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Vitamin D Levels and Risk of Juvenile Idiopathic Arthritis: A Mendelian Randomization Study

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Objectives. Observational studies report mixed findings regarding the association between vitamin D and juvenile idiopathic arthritis (JIA) incidence or activity; however, such studies are susceptible to considerable bias. Because low vitamin D levels are common within the general population and easily corrected, there is potential public health benefit in identifying a causal association between vitamin D insufficiency and JIA incidence. To limit bias due to confounding and reverse causation, we examined the causal effect of the major circulating form of vitamin D, 25-hydroxy vitamin D (25-[OH]D), on JIA incidence using Mendelian randomization (MR).

Methods. In this 2-sample MR analysis, we used summary level data from the largest and most recent genome-wide association study of 25-(OH)D levels (sample size 443,734), alongside summary data from 2 JIA genetic studies (sample sizes 15,872 and 12,501), all from European populations. To test and account for potential bias due to pleiotropy, we employed multiple MR methods and sensitivity analyses.

Results. We found no evidence of a causal relationship between genetically predicted 25-(OH)D levels and JIA incidence (odds ratio 1.00 [95% confidence interval (95% CI) 0.76, 1.33] per SD increase in standardized natural-log transformed 25-[OH]D levels). This estimate was consistent across all methods tested. Additionally, there was no evidence that genetically predicted JIA causally influences 25-(OH)D levels (–0.002 SD change in standardized natural-log transformed 25-[OH]D levels per doubling odds in genetically predicted JIA [95% CI –0.006, 0.002]).

Conclusion. Given the lack of a causal relationship between 25-(OH)D levels and JIA, population level vitamin D supplementation is unlikely to reduce JIA incidence.

INTRODUCTION

Juvenile idiopathic arthritis (JIA) is the most common rheumatic disease affecting children, with a prevalence of approximately 1 of 1,000 (1). JIA is considered a complex autoimmune disease, influenced by both environmental and genetic factors. Although there is a growing appreciation of genetic influences (2–4) on JIA, we have much less clarity regarding environmental effects on disease risk (5). Identification of modifiable environmental factors that predispose to JIA or can be used to risk stratify or alter disease outcome has huge potential benefit for patients.

Observationally, low vitamin D levels have been associated with increased incidence and disease activity of many other autoimmune disorders (6,7). Vitamin D is a fat-soluble steroid hormone for which the synthesis requires multiple steps involving biologically inactive precursors (Figure 1). The major circulating form of vitamin D measured in blood is 25-hydroxy vitamin D (25-[OH]D); however the downstream derivative 1,25-$(OH)_2$D is the biologically active form. Vitamin D levels are predominantly influenced by sunlight; ultraviolet light induces conversion of 7-dehydrocholesterol to previtamin D3 in the skin. Some vitamin D is also obtained via the gut from diet and exogenous supplementation. Vitamin D acts via the vitamin D receptor (VDR) and plays a key role in calcium homeostasis and bone metabolism. However, the VDR is also extensively expressed on immune cells, and vitamin D has been shown to have a number of immune-modulatory effects (7,8).

The role of vitamin D in JIA has recently been examined in a scoping review (9). The studies included in this review predominantly 1) examine the role of vitamin D in JIA activity/disease course or 2) report vitamin D status of JIA patients versus healthy

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SIGNIFICANCE & INNOVATIONS

- Observational data regarding the association between vitamin D levels and juvenile idiopathic arthritis (JIA) are mixed and are susceptible to confounding and reverse causation.
- Mendelian randomization is a method of causal inference that is less susceptible to these biases and was used to examine the causal relationship between vitamin D and JIA risk.
- We found no evidence of a causal relationship between genetically predicted vitamin D levels and JIA, suggesting observational data supporting this association could be confounded.
- Our findings suggest that population-level vitamin D supplementation will not reduce JIA incidence.

controls after disease onset, with mixed results. Further literature review identified few published studies examining the vitamin D status of JIA patients before disease onset. Thorsen et al (10) found no evidence of association between cord blood 25-(OH)D levels and, later, JIA risk (adjusted odds ratio [OR] 1.2 [95% confidence interval (95% CI) 0.9, 1.6]) in 300 matched case–control pairs; however, observational studies of vitamin D levels and JIA risk at other predisease time points are lacking. Conversely, a recent study of 202 JIA case–control pairs found a potentially protective association between maternal prenatal or early childhood ultraviolet radiation exposure and JIA, with vitamin D proposed as a mediator (11). Thus, the role of vitamin D in JIA incidence remains unclear.

Vitamin D insufficiency (25-[OH]D <50 nmoles/liter) is increasingly common within the general population, with children being at particular risk in part because of the nutritional demands associated with periods of rapid growth (12). Therefore, establishing a causal relationship between vitamin D and JIA has important implications. Determining causal relationships from observational data is challenging; associations may be confounded (e.g., by medication use such as steroids), subject to reverse causation (e.g., patients with JIA may have altered behavior and spend less time outdoors because of their disease state), or subject to recall bias. Mendelian randomization (MR) is a method of causal inference that relies on genetic data to interrogate the causal relationship between an exposure and outcome of interest in which single

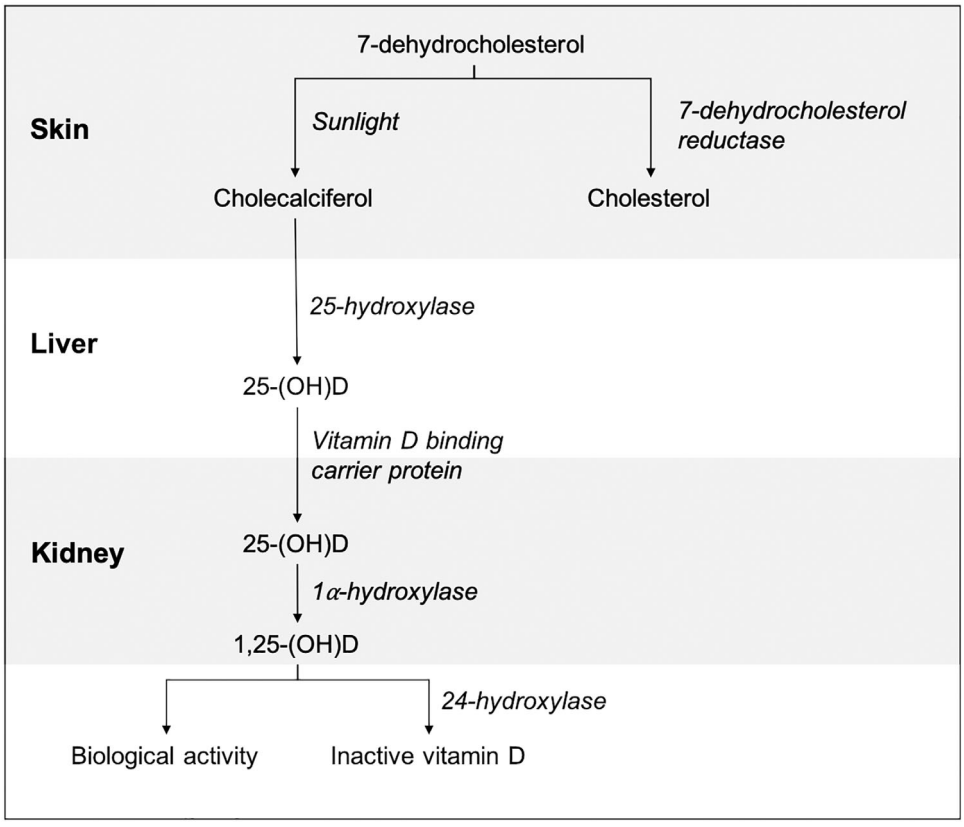

Figure 1. Major steps in the vitamin D synthetic pathway. 7-dehydrocholesterol in the skin is converted to cholecalciferol (previtamin D) in the presence of sunlight. Cholecalciferol is then hydroxylated in the liver to 25-hydroxy vitamin D (25-[OH]D) (calcidiol). 25-(OH)D is bound by vitamin D binding protein and transported to the kidneys, where it undergoes 1-hydroxylation to its active form—1,25-(OH)D (calcitriol). Downstream effects of biologically active vitamin D are mediated via the vitamin D receptor. Vitamin D is catabolized through a variety of oxidation steps involving 24-hydroxylase.

nucleotide polymorphisms (SNPs) are used as genetic proxies for the exposure of interest. The reliance of MR on genetic variants, assigned at the point of conception and not influenced by lifetime environmental exposures and experiences, makes these analyses robust to reverse causation and confounding compared with traditional epidemiologic studies. The value of this approach can be seen in relation to multiple sclerosis (MS) in which observation associations between MS and vitamin D, examined as latitudinal gradient (13), vitamin D levels (14), and VDR polymorphisms (15), have been replicated in MR studies of MS and vitamin D (16–18). The aim of this bidirectional, 2-sample MR study is to examine the causal relationship between vitamin D and JIA using the latest genome-wide association study (GWAS) summary data.

MATERIALS AND METHODS

Data sources and selection of genetic instruments. The first assumption of MR is that genetic variants are strongly associated with the exposure of interest (Figure 2). Therefore, the genetic variants selected for vitamin D comprised the 69 lead, common, conditionally independent SNPs that were robustly associated with 25-(OH)D levels ($P < 6.6 \times 10^{-9}$) from a large GWAS meta-analysis of 25-(OH)D in European individuals (n = 443,734; 52.7% female participants) adjusted for age, sex, genotype array, genotype batch, vitamin D supplementation, and latitude (19). The more stringent P value threshold of 6.6×10^{-9}, rather than the traditional 5×10^{-8}, was derived from an earlier bespoke analysis of the vitamin D GWAS cohort (20) and thus was retained for this analysis. Publicly available summary data from the most recent JIA GWAS study were used as the JIA outcome data set in this analysis because of their broad genomic coverage and inclusivity of JIA subtypes (2). This data set comprises 3,305 JIA patients (all subtypes) and 9,196 healthy controls of European ancestry.

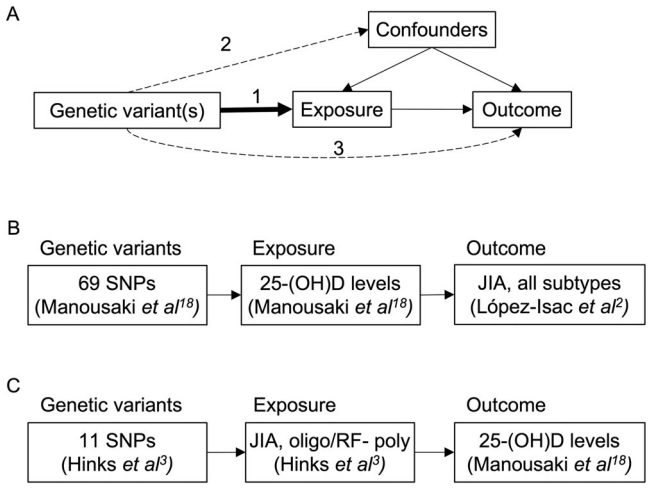

Figure 2. Mendelian randomization (MR) analyses undertaken in this study. **A**, Diagram of the assumptions for an MR analysis. In order to be valid MR instruments, genetic variants must: 1) be strongly associated with the exposure of interest (relevance assumption), 2) not be associated with confounders of the exposure or the outcome of interest (independence assumption), and 3) not influence the outcome except via the exposure (exclusion restriction). **B**, Forward MR analysis examining the causal association between genetically predicted vitamin D levels and juvenile idiopathic arthritis (JIA). **C**, Reverse MR analysis examining the association between JIA and vitamin D levels. Data sources are shown in parentheses. 25-(OH)D = 25-hydroxy vitamin D; Oligo = oligoarticular; RF- poly = rheumatoid factor negative polyarticular; SNP = single nucleotide polymorphism.

To investigate the causal effect of JIA genetic liability on vitamin D levels, we derived a genetic instrument for JIA as an exposure from an Immunochip study comprising 2,816 oligoarticular and rheumatoid factor (RF)–negative polyarticular JIA cases (76.6% female participants) and 13,056 healthy controls (55.1% female participants) of European ancestry (3). This data set comprises the largest sample size of any publicly available JIA genetic data set and identifies the greatest number of replicated JIA-associated loci. The instrument comprised 11 independent SNPs ($r^2 < 0.001$) strongly associated with JIA ($P < 5 \times 10^{-8}$) using an additive genetic model. Full summary data from the vitamin D GWAS study (19) were made available by the authors to allow for bidirectional MR analysis with vitamin D as an outcome. Details of the genetic variants used for each instrument can be found in Supplementary Table 1, available on the *Arthritis Care & Research* website at http://onlinelibrary.wiley.com/doi/10.1002/acr.24815.

Statistical analyses. Two-sample MR, implemented in the *TwoSampleMR* package (21) in the R software platform (version 4.0.2) (22), was used to examine the causal relationship between genetically predicated vitamin D levels and JIA. Effect estimates for the selected vitamin D SNPs were extracted from the JIA outcome data set. Where an SNP was not available in the outcome data set, suitable LD proxies ($r^2 > 0.8$) were identified using the *LDLinkR* package (23) from LDLink (24) (see Supplementary Table 2, available on the Arthritis Care & Research website at http://onlinelibrary.wiley.com/doi/10.1002/acr.24815). Alleles were harmonized between exposure and outcome data sets to ensure SNP-exposure and SNP-outcome effects correspond to the same allele. We aligned palindromic SNPs using default thresholds; noninferable SNPs were excluded to limit effect allele ambiguity between data sets. We used the inverse variance–weighted (IVW) method to estimate the causal effect of 25-(OH)D on JIA susceptibility (OR per 1 SD increase in standardized natural-log transformed 25-[OH]D levels). Briefly, the IVW method pools the SNP-exposure and SNP-outcome associations in a random effects meta-analysis weighted by the inverse variance of the SNP-outcome association. For clinical context, we have expressed 1 SD increase in natural-log transformed 25-(OH)D levels as the corresponding change in nmoles/liter at clinically relevant 25-(OH)D levels vitamin D deficient (<25 nmoles/liter), insufficient (25–50 nmoles/liter), and sufficient (50–70 nmoles/liter),

Table 1. Effect of a 1 SD increase in standardized natural-log transformed 25-(OH)D levels at clinically relevant vitamin D thresholds*

Clinically relevant vitamin D threshold	Effect of 1 SD change in standardized natural-log transformed 25-(OH)D at given threshold in, nmoles/liter
Vitamin D deficient (<25 nmoles/liter)	14.61
Vitamin D insufficient (25–50 nmoles/liter)	29.22
Vitamin D sufficient (50–70 nmoles/liter)	40.91

* For vitamin D insufficient individuals (25–50 nmoles/liter), a 1 SD increase in standardized natural-log 25-(OH)D would result in normalization of the vitamin D level (>50 nmoles/liter). 25-(OH)D = 25-hydroxy vitamin D.

(Table 1). The analysis was also performed in reverse, that is, with JIA as the exposure and vitamin D levels as the outcome, using the JIA genetic instrument and the vitamin D GWAS summary data set, to estimate the causal effect of JIA genetic risk on vitamin D levels. Because JIA is a binary exposure, the MR estimate refers to the SD change in standardized natural-log transformed 25-(OH)D levels per log odds ratio (logOR) increase in genetically predicted JIA. To aid interpretability, these MR estimates were multiplied by 0.693 to represent SD change in standardized natural-log transformed 25-(OH)D levels per doubling in odds of genetic liability to JIA (25). Instrument strength was measured using the F statistic, whereby the higher the F statistic, the lower the risk of weak instrument bias. This was calculated as

$$F = \left(\frac{N-K-1}{K}\right)\left(\frac{R^2}{1-R^2}\right),$$

where N indicates sample size, K indicates number of SNPs in the instrument, and R^2 indicates the proportion of the variance in the exposure explained by the instrument.

The second MR assumption requires that genetic instruments are not associated with confounders that could bias the association between the exposure and the outcome (Figure 2). To assess this, we queried both the vitamin D and the JIA genetic instruments in the PhenoScanner database (26). We defined strong associations as those traits associated with an SNP below a P value threshold Bonferroni-corrected for the number of SNPs in the genetic instrument (i.e., $P < 7.25 \times 10^{-4}$ [0.05/69] for vitamin D and $P < 4.54 \times 10^{-3}$ [0.05/11] for JIA). Traits identified below the defined P value thresholds in PhenoScanner ("secondary traits") were grouped into 21 trait categories. None of the SNP-trait associations identified with PhenoScanner were felt to be potential confounders of the association between vitamin D and JIA in our analyses and therefore violate the second MR assumption.

The third MR assumption states that genetic variants must be associated with the outcome only via the exposure (exclusion restriction), that is, that there are no horizontally pleiotropic effects. The inclusion of multiple SNPs within the genetic instrument increases the statistical power of the analysis. However, this also increases the risk of including more pleiotropic genetic variants and violating the exclusion-restriction criteria. We undertook sensitivity analyses to attempt to detect and account for potential pleiotropic effects. Heterogeneity can indicate pleiotropy, which would violate the core assumptions of MR; thus, heterogeneity was assessed using Cochran's Q statistic (27). To further investigate potential horizontal pleiotropy, we undertook MR-Egger regression (28), weighted median estimation (29), and radial MR (30). MR-Egger regression can provide an accurate estimate of causality even if all instrumental variables are invalid. MR-Egger analysis uses the inverse variance of SNP-outcome associations as weights in a weighted linear regression of SNP-outcomes effects on SNP-exposure effects; however, unlike the IVW method, the intercept is not constrained to zero. The slope of this regression represents the causal effect estimate and, in the absence of pleiotropy, equals the IVW estimate. The intercept can be interpreted as the average pleiotropic effects of the instrumental variables, with directional pleiotropy being indicated by an intercept other than zero.

Regression dilution I^2_{GX} (31) was assessed to ensure the MR-Egger analyses were robust, with a value of more than 90% considered low risk of bias. The weighted median estimator offers protection against invalid instrumental variables/horizontal pleiotropy by providing a consistent estimate of causality when up to 50% of the weight comes from invalid instrumental variables; it estimates the weighted median value of the Wald ratio. Radial MR is used to identify potentially outlying SNPs within the genetic instrument and provide MR estimates using alternative weights. We note the caution from the authors of the vitamin D meta-analysis about potential pleiotropic effects when using the 69 vitamin D–associated SNPs in an MR context.

To further examine whether pleiotropic SNPs may be biasing the association between vitamin D and JIA, we undertook a sensitivity analysis excluding all nonspecific genetic instruments (SNPs that are associated with any secondary trait are identified in PhenoScanner, see Supplementary Tables 1 and 3, available on the *Arthritis Care & Research* website at http://onlinelibrary.wiley.com/doi/10.1002/acr.24815). Additionally, we undertook a second sensitivity analysis using only the 7 SNPs from 6 loci previously robustly associated with vitamin D, 5 of which are in close proximity to genes that are directly involved in the vitamin D biologic pathway (Figure 1 and Supplementary Table 1). Consistent estimates across all MR methods (IVW, MR-Egger, weighted median, and radial MR) and vitamin D instruments make bias due to pleiotropy less probable.

Data availability. The data sets analyzed during the current study are available in the GWAS Catalog repository (Studies GCST90010715, GCST010144, and GCST005528) at www.ebi.ac.uk/gwas/studies/GCST90010715, www.ebi.ac.uk/

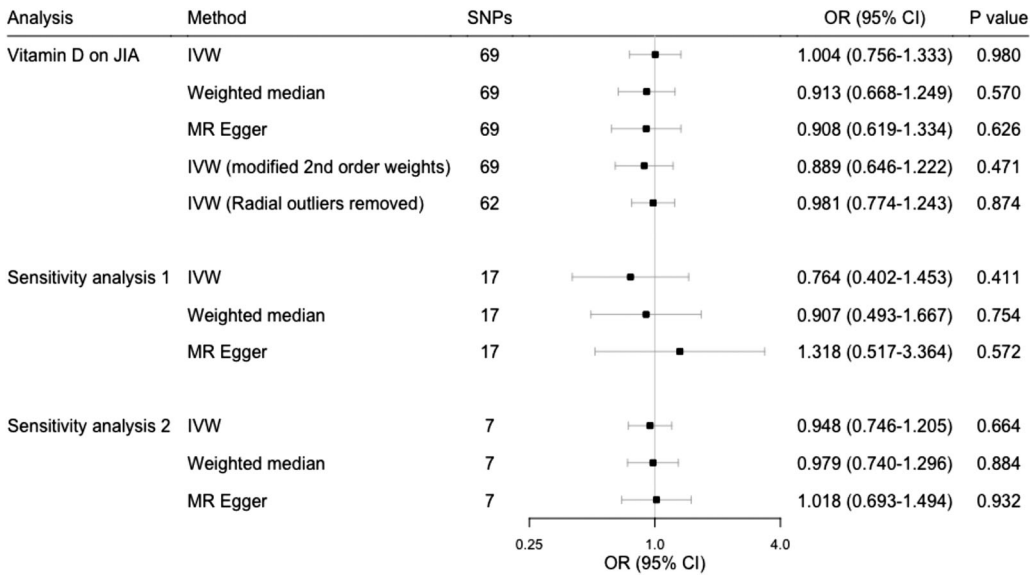

Figure 3. Two-sample Mendelian randomization (MR) analysis showing the effects of genetically predicted 25-hydroxy vitamin D (25-[OH]D) levels on juvenile idiopathic arthritis (JIA). Sensitivity analysis 1 is restricted to single nucleotide polymorphisms (SNPs) not associated with a secondary trait using PhenoScanner, and sensitivity analysis 2 is restricted to SNPs previously associated and predominantly with the vitamin D synthetic pathway. Odds ratios (ORs) represent the odds of JIA per 1 SD increase in natural-log transformed 25-(OH)D. 95% CI = 95% confidence interval; IVW = inverse variance–weighted.

gwas/studies/GCST010144, and www.ebi.ac.uk/gwas/studies/GCST005528 (2,3,19).

RESULTS

Two-sample MR analysis examining the causal effect of vitamin D on JIA risk. Sixty-nine vitamin D–associated SNPs were used as instruments with sufficient strength for MR analysis (F statistic = 115.7, R^2 = 0.018). There was no evidence that higher genetically predicted 25-(OH)D levels are causally associated with reduced JIA risk (OR 1.00 [95% CI 0.76, 1.33] per SD increase in standardized natural-log transformed 25-(OH)D levels (Figure 3 and Supplementary Table 4, available on the Arthritis Care & Research website at http://onlinelibrary.wiley.com/doi/10.1002/acr.24815). We also conducted a "leave-one-out" analysis that found that no single SNP was driving the MR estimates (see Supplementary Figure 1, available at http://onlinelibrary.wiley.com/doi/10.1002/acr.24815). Based on the JIA sample size of 12,501, alpha of 0.05, case fraction of 0.264, and R^2 of 0.018, this study has 80% power to detect effects as small as an OR of 1.47 per SD change in standardized natural-log transformed 25-(OH)D levels and has 100% to detect an OR of 1.80 per SD change in standardized natural-log transformed 25-(OH)D levels.

Assessment of heterogeneity and control for pleiotropy. We found no strong evidence of pleiotropy when examining the MR-Egger intercept (intercept 0.005, P = 0.45) (see Supplementary Table 5, available at http://onlinelibrary.wiley.com/doi/10.1002/acr.24815) and no evidence of asymmetry in the funnel plot of single SNP effects (see Supplementary Figure 2, available at http://onlinelibrary.wiley.com/doi/10.1002/acr.24815). However, Cochran's Q statistic indicated evidence of heterogeneity (Q statistic = 99.33, P = 0.008) (see Supplementary Table 6, available at http://onlinelibrary.wiley.com/doi/10.1002/acr.24815), which can suggest pleiotropy; therefore, we undertook additional analyses to assess and account for potential pleiotropic effects. MR-Egger regression and the weighted median estimator provided MR estimates comparable to the IVW estimate (Figure 3). Radial MR was used to identify and adjust for outlying and thus potentially pleiotropic SNPs; the IVW estimate was recalculated using 1) first-order weights with removal of 7 outlying SNPs identified using radial regression and 2) modified second-order weights with all 69 SNPs (see Supplementary Tables 4 and 7, available at http://onlinelibrary.wiley.com/doi/10.1002/acr.24815). In both cases, MR estimates were comparable to the original IVW estimate (Figure 3).

To further validate the effect of genetically predicted vitamin D on JIA, sensitivity analyses were undertaken using subsets of the genetic instrument for vitamin D levels. First, we used a 17 SNP vitamin D instrument (see Supplementary Table 1) in which all SNPs strongly associated with any secondary trait within PhenoScanner ($P < 7.24 \times 10^{-4}$) were excluded (i.e., high stringency removal of nonspecific genetic instruments, sensitivity analysis 1). Second, we used an instrument of 7 25-(OH)D genetic variants robustly associated with vitamin D levels from earlier GWAS studies (see Supplementary Table 1), which are also predominantly involved in the vitamin D biologic (sensitivity analysis 2).

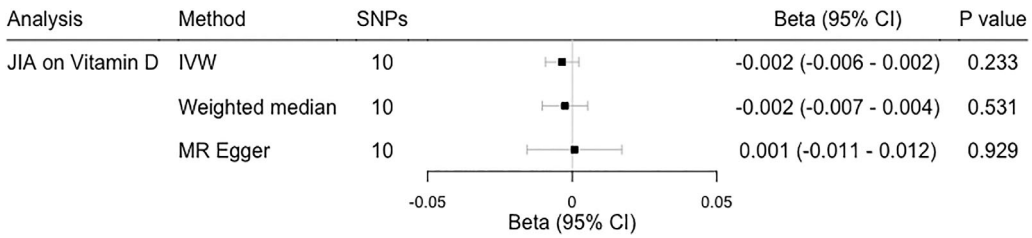

Figure 4. Two-sample Mendelian randomization (MR) analysis showing the effects of genetically predicted juvenile idiopathic arthritis (JIA) on 25-hydroxy vitamin D (25-[OH]D) levels. Beta coefficients represent SD change in standardized natural-log transformed 25-(OH)D levels per doubling in odds of genetic liability to JIA. 95% CI = 95% confidence interval; IVW = inverse variance–weighted; SNP = single nucleotide polymorphism.

Again, comparable MR estimates to the main IVW estimate were obtained (Figure 3).

Reverse 2-sample MR analysis examining the causal effect of JIA on vitamin D levels. To assess whether the absence of a causal relationship between vitamin D levels and JIA in our previous analysis, despite some observational evidence to the contrary, could be due to reverse causation (i.e., JIA causes changes in vitamin D levels), we used a genetic instrument comprising 11 SNPs associated with oligoarticular and RF negative polyarticular JIA (see Supplementary Table 1). This instrument has sufficient strength for MR analysis (F statistic = 52.6, R^2 = 0.035). It comprises SNPs that were not associated with a confounding secondary trait in PhenoScanner (see Supplementary Table 8, available on the Arthritis Care & Research website at http://onlinelibrary.wiley.com/doi/10.1002/acr.24815). There was no evidence that genetically predicted oligoarticular and RF negative polyarticular JIA is associated with changes in 25-(OH)D levels (–0.002 SD change in standardized natural-log transformed 25-[OH]D levels per doubling odds in genetically predicted JIA [95% CI –0.006, 0.002]) (Figure 4 and Supplementary Table 9, available on the Arthritis Care & Research website at http://onlinelibrary.wiley.com/doi/10.1002/acr.24815). Cochran's Q statistic found no evidence of heterogeneity (Q statistic = 9.35, P = 0.75) (see Supplementary Table 5), and the MR-Egger intercept indicated low risk of bias due to pleiotropy (intercept –0.001, P = 0.60) (see Supplementary Table 5). MR estimates were comparable across the IVW, weighted median estimator, and MR-Egger methods. Leave-one-out analysis showed that no single SNP was biasing the MR estimates (see Supplementary Figure 3).

DISSCUSSION

Using summary-level data from the most recent 25-(OH)D (19) and JIA (2,3) GWASs in European populations, we undertook the first 2-sample MR study in JIA to investigate the presence and magnitude of the causal effect of vitamin D on disease risk. Despite using multiple, complementary MR methodologies and sensitivity analyses, we found no evidence of a causal relationship between genetically predicted vitamin D levels and JIA (all subtypes). We were also unable to demonstrate a causal effect of genetically predicted oligoarticular and RF negative polyarticular JIA on circulating vitamin D levels.

A causal role for low vitamin D in increasing MS risk has been robustly established using MR methodology (16–18); however, the results of our study are in keeping with the growing number of other MR studies, which have found no causal relationship between vitamin D and other immune-mediated disorders such as rheumatoid arthritis (32), systemic lupus erythematosus (32), inflammatory bowel disease (33), type 1 diabetes mellitus (34), childhood asthma (35,36), and eczema (36,37). Therefore, although we cannot exclude small effects of vitamin D levels on JIA incidence, our results show that higher genetically predicted vitamin D levels do not reduce JIA risk and that attempts to increase circulating vitamin D levels, for example, through supplementation, are unlikely to substantially reduce the incidence of JIA in European populations.

The role of vitamin D in JIA outcome has also been considered within the literature with regard to the level of disease activity (38,39) and incidence of JIA-associated complications, for example, uveitis (38). However, corresponding trial data of vitamin D supplementation are lacking with regard to reducing disease activity (40). Accordingly, our study has not demonstrated a causal effect of genetically predicted oligoarticular and RF negative polyarticular JIA on circulating vitamin D levels. However, exploring the precise role of vitamin D in JIA disease activity in a 2-sample MR context would require data sets that identify genetic variants proxying JIA outcome within a JIA population. Such data sets are not currently available; thus, this study cannot exclude a role for vitamin D in JIA severity, disease activity, or prognosis, and thus, vitamin D supplementation may have a role as a treatment adjunct in JIA.

The major strength in this study lies within the rigorous interrogation of the association between vitamin D and JIA across multiple analyses, using the most up-to-date and comprehensive data sets available for vitamin D and JIA. We used stringent criteria for inclusion of SNPs as instrumental variables in our analyses (MR assumption 1) and undertook comprehensive assessment for confounding (MR assumption 2). Furthermore,

we employed multiple pleiotropy robust MR methods and vitamin D instruments to attempt to identify and account for any biasing effects of horizontal pleiotropy on our findings (MR assumption 3).

Nevertheless, this study has its limitations. First, the data sets we used derive from different age populations; the vitamin D data set derives from an adult population, and the JIA data sets from pediatric ones. Therefore, this study is assuming that the genetic effects on the exposure of interest are fixed and constant across the life course. Although large-scale pediatric vitamin D GWAS data are not available to formally test this assumption, the replication of some of the adult GWAS vitamin D associations in a small study of children from the general population at age 6 years and age 14 years provides reassurance that vitamin D genetic risk is constant across the ages (41). The generalizability of these data to non-European populations is also limited given the sparsity of ethnically diverse GWAS summary data; therefore, extrapolation of these findings to populations of non-European ancestry should be cautious. Although we have attempted to identify and account for bias due to pleiotropy, we cannot exclude residual bias in which the function of the included SNPs largely remains unknown.

The sample size of the current JIA outcome data set (total sample size 12,501), although considerable for a rare disorder, does limit the power of this analysis; thus, it is possible that vitamin D has a causal effect on JIA that is too small to be detectable by our study. However, the clinical relevance and utility of identifying such a small causal effect, given the overall population prevalence of JIA, is questionable. Additionally, the JIA outcome data set is heterogenous, including all JIA subtypes to maximize sample size and power. This makes it possible that any causal effect of vitamin D on JIA risk that is either subtype specific and/or has opposing effects by subtype would be masked within this study; subtype specific JIA data sets suitable for MR analysis are not currently available. Finally, because this study examines the effect of the major circulating form of vitamin D, 25-(OH)D, on JIA, we are unable to interrogate a causal relationship between other components of the vitamin D pathway, particularly biologically active vitamin D, and JIA.

In conclusion, this study represents the first MR study to investigate a potential causal effect of vitamin D on JIA disease risk. This study demonstrates no evidence of a causal relationship between genetically predicted 25-(OH)D levels and JIA. Similarly, there was no evidence that genetically predicted oligoarticular and RF negative polyarticular JIA are causally associated with altered 25-(OH)D levels. Accordingly, this study suggests that low vitamin D levels do not increase JIA risk and that vitamin D supplementation would not reduce the incidence of JIA in the general population. Further interrogation of these findings with larger, ethnically diverse, and subtype-specific data sets would be beneficial. In addition, the role of vitamin D in JIA disease activity and thus the utility of vitamin D supplementation as a treatment adjunct warrant further investigation.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be submitted for publication. Dr. Clarke had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study conception and design. Clarke, Mitchell, Sharp, Ramanan, Relton.

Acquisition of data. Clarke, Mitchell.

Analysis and interpretation of data. Clarke, Mitchell.

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