**Genetically predicted vitamin K levels and risk of osteoarthritis: Mendelian randomization study**

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**Abstract**

**Objective.** Osteoarthritis (OA) is a progressive disease for which there is no disease modifying therapy. Vitamin K levels and vitamin K antagonism have been associated with risk and progression of OA which may have direct implications for clinical management, but these observational findings are susceptible to confounding. We aimed to estimate the causal association between vitamin K and OA risk using Mendelian randomisation (MR).

**Methods.** We used data from the largest genome-wide association study (GWAS) of OA to date (up to 826,690 individuals) to estimate the effect of genetically predicted vitamin K level (instrumented using four variants derived from a GWAS of 2,138 individuals) on risk of all OA types, knee, hip, spine, hand OA, and total joint replacement. We employed the inverse-variance weighted method for the primary analysis and, in a series of sensitivity analyses, adjusted for sub-genome wide significant instruments and tested for potential bias from pleiotropy.

**Results.** We showed that genetically predicted vitamin K levels were not causally associated with risk of OA overall (OR 0.98 per unit increase in log-transformed vitamin K1; 95%CI 0.96-1.01), knee (OR 0.98; 0.92-1.03), hip (OR 0.97; 0.88-1.07), spine (OR 0.97; 0.90-1.04), hand OA (OR 0.97; 0.91-1.04) or joint replacement (OR 0.96; 0.89-1.04). Results were similar across all sensitivity analyses.

**Conclusion.** We found little evidence of a causal association between genetically predicted vitamin K and OA risk. Larger genetic and interventional studies of vitamin K are required to confirm our findings.

**Keywords:** vitamin K, phylloquinone, osteoarthritis, Mendelian randomization, genome-wide association study

**Introduction**

Osteoarthritis (OA) is a leading cause of disability in older adults with considerable personal, economic, and societal costs [1]. There are no effective disease modifying treatments; therefore, identifying modifiable risk factors is paramount.

Vitamin K is hypothesised to play a role in OA pathogenesis through its role in generating gamma-carboxyglutamic acid (Gla), which confers functionality to several potentially relevant proteins in bone and joint health [2]. Observational studies have shown that low vitamin K, in serum or dietary intake, is associated with increased incidence and progression of knee OA [3,4].

These findings are important for public health since vitamin K deficiency is not uncommon. Nearly 1 in 10 participants in the Health, Aging and Body Composition Study had undetectable phylloquinone (vitamin K1) levels (<0.2nmol/L) [3]. Furthermore, millions are long-term users of vitamin K antagonists (e.g., warfarin), which have been associated with increased incidence and progression of, and joint replacement (as an indicator of severity) in, knee and hip OA [5,6].

These observational designs are susceptible to confounding, i.e., unmeasured characteristics that influence both vitamin K levels and OA risk. A randomised controlled trial of vitamin K supplementation over 3 years showed no effect on radiographic hand OA, joint space narrowing or osteophytes [7]. However, this trial was not primarily designed to study OA. It may have been limited by sample size and, importantly, duration, since vitamin K levels may influence OA risk over years if not decades. The causal role of vitamin K on OA is therefore unclear and will remain challenging for interventional studies to elucidate.

Mendelian randomization (MR) is an observational design that uses genetic variants as instrumental variables to estimate the causal effect of an exposure on an outcome. Since variants are randomly allocated at conception, MR is less susceptible to confounding than other observational designs. Our aim was to use two-sample MR to investigate the effect of vitamin K on OA risk.

**Methods**

Genetic associations

To instrument vitamin K concentration, we used data from a GWAS meta-analysis of circulating phylloquinone (vitamin K1) comprising 2,138 individuals of European descent [8]. Circulating phylloquinone concentration (plasma or serum in nmol/L) was natural log transformed. Genetic associations were tested using linear models adjusted for age, sex, principal components, and study-specific covariates (e.g., study site, principal components, when applicable). The same phylloquinone GWAS has been used to instrument vitamin K in prior MR studies that demonstrated positive associations with ischaemic stroke [9] and coronary artery disease [10].

Genetic associations for OA were derived from the largest meta-analysis to date including all OA types (177,517 cases and 649,173 controls), knee (62,497 and 333,557), hip (36,445 and 316,943), spine (28,372 and 305,578), hand (20,901 and 282,881), finger (10,804 and 255,814), thumb (10,536 and 236,919), early age-at-onset OA (defined as <45 years; 6,838 cases 41,449 controls) and, as indicators of severe OA, total joint replacement (40,887 and 327,689), total hip replacement (23,021 and 296,016) and total knee replacement (18,200 and 233,841) [11]. OA phenotypes were defined using various combinations of International Classification of Diseases codes, joint replacement surgery, imaging or self-report; definitions along with demographics and genotyping methods are provide in supplementary materials of the original publication [11].

Since these data include non-European ethnicities, we also restricted analyses to an earlier GWAS of European ancestry individuals in the UK Biobank and arcOGEN, comprising 39,426 cases (24,955 knee and 15,704 hip) and 378,169 controls [12]. The current analysis used publicly available GWAS summary statistics, therefore did not require additional ethical approval.

Instrument identification and data harmonization

To instrument circulating phylloquinone, we selected independent genetic variants – lead variant from each locus to reduce “double counting” from correlated variants – from the 11 originally reported single nucleotide polymorphisms (SNPs) with p<1x10-6 (supplementary Table S1 and S2). None of the SNPs reached genome-wide significance [8]. Two SNPs near *CTNAA2* were not present in the OA GWAS and were excluded given no suitable proxies (r2>0.8) were available. Four variants were selected: rs2108622 (missense variant in the *CYP4F2* gene), rs4645543 (intergenic nearest to *KCNK9*), rs6862071 (intergenic nearest to *CDO1*) and rs964184 (3 prime untranslated region *ZNF259*). We performed sensitivity analyses without the potentially pleiotropic variant rs964184. We obtained the instruments from a discovery GWAS which is susceptible to “winner’s curse.” In the second-stage replication analysis of 265 individuals, the original authors reported suggestive (p<0.05) replication for two variants in the locus nearest to *CDO1*; we restricted to the lead variant rs6862071 in further sensitivity analyses.

Variance explained (r2) was calculated using 2EAF(1-EAF)β2, where EAF is the effect allele frequency. F statistic was approximated using the chi-square approximation [13]. F statistics >10 is considered suggestive of adequate instrument strength [14].

Statistical analysis

Assuming alpha of 0.05, analysis for all OA types (case control ratio 1:3.7) had >80% power to detect >3.5% lower odds (i.e., OR<0.965) of OA for each unit increase in vitamin K [15]. The analysis with the smallest sample size (early onset OA; case control ratio 1:10.8) was powered to detect >8% lower odds.

We used the inverse-variance weighted method [16] to combine effect estimates from each SNP using fixed-effect meta-analysis. For sensitivity analyses using a single variant, we used the Wald ratio method. Effect sizes are interpreted as per unit increase in log-transformed phylloquinone. The above-mentioned trial of vitamin K supplementation (0.5mg per day over 3 years) increased levels by 1.5 (SD 2.4) nmol/L in males and 2.3 (SD 2.7) nmol/L in females, which is numerically comparable to a 1 unit increase in log-transformed phylloquinone [8] (e.g., from 1.0 to 2.7 nmol/L).

We used the weighted median, weighed mode and MR Egger methods to evaluate the robustness of IVW estimates to horizontal pleiotropy [16] (whereby genetic variants influence the exposure and outcome via two separate biological pathways [17]). Lastly, we applied MR with robust adjusted profile score (MR-RAPS), which allows for the use of sub-genome wide significant variants and increases power to detect a significant effect [18]. Analyses were performed in R using the TwoSampleMR package [19].

**Results**

The four selected variants explained 5.5% of the variance for circulating phylloquinone, corresponding to a mean F statistic of 27 (range 18 to 33). Genetically predicted vitamin K1 levels were not associated with all OA types (OR 0.98; 95%CI 0.96, 1.01), knee (OR 0.98; 0.92, 1.03), hip (OR 0.97; 0.88, 1.07), spine (OR 0.97; 0.90, 1.04), hand (OR 0.97; 0.91, 1.04), early onset OA (OR 1.01; 0.90, 1.14), total joint (OR 0.96; 0.89, 1.04), total hip (OR 0.95; 0.85, 1.07), or total knee replacement (OR 0.94; 0.88, 1.01) (Figure 1). Scatter plots are shown in supplementary Figure S1.

Analysis excluding the potentially pleiotropic variant rs964184 produced similar results (supplementary Figure S2). Restricting to rs6862071 produced similar results (supplementary Figure S3) Analysis restricted to European populations (using UK Biobank and arcOGEN) showed similar results (supplementary Figure S4). Results of sensitivity analyses were consistent with the primary analysis and demonstrated no significant directional pleiotropy (supplementary Tables S3-4).

**Discussion**

We aimed to estimate the effect of genetically predicted vitamin K on risk of OA. Using the largest GWAS of OA to date, we found little statistical evidence of a causal association between genetically predicted vitamin K and risk of OA.

Our findings are concordant with those from a small RCT of vitamin K supplementation, which demonstrated no overall effect on radiographic hand OA [7]. In this RCT, a suggestive benefit on joint space narrowing (but not radiographic OA or osteophytes) was observed among trial participants who successfully corrected baseline vitamin K insufficient. These results should be interpreted with caveats of multiple testing and selection bias in mind. Nevertheless, potential non-linear or threshold effects of vitamin K should be a focus of future studies. MR estimates the effect of life-long levels of the exposure, thus effect sizes are typically larger than shorter-term therapeutic trials of the same [20]. If vitamin K had a causal role in OA pathology [2] our results suggest that the magnitude of any such effects may not be clinically meaningful.

By contrast, several observational studies reported large positive associations between vitamin K or vitamin K antagonist-use and OA. For example, individuals with very low vitamin K (<0.2nmol/L) had higher odds of MRI cartilage damage (OR 1.7; 95%CI 1.0, 3.0) and meniscus damage (OR 2.6; 1.3, 5.2) compared to those with sufficient levels (>1.0nmol/L) over three years [3]. Sub-clinical vitamin K deficiency (<0.5nmol/L) at baseline was associated with 56% higher risk (95%CI 1.08, 2.25) of incident radiographic (MRI) knee OA compared to those not deficient [4]. Discordance between the large observational effect sizes and the null findings from randomised studies (including RCT and MR) suggest that residual confounding may explain the observational association. For example, vitamin K1 is diet (predominantly leafy green vegetables) derived, thus dietary and other related lifestyle factors are all plausible confounders that are difficult to measure in observational settings. By analogy, there have been a plethora of strong observational associations reported between vitamins and health-related outcomes (e.g., vitamin C, D or E, each with equally plausible biology and more cumulative evidence) that failed to replicate in large RCTs [21]. It is for these reasons that evidence triangulation – including from MR – is required.

MR estimates relate to the potential effect of interventions on the naturally occurring range of vitamin K, but not the potential therapeutic effect of high-dose clinical interventions nor vitamin K antagonism. A cohort study showed new-users of acenocoumarol (a vitamin K antagonist) had 2.5 fold higher odds (95%CI 1.94, 3.20) of incidence or progression of knee and/or hip OA compared to non-users [5]. These findings may reflect vitamin K’s potential role in OA progression which we did not examine. However, non-users will include individuals with no indication for or contraindication to acenocoumarol; causal inference from such comparisons is problematic since overlap in measured characteristics is likely to be poor and unmeasured confounding may be substantial [22]. In a case-control study published alongside the above, knee or hip replacement (an indicator for end-stage OA) were compared between new-users of warfarin and direct oral anticoagulants (DOACs) [6]. The effect estimates were more modest (OR 1.59; 95%CI 1.31, 1.92) but nevertheless statistically significant. Although use of an active comparator helps to reduce confounding, there may still be channelling; for example, the greater ease (accessibility and lower cost) with which the effects of warfarin can be reversed, relative to DOACs, may make it more attractive for individuals who are likely to have joint replacement surgery in the near future. Matching in case-control designs may also introduce bias [23]. Further cohort studies using active comparators are needed to replicate these findings.

A strength of MR is its relative robustness to confounding and reverse causation compared to traditional observational designs, but we note some limitations to the current work. Current GWAS of vitamin K concentration have discovered only a handful of associated variants at sub-genome-wide significance. A key concern during peer review was the issue of sufficient instrument strength, for which we offer the following points to consider. First, the F statistic was above the rule-of-thumb F>10 for all four variants, including the replicated variant rs6862071. Second, prior MR studies reported positive associations between vitamin K levels and cardiovascular disease [9,10] using these instruments, which serve as positive controls (i.e., vitamin K antagonists are used to reduce cardiovascular risk in certain settings). Third, we applied sensitivity methods that account for sub-genome-wide significant instruments further lend support to the robustness of our findings. Nevertheless, future studies are needed to identify stronger instruments for vitamin K. Such studies are also required to mitigate potential for “winners’ curse”, as was the case in our study where the same GWAS was used for instrument discovery and effect estimate. This may lead to overestimation of the variance explained in the discovery cohort and subsequent power calculations. The current analysis estimates the effect of vitamin K on OA susceptibility and may not generalise to OA progression. We excluded potentially pleiotropic SNPs but cannot guarantee against bias from horizontal pleiotropy. Pleiotropy robust sensitivity analyses were overall reassuring but may have limited utility with the small number of SNPs. Lastly, the study population included mainly participants of European ancestry, future study among other ethnic populations may be helpful in confirming the generalizability of the current findings.

In summary, we found little statistical evidence of a causal association between genetically predicted vitamin K and risk of OA development. These results suggest that population level vitamin K supplementation is unlikely to reduce OA incidence. Larger genetic and interventional studies of vitamin K and OA are needed.

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**Author contributions**

All authors – SZ, JB, DH, TS, CZ, HL – have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted. SZ takes responsibility for the integrity of the work as a whole, from inception to finished article.

**Conflict of interests statement**

The authors declare no conflicts of interest.

**Declaration of funding**

SSZ was funded by a National Institute for Health Research (NIHR) Academic Clinical Lectureship. JB is presently an employee and shareholder of Regeneron Pharmaceuticals Inc, and the presented work was performed independently from this employment. HL was funded by the Excellent Young Scholars Training Program from The Chinese PLA General Hospital (2020-YQPY-001) and the China Postdoctoral Science Foundation (2020M682593). Remain authors do not have disclosures.

**Role of the funding source**

The funding sources had no input into study design, in the collection, analysis and interpretation of data; in the writing of the manuscript; or in the decision to submit the manuscript for publication.

**Availability of data**

Summary statistics for the osteoarthritis GWAS was made publicly available by the study authors at msk.hugeamp.org

**Figure legends:**

Figure 1. Two-sample Mendelian randomization estimate for effect of vitamin K on risk of OA (n=number of cases). Estimates presented as odds ratios (OR) of OA, per unit increase in log-transformed vitamin K.

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