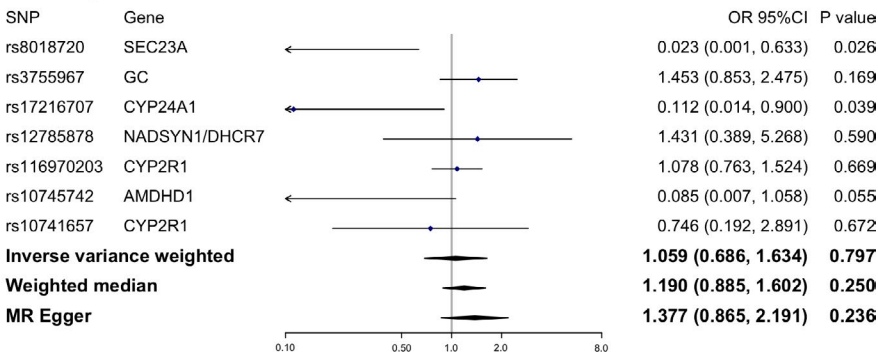
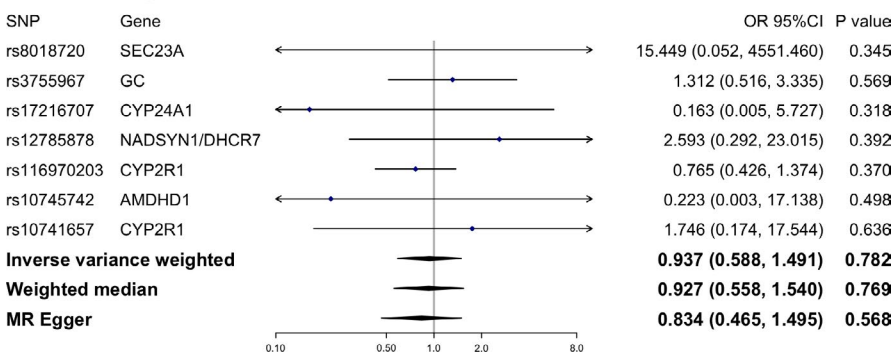
(A) Allergic rhinitis.**(B) Allergic sensitization.****(C) Non-allergic rhinitis**

FIGURE 1 The forest plots showing the association between 25(OH)D and allergic rhinitis, allergic sensitization and non-allergic rhinitis in Mendelian randomization analysis. A, Allergic rhinitis. B, Allergic sensitization. C, Non-allergic rhinitis. 25(OH)D, 25-hydroxyvitamin D; OR, odds ratio; SNP, single nucleotide polymorphism. Using meta-analysis with random-effects model as outcome data source

D decreases AR-related inflammation and elicits an anti-AR effect by inhibiting the proliferation of T cells, promoting development of regulatory T cells and suppressing the differentiation, maintenance, bioactivity and transcription of Th17 cells.^{4,6,54} On the other hand, vitamin D has a pro-AR effect and induces a switch from Th1 to Th2 by decreasing Th1 cell response (less cytokine secretion) via regulation of antigen-presenting cells and enhancing Th2 cell development and response.^{4,6,54} Simultaneously, vitamin D inhibits the immunostimulatory capacity of differentiated dendritic cells, macrophage inflammation and T-cell stimulation.^{6,54,55} Therefore, the overall effect is controlled by the immunoregulation of the two directions (anti-AR vs pro-AR) and determined by their relative strength. Additionally, vitamin D regulates genes with vitamin D response elements in promoter regions, which is widely distributed in various tissues, and this further complicates the overall physiological effect of vitamin D.⁵⁶ It is likely that the overall effect converges to null, or very minor, because the OR

point estimates in both primary analysis and sensitivity analyses of our MR study were found to be close to 1. Nevertheless, our study alone did not provide definitive evidence to the absence of a causal association. Alternatively, there is possibility that the effect is non-linear. Nevertheless, previous studies have shown controversial findings regarding this, with evidence of both U-shaped⁵⁷ and inverted U-shaped⁵⁸ associations.

MR studies have demonstrated that serum 25(OH)D level was not associated with asthma, eczema and allergic sensitization.⁵⁹⁻⁶¹ Incorporating all the evidence together, we may conclude that the overall effect of vitamin D on allergy-related immunomodulation is null or very minor, and this probably further undermines the postulated potential effect of vitamin D supplementation on prevention of allergic diseases. This finding is consistent with recent randomized trials.^{52,53,62} For example, Litonjua et al found that antenatal vitamin D supplementation insignificantly reduced Immunoglobulin E levels, but did not reduce the incidence of

TABLE 3 Results of Mendelian randomization analysis and sensitivity analyses by changing Mendelian randomization method and outcome data source

Outcome data source	Number of SNPs	Sample size	Inverse-variance weighted		Weighted median		MR-Egger	
			OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Allergic rhinitis								
Meta-analysis ^a	7	297 526	0.960 (0.779, 1.184)	.703	0.979 (0.803, 1.194)	.836	1.038 (0.823, 1.308)	.768
EAGLE	7	38 838	0.962 (0.647, 1.432)	.850	0.996 (0.755, 1.315)	.977	1.000 (0.580, 1.725)	.999
UK Biobank	6	258 688	1.020 (0.827, 1.258)	.854	1.021 (0.897, 1.162)	.756	1.068 (0.812, 1.405)	.662
Meta-analysis ^b	7	297 526	1.005 (0.861, 1.175)	.945	1.022 (0.906, 1.152)	.726	1.052 (0.864, 1.281)	.636
Allergic sensitization	7	24 481	1.059 (0.686, 1.634)	.797	1.190 (0.885, 1.602)	.250	1.377 (0.865, 2.191)	.236
Non-allergic rhinitis	7	11 634	0.937 (0.588, 1.491)	.782	0.927 (0.558, 1.540)	.769	0.834 (0.465, 1.495)	.568

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; SNP, single nucleotide polymorphism.

^aMeta-analysis with random-effects model, as primary analysis

^bMeta-analysis with fixed-effect model, as sensitivity analysis.

asthma at both 3- and 6-year follow-up.^{62,63} This MR study is the first focusing on AR to our knowledge. With the availability of genomic data on large well-characterized populations, it is now possible to evaluate the plausibility of hypotheses before embarking on large randomized clinical trials. Our study findings call for urgent wider dissemination, because new randomized trials testing vitamin D for prevention of allergic disorders are still being designed and conducted.^{64,65} For the sake of evidence-based research⁶⁶ and reducing research waste,⁶⁷ future clinical trials on reducing AR using vitamin D supplementation should be scrutinized carefully for their usefulness.

This study has limitations. First, there was heterogeneity between the EAGLE Consortium and the UK Biobank, such as in the definition of AR and the population characteristics. AR was self-reported physician diagnosis in UK Biobank, while EAGLE additionally included self-reported symptomatic experience, which might yield a higher incidence and prevalence. This introduces classification bias and may cause underestimation. UK Biobank focuses on outcomes during adulthood, while EAGLE Consortium mainly focuses on early life and childhood outcomes. It is likely that the effect of vitamin D on allergic outcomes may differ in children and adults. However, the results remained stable when using different outcome data sources, so the effect of heterogeneity between EAGLE Consortium and UK Biobank should be minor. Second, the selected SNPs explained only 5.3% of phenotypic variation, which in itself may seem small but these relatively small contributions are actually common in most MR studies. The SNPs selected formed a strong composite instrumental variable, and our analysis had high statistical power (see Figure S1). Third, the statistical power in our study for AS and NAR was lower compared to that for AR, but the results were consistent with a previous MR study.⁵⁹ Fourth, our MR analysis assumes a linear association, thus not effective in evaluating non-linear causal effect. Fifth, all participants in this MR study were of European ancestry,

and extra caution is required when interpreting and generalizing our conclusions to other ethnicities, for which further research is warranted. In particular, we included rs3755957 near the GC gene, which shows large racial differences in European and African Americans.⁶⁸ Sixth, we only studied the effect of serum 25(OH)D level, but not its biologically active metabolite 1,25-dihydroxyvitamin D (1,25(OH)₂D). However, 1,25(OH)₂D is difficult to measure, given its very short half-life (7 hours, compared to 25 days of 25(OH)D), lack of a standard method for measurement and poor concordance across methods.⁶⁹ More importantly, 1,25(OH)₂D is insensitive to vitamin D status and is often within normal range even in people with known vitamin D deficiency. So far, 25(OH)D is still the best reflection of overall vitamin D status.⁷⁰ Seventh, we did not examine this association in sex-specific subgroups, as was suggested by previous observational studies.¹³ Therefore, we cannot exclude the possibility that vitamin D may affect men and women differently.

5 | CONCLUSION

This MR study found no evidence of a causal relationship between serum vitamin D level and the risk of AR, AS and NAR in European-ancestry populations, suggesting that previously reported association in observational studies may be subject to confounding. Our findings argue against the previous postulation that vitamin D supplementation may be effective in prevention of allergic diseases.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

QF and BMYC conceived the research idea; KB and WEE provided data; QF conducted data analysis; SY and GHYL provided methodological guidance; QF, KB, WEE and BLC interpreted results; QF drafted the manuscript; and all authors critically reviewed the manuscript.

DATA AVAILABILITY STATEMENT

Data and analysis code are available upon request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Feng Q, Bønnelykke K, Ek WE, et al. Null association between serum 25-hydroxyvitamin D levels with allergic rhinitis, allergic sensitization and non-allergic rhinitis: A Mendelian randomization study. *Clin. Exp. Allergy*. 2020;00:1-9. <https://doi.org/10.1111/cea.13739>

Null association between serum 25-hydroxyvitamin D levels with allergic rhinitis, allergic sensitization and non-allergic rhinitis: A Mendelian randomization study

Supplementary file 1: Data analysis methods

Supplementary figure 1: Statistical power analyses for allergic rhinitis, allergic sensitization and non-allergic rhinitis.

Supplementary table 1: Related traits of 25(OH)D-related SNPs (identified from PhenoScanner v2 database)

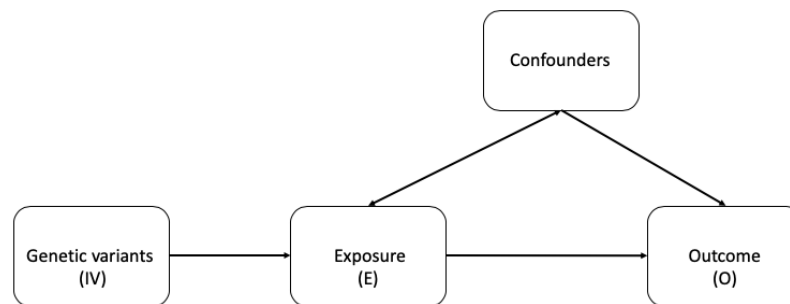
Supplementary table 2: Sensitivity analysis by changing the order of data analysis (performing Mendelian randomization analysis first and meta-analysis later)

Supplementary file 1: Data analysis methods

Mendelian randomization (MR) is defined as “any study that uses genetic variants that served as a robust proxy for an environmentally modifiable exposure in order to make causal inference about the outcomes of the modifiable exposure”. (1)

MR is based on instrumental variable (IV) analysis, in which at least three variables are involved: exposure (E), outcome (O), and IV. The objective is to estimate the effect of environmentally modifiable exposure on outcome (β_{E-O}), from the association between IV with exposure (β_{IV-E}) and that between IV with outcome (β_{IV-O}). In a simplest case of linear association and a continuous outcome, their relationship is shown in the formula $\beta_{E-O} = \frac{\beta_{IV-O}}{\beta_{IV-E}}$. (1)

MR uses genetic variants (single nucleotide polymorphisms (SNPs)) as instrumental variables (see the directed acyclic graph below). The associations between genetic variants with exposure (β_{IV-E}) and outcome (β_{IV-O}) are estimated in genome-wide association (GWA) studies.

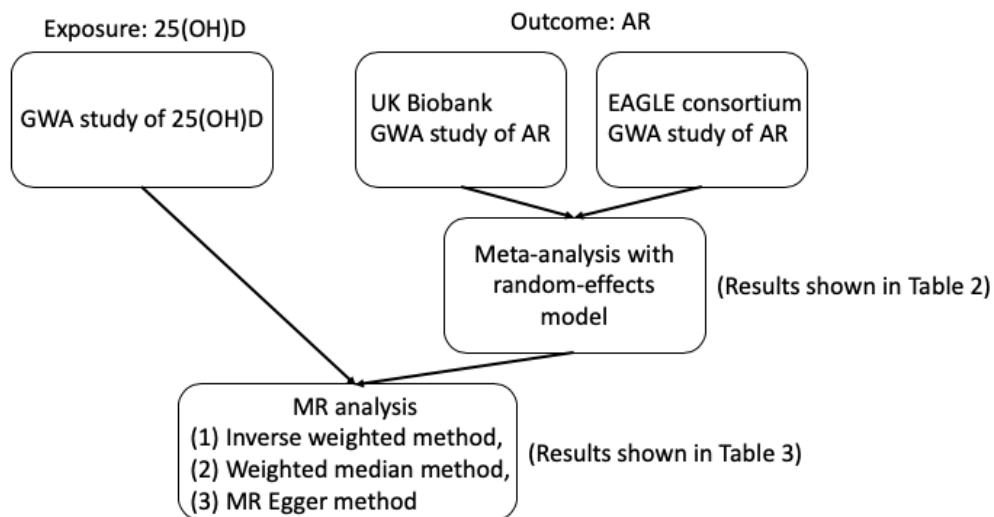


In our study, we used a two-sample MR approach, in which β_{IV-E} and β_{IV-O} were estimated in different populations. The exposure was serum 25-hydroxyvitamin D (25(OH)D) levels. The primary outcome was allergic rhinitis (AR), while the secondary outcomes were allergic sensitization (AS) and non-allergic rhinitis (NAR). The EAGLE consortium and UK Biobank GWA studies were the original data sources for AR, which only EAGLE consortium GWA study provided data on AS and NAR. The associations between genetic variants with exposure (β_{IV-E}) and with outcome (β_{IV-O}) were extracted from these GWA studies and used to compute the effect of exposure on outcome (β_{E-O}). The data sources for exposure and outcomes are shown below.

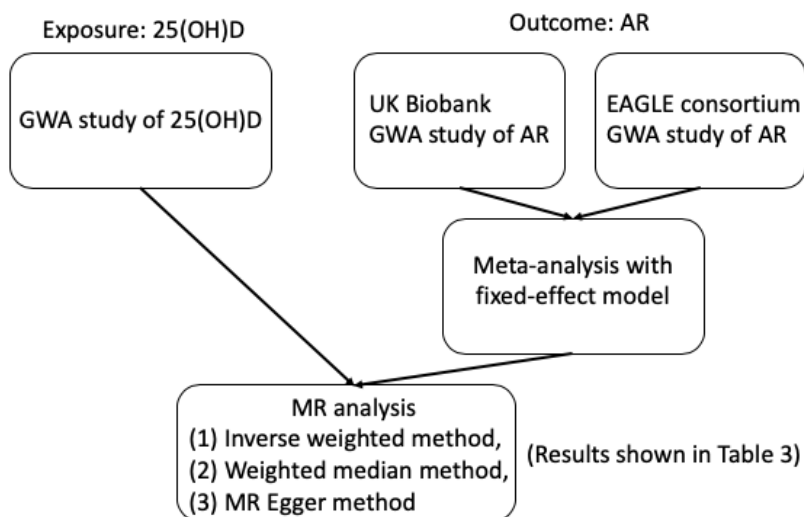
Exposure/outcomes	Data source
Serum 25(OH)D levels	GWA studies of serum 25(OH)D levels (7 SNPs in total as IVs, 6 of which was extracted from Jiang X <i>et al.</i> (2), which the other one from Manousaki D <i>et al.</i> (3)
AR	EAGLE consortium GWA study of AR (4) UK Biobank GWA study of AR (5)
AS	EAGLE consortium GWA study of AS (4)
NAR	EAGLE consortium GWA study of NAR (4)

Since multiple SNPs were selected as instrumental variables, the estimates of β_{E-O} for all the SNPs would be combined, for which we performed inverse-variance weighted method as the primary analysis, while weighted median method and MR Egger method as sensitivity analysis.

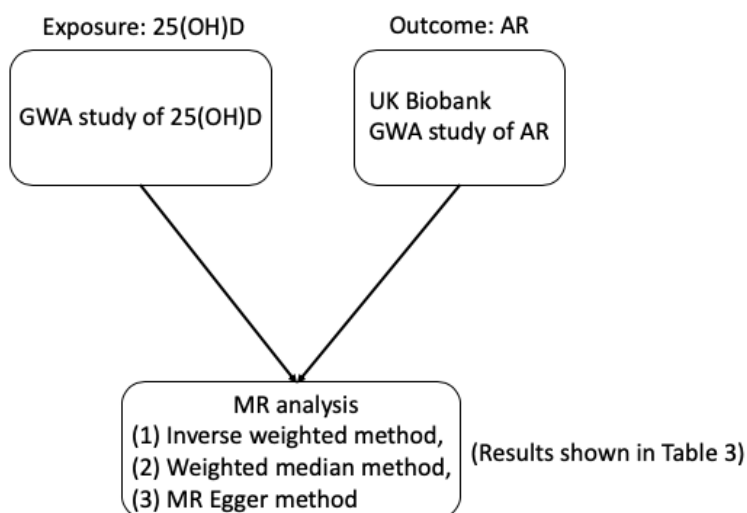
For the outcome of AR, since two original data sources (EAGLE and UK Biobank) were available, we combined the effect estimates from the two data sources using meta-analysis with random-effects model as primary analysis, while fixed-effect model as sensitivity analysis. Additionally, we also estimated the effect in EAGLE and in UK Biobank separately. Below are the flowcharts of data analyses.



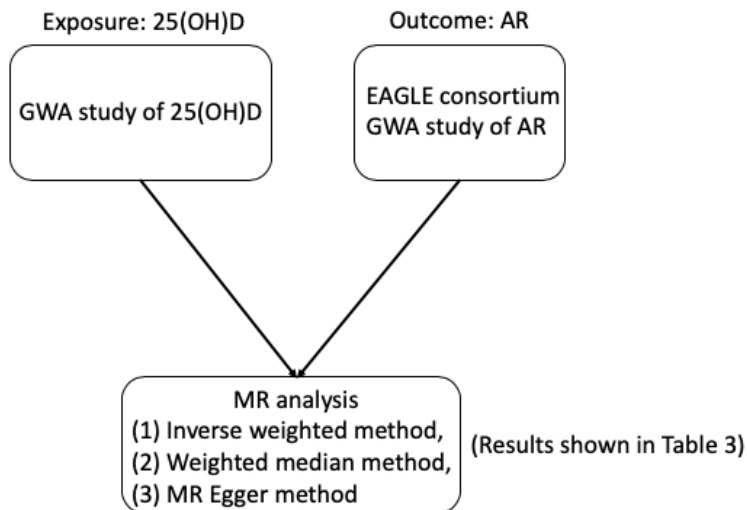
(1) Primary analysis for AR



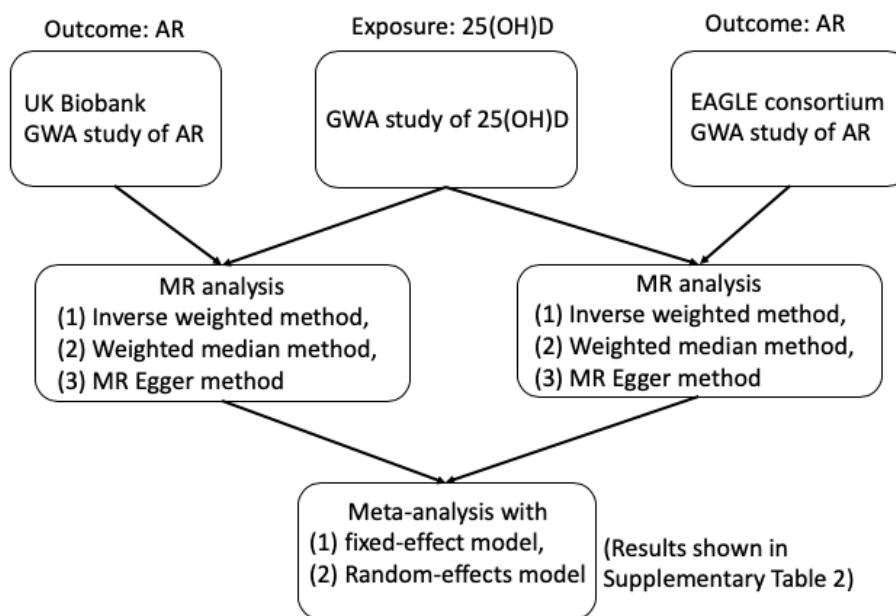
(2) Sensitivity analysis 1 for AR



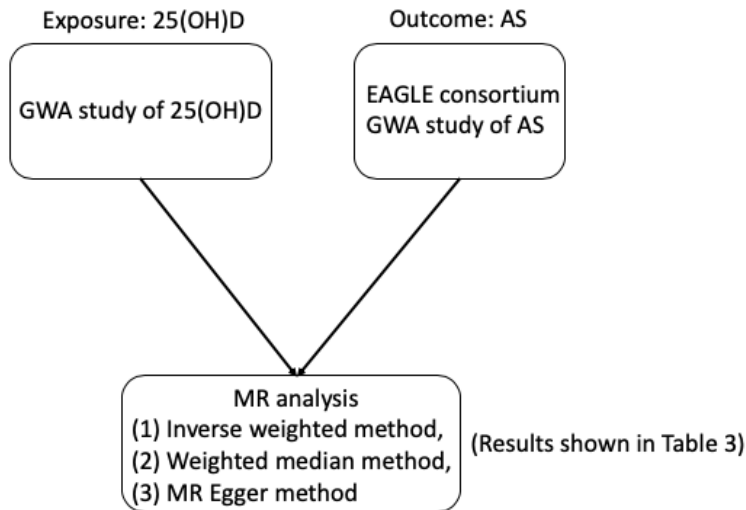
(3) Sensitivity analysis 2 for AR



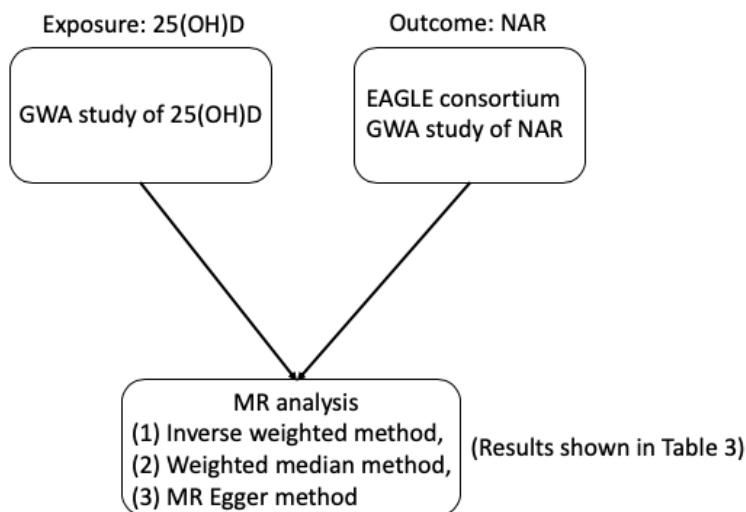
(4) Sensitivity analysis 3 for AR



(5) Sensitivity analysis 4 for AR



(6) Primary analysis for AS



(7) Primary analysis for NAR

Using SNPs as valid IVs in MR relies on three assumptions: (1) the SNPs are strongly association with exposure, (2) the SNPs affect the outcome only through their effect on the exposure, and (3) the SNPs are independent of confounders of the association between the exposure and the outcome. Pleiotropy is the potential for a SNP to have more than one specific phenotypic effect (1). Presence of pleiotropic effect that influences the outcome would violate the assumption (2) and/or (3), and underestimate the overall effect; therefore, it is important to examine potential pleiotropy. There are two common methods. First, to perform a statistical test for the intercept in MR Egger method, i.e., to test whether the intercept is zero, and rejecting the null hypothesis ($p < 0.05$) suggests pleiotropy. Second, to search if the SNPs are significantly associated with other phenotypic traits in databases, such as PhenoScanner. Power calculation for MR analysis was based on the method proposed by Brion *et al*(6). More discussion on the principles, methodological details and limitations of MR analysis has been previously published (1, 6-11).

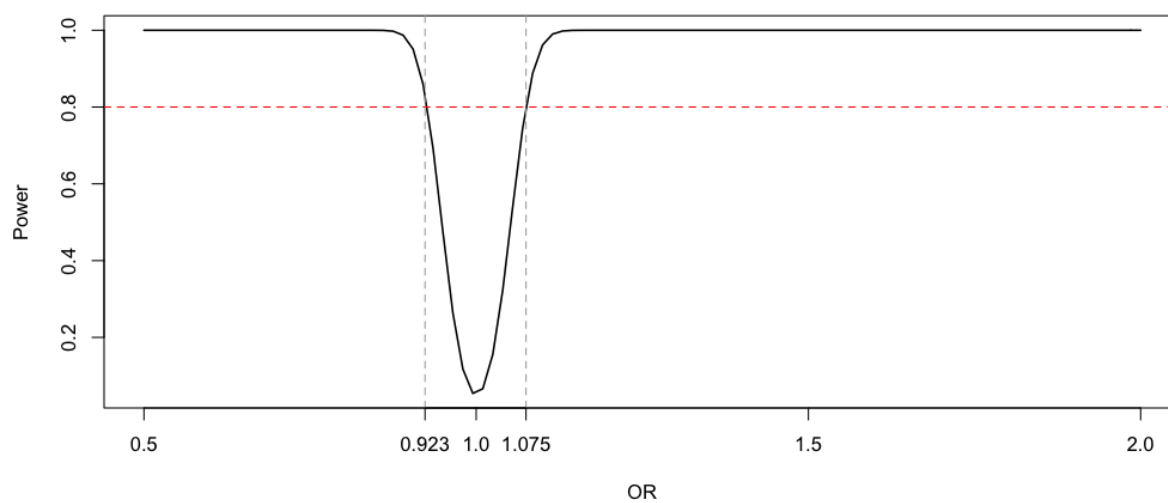
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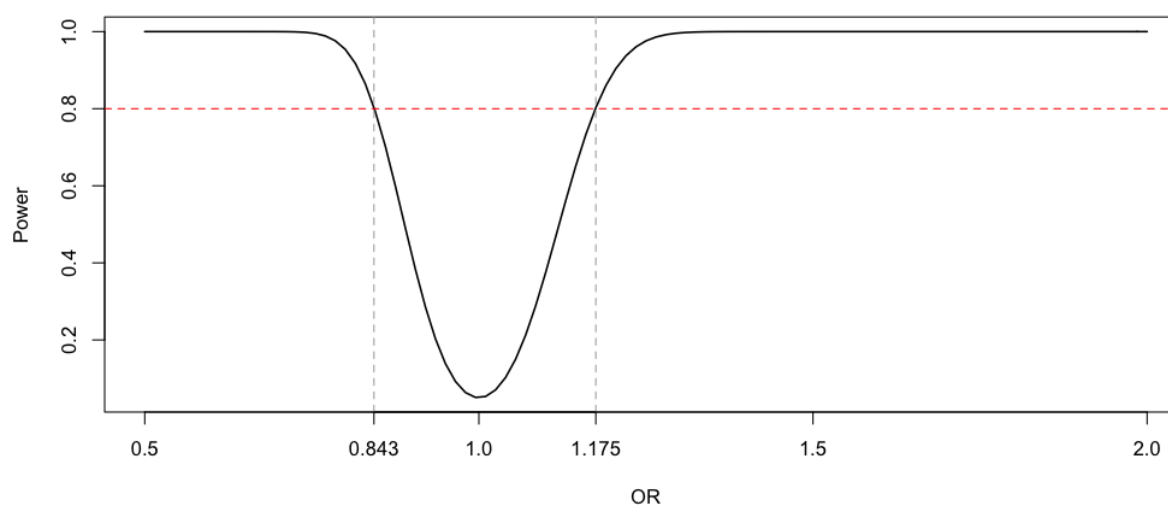
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11. Ebrahim S, Davey Smith G. Mendelian randomization: can genetic epidemiology help redress the failures of observational epidemiology? *Hum Genet.* 2008;123(1):15-33.

Supplementary figure 1: Statistical power analyses for allergic rhinitis, allergic sensitization and non-allergic rhinitis.

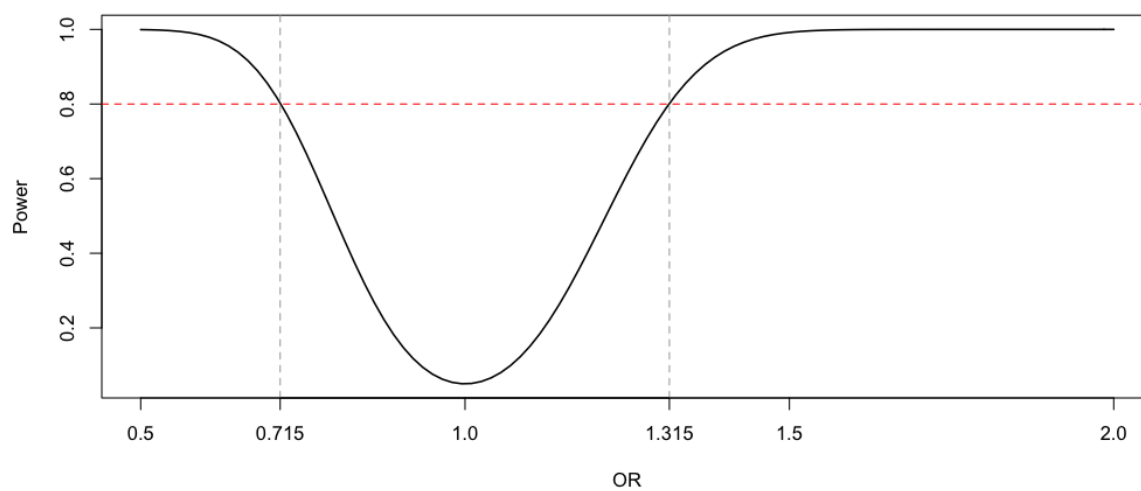
(A) Allergic rhinitis



(B) Allergic sensitization



(C) Non-allergic rhinitis



Supplementary table 1: Related traits of 25(OH)D-related SNPs (identified from PhenoScanner v2)

SNP	Chr:Pos	Gene	Effect allele	Related trait	Beta	P value
rs8018720	14:39086981	<i>SEC23A</i>	G	NA	NA	NA
rs3755967	4:71743681	<i>GC</i>	C	Blood white cell count	0.0261	3.61×10^{-11}
				Myeloid white cell count	0.0257	8.05×10^{-11}
				Sum basophil neutrophil counts	0.0251	1.85×10^{-10}
				Granulocyte count	0.0250	2.32×10^{-10}
				Neutrophil count	0.0247	3.22×10^{-10}
				Sum neutrophil eosinophil count	0.0247	3.26×10^{-10}
rs17216707	20:54115823	<i>CYP24A1</i>	T	Estimated glomerular filtration rate (creatinine)	0.0077	9.00×10^{-15}
rs12785878	11:71456403	<i>NADSYN1</i> <i>/DHCR7</i>	T	NA	NA	NA
rs116970203	11:14855172	<i>CYP2R1</i>	G	NA	NA	NA
rs10745742	12:95964751	<i>AMDHD1</i>	T	NA	NA	NA
rs10741657	11:14893332	<i>CYP2R1</i>	A	Height	0.0098	2.30×10^{-8}

25(OH)D: 25-hydroxyvitamin D. SNP: single nucleotide polymorphism.

Supplementary table 2: Sensitivity analysis for allergic rhinitis by changing the order of data analysis (performing Mendelian randomization analysis first and meta-analysis later)

MR method	Meta-analysis model	OR (95%CI)	I ²
Inverse variance weighted method	Random-effects model	1.007 (0.836, 1.212)	0
Inverse variance weighted method	Fixed-effect model	1.007 (0.836, 1.212)	0
Weighted median method	Random-effects model	1.016 (0.904, 1.143)	0
Weighted median method	Fixed-effect model	1.016 (0.904, 1.143)	0
MR Egger method	Random-effects model	1.054 (0.825, 1.347)	0
MR Egger method	Fixed-effect model	1.054 (0.825, 1.347)	0