Running title: Vitamin K and osteoarthritis

**Title: Vitamin K and risk of osteoarthritis: Mendelian randomization study**

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Funding and disclosures:

SSZ was funded by a National Institute for Health Research (NIHR) Academic Clinical Lectureship. JB is employed by Regeneron Genetics Center.

Word count: 1500

**Abstract 217/250**

**Objective.** Vitamin K levels and vitamin K antagonism are consistently associated with risk and progression of osteoarthritis (OA) in observational studies that are susceptible to confounding and reverse causation. The sole randomised trial of vitamin K supplementation did not show an effect on OA progression. We examined the causal association between vitamin K and OA risk using mendelian randomisation (MR).

 **Methods.** In this two-sample MR analysis, 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 on risk of all OA types, knee, hip, spine and hand OA. We employed multiple MR methods and sensitivity analyses to test for potential bias due to pleiotropy.

**Results.** We showed that genetically predicted vitamin K levels was not causally associated with risk of all OA types (OR 1.00 per unit increase in log-transformed vitamin K1; 95%CI 0.97, 1.02). Results were similar for all OA phenotypes and across all sensitivity analyses.

**Conclusion.** Thelack of causal relationship between vitamin K and OA suggests that prior positive observational findings were likely influenced by confounding or reverse causation. Population level vitamin K supplementation is unlikely to reduce OA incidence. Equally, vitamin K antagonism with warfarin is unlikely to have any meaningful detrimental effects.

**Keywords:** vitamin K, osteoarthritis, mendelian randomisation

**Introduction**

Observational studies suggest that vitamin K may play a role in the pathogenesis of osteoarthritis (OA). Low vitamin K level, in serum or dietary intake, were each associated with increased prevalence of radiographic OA features [1,2]. In longitudinal studies, low vitamin K was linked to 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 the vitamin K antagonist warfarin. Warfarin-use has been associated with increased incidence and progression of, and joint replacement (as an indicator of advanced disease) in, knee and hip OA [5,6].

These observational designs are susceptible to bias from reverse causation (OA or its sequelae may influence dietary intake/absorption) and confounding (unmeasured characteristics that channel anticoagulant use or choice). A randomised controlled trial of vitamin K supplementation (n=193 vs 185 placebo) over 3 years showed no effect on radiographic hand OA, joint space narrowing or osteophytes [7]. The trial may have been limited by sample size and, importantly, duration. Whether by deficiency or antagonism, vitamin K levels are likely to influence OA risk over years if not decades. The causal role of vitamin K on OA is therefore unclear and will remain challenging to elucidate.

Mendelian randomization (MR) is an observational design that uses genetic instrumental variables to estimate the causal effect of the exposure on the outcome. Since variants are randomly allocated at conception, MR is less susceptible to reverse causation and confounding than other observational designs. Here, it can be conceptualised as a quasi-randomised natural experiment comparing OA risk in subpopulations with higher vs lower genetically predicted life-long vitamin K levels. Our aim was to use two-sample MR to investigate the effect of vitamin K on knee or hip OA risk.

**Patients and Methods**

Genetic associations

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 (to account for potential confounding from underlying population structure), and triglyceride concentration (phylloquinone is transported on triglyceride-rich lipoproteins).

Genetic associations for OA were derived from the largest meta-analysis to date of up to 826,690 individuals, 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) and early age-at-onset OA (defined as <45 years; 6,838 cases 41,449 controls) [9]. 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 [10]. All OA phenotypes were defined by radiograph, joint replacement or hospital diagnosis.

Instrument identification and data harmonization

We selected genetic instruments for phylloquinone from the 11 originally reported single nucleotide polymorphisms (SNPs) with p<1x10-6; none of the SNPs reached genome-wide significance [8]. We retained three SNPs for analysis rs2108622 (missense variant in the CYP4F2 gene), rs4645543 (intergenic nearest to KCNK9), rs6862071 (intergenic nearest to CDO1). We excluded the intron SNPs in CTNAA2 (rs4852146, rs2192574) that were not present in the OA GWAS, and rs964184 which was highly pleiotropic. Effect alleles were checked to be on the forward strand. The F statistic is derived from the variance explained (r2) by (r2/K)/[(1-r2)(N-K-1)], where K is the number of SNPs and N the sample size. F statistics >10 is considered suggestive of adequate instrument strength [11].

Statistical analysis

We used the inverse-variance weighted method [12] to combine effect estimates from each SNP using fixed-effect meta-analysis. 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 1 unit increase in log-transformed phylloquinone (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 [12]. Horizontal pleiotropy is a main source of bias in MR, whereby genetic variants influence the exposure and outcome via two separate biological pathways [13]. Analyses were performed in R using the TwoSampleMR package [14].

**Results**

The three selected SNPs explained 4.6% of the variance for circulating phylloquinone, corresponding to an F statistic of 34. Genetically predicted vitamin K1 levels did not have an effect on all OA types (OR 1.00; 95%CI 0.97, 1.02), knee (OR 0.99; 0.94, 1.04), hip (OR 1.00; 0.92, 1.08), spine (OR 1.00; 0.94, 1.06), hand (OR 0.98; 0.91, 1.06), or early onset OA (OR 1.03; 0.91, 1.17) (Figure 1).

Analysis restricted to European populations showed the same results (supplementary table 1). Results of sensitivity analyses were consistent with the primary analysis and demonstrated no significant directional pleiotropy (supplementary table 2).

**Discussion**

We performed the first two-example MR study to investigate the causal effect of vitamin K on risk of OA. Using the largest GWAS of OA to date, we showed that genetically predicted vitamin K levels was not associated with risk of OA. These results suggest that population level vitamin K supplementation is unlikely to reduce OA incidence. Equally, vitamin K antagonism with warfarin is unlikely to have any meaningful detrimental effects.

Our findings are concordant with those from the small RCT of vitamin K supplementation, which demonstrated no effect on radiographic hand OA [7]. 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 [15]. Even if vitamin K had a causal role in OA pathology (vitamin K is involved in the post-translational carboxylation that confers functionality to proteins potentially for bone health [16]) the magnitude of any such effects is unlikely to be clinically meaningful.

By contrast, prior observational studies reported large effect sizes. 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]. 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]. Similarly, a case control study of individuals with atrial fibrillation showed that new-users of warfarin (compared to direct oral anticoagulants, DOAC) had 59% higher odds (95%CI 1.31, 1.92) of knee or hip replacement (an indicator for end-stage knee OA) [6].

The totality of observational evidence would strongly suggest interventions to supplement vitamin K and change vitamin K antagonists to other anti-coagulants. The latter has important clinical implications since indications for anticoagulation such as atrial fibrillation is commonly comorbid with OA in elderly individuals. However, they were not supported by our findings. The discordance between large observational effect sizes and those from randomised (and quasi-randomised MR) designs suggest that strong residual confounding may be driving the observed effect. Vitamin K1 is diet (predominantly leafy green vegetable) derived, thus dietary and other related lifestyle factors are all plausible confounders that are difficult to measure in observational settings. Similarly, the ease with which the effects of warfarin can be reversed (in contrast to DOACs) makes it more attractive for individuals who are likely to have surgery in the near future.

They key strength of MR is its relative robustness to confounding and reverse causation compared to traditional observational designs. However, some MR assumptions required for valid causal inference are not empirically verifiable [12]. Certain assumption, such as linearity of the exposure-outcome relationship and monotonicity (i.e., a variant cannot increase the exposure in some individuals and decrease it in others), are not required to test the causal null, as in this analysis. The F statistic also suggested sufficient instrument strength, despite lack of genome-wide significant SNPs. Future studies should seek to identify stronger instruments for vitamin K. The current analysis estimates the effect of vitamin K on OA susceptibility and may not fully generalise to OA prognosis. However, it is unlikely that the proposed mechanism through which vitamin K acts on OA differs between the two. Lastly, we excluded potentially pleiotropic SNPs but cannot guarantee against bias from horizontal pleiotropy. Sensitivity analyses were overall reassuring but may have limited utility with the limited number of SNPs.

In summary, this two-sample MR study leveraged the largest OA GWAS to date to show that genetically predicted vitamin K level has not causal effect on risk of OA. These findings suggest that population level vitamin K supplementation is unlikely to reduce OA incidence. Equally, clinicians need not be concerned about detrimental effects of vitamin K antagonists on OA.

**Acknowledgements.**

We are grateful to the Genetics of Osteoarthritis (GO) consortium who made the osteoarthritis GWAS summary data publicly available.

**Author contributions**

SZ (1a, 1c, 2, 3), JB (1a, 1c, 2, 3), DH (1a, 1c, 2, 3), RS (1a, 1c, 2, 3), HL (1a, 1c, 2, 3)

**Availability of data**

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

**Conflict of interests**

The authors declare no conflicts of interest.

**Role of funding source**

SSZ was funded by a National Institute for Health Research (NIHR) Academic Clinical Lectureship. JB is employed by Regeneron Genetics Center……

**Patient and public involvement**

PPI was not included in this study using existing summary statistics.

**Figure legends:**

Figure 1. Two-sample Mendelian randomization estimate for effect of vitamin K on risk of OA (n=number of cases).



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