Circulating Phylloquinone Concentrations and Risk of Type 2 Diabetes: A Mendelian Randomization Study

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This study investigated the causal relation between circulating phylloquinone (vitamin K_1) concentrations and type 2 diabetes by using a Mendelian randomization (MR) approach. We used data from three studies: the European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct case-cohort study, Diabetes Genetics Replication and Meta-analysis (DIAGRAM), and the UK Biobank, resulting in 69,647 subjects with type 2 diabetes. We calculated a weighted genetic risk score including four genetic variants previously found to be associated with circulating phylloquinone concentrations. Inverse-variance weighted analysis was used to obtain a risk ratio (RR) for the causal relation between circulating phylloquinone concentrations and risk of type 2 diabetes. Presence of pleiotropy and the robustness of the results were assessed using MR-Egger and weighted-median analyses. Genetically predicted concentrations of circulating phylloquinone were associated with lower risk of type 2 diabetes with an RR of 0.93 (95% CI 0.89; 0.97) per every natural logarithm (Ln)-nmol/L–unit increase in circulating phylloquinone. The MR-Egger and weighted median analyses showed RRs of 0.94 (0.86; 1.02) and 0.93 (0.88; 0.98), respectively, indicating no pleiotropy. In conclusion, our study supports that higher circulating phylloquinone may be causally related with lower risk of type 2 diabetes, highlighting the importance of sufficient phylloquinone in the human diet.

Vitamin K is a fat-soluble vitamin occurring in two biologically active forms: phylloquinone (vitamin K_1) and menaquinones (vitamin K_2). Phylloquinone is the most common form in circulation and is predominantly present in green leafy vegetables (1). Higher phylloquinone intake was associated with a lower risk of type 2 diabetes in observational studies (2,3). Intervention studies also showed improved insulin sensitivity after phylloquinone supplementation, although the results were inconsistent (4–6), and these studies did not use the gold standard (hyperinsulinemic-euglycemic clamp) to measure insulin sensitivity (4–6). The underlying mechanism still is unknown, but vitamin K–dependent carboxylation of osteocalcin and a possible anti-inflammatory effect of vitamin K are suggested (7,8).

Current evidence thus points toward a beneficial role for phylloquinone in the prevention of type 2 diabetes, but the study designs used each have certain limitations. Observational studies may be hampered by reverse causation or confounding (9) because phylloquinone coexists with vitamins and phytochemicals of vegetables, which are likely to affect the risk of noncommunicable diseases in

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diverse pathways (7). Intervention studies are limited by having a short duration and focus on intermediate risk factors for type 2 diabetes, such as insulin sensitivity, instead of risk of type 2 diabetes. Due to these limitations, the causal association between phylloquinone and type 2 diabetes remains debatable.

No study to date has assessed the association between circulating phylloquinone concentrations and type 2 diabetes using a Mendelian randomization (MR) approach. This study therefore investigated the causal relation between circulating phylloquinone concentrations and type 2 diabetes using a MR approach in which we used a set of genetic variants as an instrumental variable.

RESEARCH DESIGN AND METHODS

Study Populations

For these analyses, we used data from three studies: European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct case-cohort study, Diabetes Genetics Replication and Meta-analysis (DIAGRAM), and the UK Biobank. Individual participant data were only available in EPIC-InterAct. EPIC-InterAct is a prospective case-cohort study nested within EPIC, involving eight European countries (Denmark, France, Germany, Italy, the Netherlands, Spain, Sweden, and the U.K.) (10). EPIC-InterAct identified 12,403 incident case subjects with type 2 diabetes between 1991 and 2007 and a randomly selected a subcohort of 16,154 participants (10). After exclusion of individuals with missing genetic data (n = 2,332 case subjects and n = 3,115 subcohort members) and excluding 1,528 participants from EPIC-Norfolk (which contributes to DIAGRAM), 9,400 case subjects and 12,182 subcohort members were left for analyses (this subcohort also included a set of 598 incident case subjects reflecting the case-cohort design). The mean age in the subcohort was 52.4 (SD 9.2) years, 38.4% were men, and mean BMI was 26.0 kg/m^2.

Summarized data were included from DIAGRAM and the UK Biobank. The aim of DIAGRAM is to characterize genetic information for type 2 diabetes, mainly in samples of participants of European descent. DIAGRAM included data from 23 studies, which meta-analyzed genetic variants on Metabochip (11). DIAGRAM included men and women, mainly of European ancestry, aged 43–72 years, with BMIs varying from 22.3 to 33.4 kg/m^2 among the case-control or cohort participants. Data are publicly available at www.diagram-consortium.org.

The UK Biobank is a large, population-based cohort study established to study the interrelationships between environment, lifestyle, and genes. The UK Biobank (www.ukbiobank.ac.uk) recruited more than 500,000 men and women between 2006 and 2010 (application number 9161, 9905, 12885, 20684) (12). Age varied between 37 and 73 years, and the mean BMI was 27.5 (SD 4.8) kg/m^2. The UK Biobank was approved by the North West Multi-Centre Research Ethics Committee, and all participants provided written informed consent to participate in the UK Biobank study. EPIC-InterAct, DIAGRAM, and the UK Biobank included up to 69,647 subjects with type 2 diabetes and 551,336 participants without diabetes.

Genotyping and Construction of Weighted Genetic Risk Score

For EPIC-InterAct, DNA was extracted from the buffy coat from citrated blood samples. Participants were genotyped with the Illumina Human Core Exome chip and the Illumina Human Quad 660 chip. Missing genotypes were imputed by the Haplotype Reference Consortium v1.0, using IMPUTE v2.3.2, and was performed at the Wellcome Trust Centre for Human Genetics.

Genotyping in the UK Biobank was performed using the Affymetrix UK BiLEVE Axiom array and the Affymetrix UK Biobank Axiom Array (13). DIAGRAM participants were genotyped using different assays, as described previously (14).

We included 4 single nucleotide polymorphisms (SNPs) selected from 11 SNPs that were likely to predict circulating phylloquinone (plasma or serum) among 2,138 European descents in a genome-wide meta-analysis study (rs964184, rs4645543, rs2199565, rs7018214, rs2108622, rs2192574, rs4852146, rs6862909, rs6862071, and rs4122275): these SNPs passed the threshold of $P < 5 \times 10^{-6}$ but not $P < 5 \times 10^{-8}$ (15). Among the 11 SNPs, linkage disequilibrium at $R^2 > 0.9$ was evident among rs2192574 and rs4852146 (chromosome 2); rs6862909, rs6862071, and rs4122275 (chromosome 5); rs4645543, rs2199565, and rs7018214 (chromosome 8); and rs2108622 and rs12609820 (chromosome 19). Therefore, five SNPs with the strongest association with circulating phylloquinone from each chromosome were initially selected (rs2108622, rs2192574, rs4645543, rs6862071, and rs964184).

We further excluded rs964184 with pleiotropic effects on blood lipids and coronary heart disease by searching genome-wide association study (GWAS) publications in the GWAS Catalog and PhenoScanner for the SNPs or its proxies ($R^2 > 0.8$) and querying associations of the SNPs with variables indicating pleiotropic effects with $P < 5 \times 10^{-8}$ (16,17). After these exclusions, rs2108622, rs2192574, rs4645543, and rs6862071 were retained for the current study.

In all three cohorts, we calculated a weighted genetic risk score (wGRS) by summing the number of effect alleles coded as 0, 1, or 2 at each individual SNP weighted by the respective allelic effect sizes on circulating phylloquinone concentrations from the previous genome-wide meta-analysis study (15).

Diabetes Ascertainment

For EPIC-InterAct, a detailed description of ascertainment and verification of subjects with incident diabetes has been described previously (10). Briefly, occurrence

of type 2 diabetes during follow-up was identified through self-report, linkage to primary care registers, secondary care registers, medication use, and hospital admissions and mortality data. Type 2 diabetes was determined when at least two sources identified the diagnosis of type 2 diabetes. Diagnosis of type 2 diabetes in DIAGRAM differed per study, as described previously (14). Type 2 diabetes diagnosis in the UK Biobank was self-reported at baseline, as previously described (18).

Statistical Analysis

Individual participant data analysis was performed in EPIC-InterAct. Accounting for its case-cohort design, Prentice-weighted Cox regression (19) was used to quantify the association of the individual SNPs and the wGRS with type 2 diabetes risk. Age was used as an underlying timescale. The associations were evaluated in strata of countries and adjusted for sex, study sites, principal components of ancestry, and genetic array. For each of the individual SNPs and the wGRS, we combined the hazard ratios obtained from EPIC-InterAct with odds ratios (ORs) from DIAGRAM and UK Biobank as risk ratios (RRs) using a random effect meta-analysis. Then, inverse-variance weighted (IVW) estimates for the causal association between circulating phylloquinone and type 2 diabetes risk were calculated using the formulas reported by Burgess et al. (20). The ratio estimate of the causal effect of circulating phylloquinone on diabetes risk was calculated as the association between the SNP and diabetes risk divided by the association between the SNP and circulating phylloquinone. The IVW estimate combines the ratio estimates using each variant in a fixed-effect meta-analysis model (21).

We quantified the *F* statistic to assess the strength of the genetic instrument by using the proportion of variance explained by the genetic variants, the sample size, and number of variants included (22). To assess the presence of unbalanced pleiotropy, we performed a MR-Egger and weighted-median analysis in all cohort studies using the "MendelianRandomization" package in R (23–25). Unbalanced pleiotropy was considered present if the intercept significantly deviated from zero ($P < 0.05$). In the subcohort of EPIC-InterAct only, we assessed potential pleiotropic effects of the wGRS on demographic, lifestyle, and physiological variables using logistic and linear regression analyses in strata of countries and adjusted for sex, center, principal components, and genetic array.

Phylloquinone is carried predominantly in triglyceride-rich lipoproteins, and β-coefficients for the gene-phylloquinone associations adjusted for fasting triglycerides were also reported in the previous GWAS (15). We therefore performed sensitivity analyses, in EPIC-InterAct data only, using the β-coefficients adjusted for triglycerides and corrected the association between the wGRS and diabetes incidence for triglycerides and hours of fasting. Missing values on triglycerides (5.1%) and hours of fasting (32.3%) were imputed with predictive mean matching. We used a maximum of 50 iterations and 10 imputations using the R package "mice" (26). This analysis was also performed in the complete case subject data set to assess the influence of the large number of missing values for hours of fasting. Finally, we calculated ORs in EPIC-InterAct and pooled these with the ORs in DIAGRAM and UK Biobank instead of combining the hazard ratio (HR) in EPIC-InterAct with ORs in DIAGRAM and UK Biobank. All analyses were performed using R v3.1.1 software.

RESULTS

For EPIC-InterAct participants only, baseline characteristics are described in Supplementary Table 1, and the distributions of the alleles of each phylloquinone-related SNP are shown by country in Supplementary Table 2. We did not detect any association between genetically predicted circulating phylloquinone and possible pleiotropic traits (Supplementary Table 3).

The IVW analyses showed that higher genetically predicted concentrations of circulating phylloquinone were associated with a lower risk of type 2 diabetes in EPIC-InterAct, with an RR of 0.88 (95% CI 0.78; 0.99) per natural logarithm (Ln)-nmol/L increase in circulating phylloquinone (Fig. 1). Similar results were observed in DIAGRAM (RR 0.90 [95% CI 0.81; 1.00]) and UK Biobank (RR 0.95 [95% CI 0.91; 1.00]). Pooling the three data sets (EPIC-InterAct, DIAGRAM, and UK Biobank) resulted in an RR for the wGRS of 0.93 (95% CI 0.89; 0.97) per Ln-nmol/L increase in circulating phylloquinone. There was no heterogeneity between the studies (I^2 = 0.3%, P = 0.96). No association was observed for the individual SNPs; however, the directions of genetic associations with the outcome were consistent across the four SNPs, and there was some suggestion of a dose-response relationship in the genetic associations with the risk factor and outcome.

The included SNPs explained 4.7% of the variation in circulating phylloquinone in the GWAS, resulting in an *F* statistic of 208.9 in our study. The MR-Egger method indicated no pleiotropic effects (intercept = −0.02, SE = 0.01, P = 0.88) and resulted in an RR of 0.94 (95% CI 0.86; 1.02) per Ln-nmol/L in circulating phylloquinone (Supplementary Fig. 1). The weighted median method resulted in an RR of 0.93 (95% CI 0.88; 0.98) per Ln-nmol/L in circulating phylloquinone.

In EPIC-InterAct data only, additional adjustments for triglycerides and hours of fasting did not alter the results and resulted in an HR for the wGRS of 0.87 (95% CI 0.78; 0.98) in the IVW analyses (Supplementary Table 4). Similar results were observed in a complete case subjects analysis (HR wGRS 0.82 [95% CI 0.71; 0.95]). Finally, calculating ORs in EPIC-InterAct resulted in comparable pooled results (OR wGRS 0.93 [95% CI 0.89; 0.98)].

DISCUSSION

Our study supports that higher circulating phylloquinone may be causally related with lower type 2 diabetes risk.

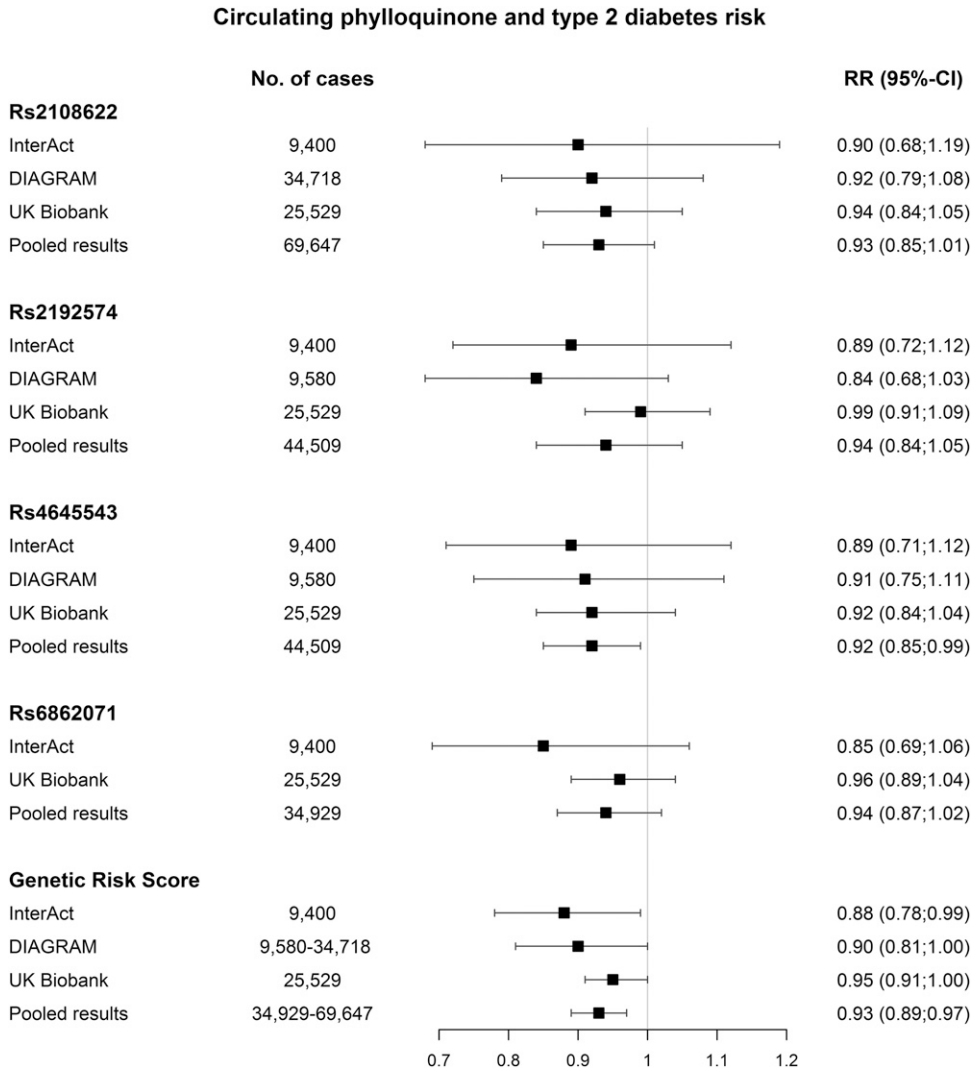

Figure 1—Causal estimates of circulating phylloquinone and diabetes risk: the MR analyses in EPIC-InterAct, DIAGRAM, and UK Biobank. wGRS represents the summary genetic effect of the four variants. RR and 95% CIs were per 1-unit increase in Ln of circulating phylloquinone (nmol/L).

This is the first MR study linking circulating phylloquinone concentrations to the risk of type 2 diabetes. Our results are in line with the current evidence on this association from observational and intervention studies. Two observational studies found that higher phylloquinone intake was associated with a lower type 2 diabetes incidence (2,3). Current evidence from trials is not conclusive on the effect of phylloquinone supplementation on insulin sensitivity and insulin resistance (4–6). Two studies observed improved insulin sensitivity after phylloquinone supplementation (4,6) after interventions of 4 weeks (6) or 3 years (4). By contrast, another small-scale study did not find improved insulin sensitivity after 1 year of phylloquinone supplementation (5). Although the inconsistency should be acknowledged, altogether, the available evidence from MR, observational studies, and trials supports a causal relation between higher circulating phylloquinone concentrations and a reduced risk of type 2 diabetes. This evidence highlights the need of sufficient phylloquinone in the diet and supports the further development of phylloquinone requirements.

The mechanism is still unknown, however, and it is possible that the causation is due to a biological pathway retaining high phylloquinone concentrations but not due to phylloquinone itself. Furthermore, because phylloquinone concentrations and phylloquinone intake are only modestly correlated (r = 0.51) (27), our results cannot be directly translated to phylloquinone intake. Nonetheless, these results highlight the need for further work relating phylloquinone metabolism to the etiology of type 2 diabetes.

MR studies only provide evidence about the causality of an association when three assumptions are met. We will discuss each assumption with its strengths and limitations. The first assumption implies a strong association between the instrumental variable (wGRS) and the risk factor, in

our case, circulating phylloquinone. None of the SNPs selected for this study reached genome-wide significance in the phylloquinone genome-wide meta-analysis study (15). rs2108622 is a *CYP4F2* variant that functions as a phylloquinone oxidase (28). Previous studies showed that rs2108622 was associated with altered phylloquinone metabolism and warfarin dosage (29). Because of the biologically plausible effect of rs2108622 on circulating concentrations of phylloquinone, we considered this functional SNP causally altering circulating phylloquinone. However, this genetic variant did not have a genome-wide significant association with phylloquinone concentrations in the GWAS (15). Presumably, this was due to the relatively small sample size (n = 2,138) of the meta-analysis and the low minor allele frequency (15). Although the F statistic showed no indication of weak instrument bias (288.2 in EPIC-InterAct) and the included SNPs explained 4.7% of the variation in circulating phylloquinone in the small-scale GWAS, the F statistic might be an overestimation of the true effect, because there might be an overestimation of the R^2 of the GWAS due to winner's curse resulting in effect size estimates that are upwardly biased (30). The F statistic might be an overestimation, but there is still no indication of weak instrument, because the F statistic is much higher than the arbitrary threshold of 10. The wGRS has not been validated in an external data set because no large scale cohort studies have performed a GWAS and assessed circulating phylloquinone levels apart from those included in the GWAS by Dashti et al. (15). Because of this lack of validation and because none of the SNPs reached genome-wide significance, our findings need to be interpreted with caution. Additional GWAS data on circulating phylloquinone or other markers of vitamin K status are warranted to further investigate causal roles of vitamin K and its metabolic pathway in the development of type 2 diabetes.

The second and third assumption comprises no association between the instrumental variable and outcome via confounders or other mechanisms. The MR-Egger and the linear and logistic regression analyses showed no indication of pleiotropy. Still, we note that rs2108622 is a variant in the *CYP4F2* gene and may play a role in transport and metabolism of vitamin E (31). Notably, a trial of vitamin E showed no evidence of its effect on type 2 diabetes (32), whereas vitamin E may antagonize vitamin K (33). We found no evidence of pleiotropy in the genetically predicted effect of vitamin K on diabetes risk, but genetics and the trial both support the causal role of vitamin E in vitamin K metabolism, and this nutrient-nutrient interaction is of further interest also in etiology of type 2 diabetes. Although we did not find any indication of pleiotropy, circulating phylloquinone may be a biomarker that reflects healthy lifestyle in general instead of phylloquinone specifically.

In conclusion, this study supports that higher circulating phylloquinone concentrations are causally related with lower type 2 diabetes risk, highlighting the importance of sufficient amounts of phylloquinone in the human diet.

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References

1. Bolton-Smith C, Price RJ, Fenton ST, Harrington DJ, Shearer MJ. Compilation of a provisional UK database for the phylloquinone (vitamin K1) content of foods. Br J Nutr 2000;83:389–399

2. Beulens JWJ, van der A DL, Grobbee DE, Sluijs I, Spijkerman AMW, van der Schouw YT. Dietary phylloquinone and menaquinones intakes and risk of type 2 diabetes. Diabetes Care 2010;33:1699–1705

3. Ibarrola-Jurado N, Salas-Salvadó J, Martínez-González MA, Bulló M. Dietary phylloquinone intake and risk of type 2 diabetes in elderly subjects at high risk of cardiovascular disease. Am J Clin Nutr 2012;96:1113–1118

4. Yoshida M, Jacques PF, Meigs JB, et al. Effect of vitamin K supplementation on insulin resistance in older men and women. Diabetes Care 2008;31:2092–2096

5. Kumar R, Binkley N, Vella A. Effect of phylloquinone supplementation on glucose homeostasis in humans. Am J Clin Nutr 2010;92:1528–1532

6. Rasekhi H, Karandish M, Jalali MT, et al. The effect of vitamin K1 supplementation on sensitivity and insulin resistance via osteocalcin in prediabetic women: a double-blind randomized controlled clinical trial. Eur J Clin Nutr 2015; 69:891–895

7. Shea MK, Booth SL. Concepts and controversies in evaluating vitamin K status in population-based studies. Nutrients 2016;8:8

8. Ferron M, Hinoi E, Karsenty G, Ducy P. Osteocalcin differentially regulates beta cell and adipocyte gene expression and affects the development of metabolic diseases in wild-type mice. Proc Natl Acad Sci U S A 2008;105:5266–5270

9. Smith GD, Ebrahim S. Mendelian randomization: prospects, potentials, and limitations. Int J Epidemiol 2004;33:30–42

10. InterAct Consortium, Langenberg C, Sharp S, Forouhi NG, et al. Design and cohort description of the InterAct Project: an examination of the interaction of genetic and lifestyle factors on the incidence of type 2 diabetes in the EPIC Study. Diabetologia 2011;54:2272–2282

11. Morris AP, Voight BF, Teslovich TM, et al.; Wellcome Trust Case Control Consortium; Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) Investigators; Genetic Investigation of ANthropometric Traits (GIANT) Consortium; Asian Genetic Epidemiology Network–Type 2 Diabetes (AGEN-T2D) Consortium; South Asian Type 2 Diabetes (SAT2D) Consortium; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. Large-scale association

analysis provides insights into the genetic architecture and pathophysiology of type 2 diabetes. Nat Genet 2012;44:981–990
12. Collins R. What makes UK Biobank special? Lancet 2012;379:1173–1174
13. UK Biobank: protocol for a large-scale prospective epidemiological resource [Internet], 2006. Available from www.ukbiobank.ac.uk/resources/. Accessed 20 May 2018
14. Gaulton KJ, Ferreira T, Lee Y, et al.; DIAbetes Genetics Replication And Meta-analysis (DIAGRAM) Consortium. Genetic fine mapping and genomic annotation defines causal mechanisms at type 2 diabetes susceptibility loci. Nat Genet 2015; 47:1415–1425
15. Dashti HS, Shea MK, Smith CE, et al. Meta-analysis of genome-wide association studies for circulating phylloquinone concentrations. Am J Clin Nutr 2014;100:1462–1469
16. MacArthur J, Bowler E, Cerezo M, et al. The new NHGRI-EBI Catalog of published genome-wide association studies (GWAS Catalog). Nucleic Acids Res 2017;45:D896–D901
17. Staley JR, Blackshaw J, Kamat MA, et al. PhenoScanner: a database of human genotype-phenotype associations. Bioinformatics 2016;32:3207–3209
18. Eastwood SV, Mathur R, Atkinson M, et al. Algorithms for the capture and adjudication of prevalent and incident diabetes in UK Biobank. PLoS One 2016;11: e0162388
19. Prentice RL. A case-cohort design for epidemiologic cohort studies and disease prevention trials. Biometrika 1986;73:1–11
20. Burgess S, Thompson SG. *Mendelian Randomization : Methods for Using Genetic Variants in Causal Estimation*. Didcot, U.K., Taylor & Francis, 2015
21. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol 2013;37: 658–665
22. Burgess S, Thompson SG; CRP CHD Genetics Collaboration. Avoiding bias from weak instruments in Mendelian randomization studies. Int J Epidemiol 2011; 40:755–764
23. Yavorska OO, Burgess S. MendelianRandomization: an R package for performing Mendelian randomization analyses using summarized data. Int J Epidemiol 2017;46:1734–1739
24. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol 2015;44:512–525
25. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. Genet Epidemiol 2016;40:304–314
26. van Buuren S, Groothuis-Oudshoorn K. mice: multivariate imputation by chained equations in *R*. J Stat Softw 2011;45:1–67
27. Booth SL, Tucker KL, McKeown NM, Davidson KW, Dallal GE, Sadowski JA. Relationships between dietary intakes and fasting plasma concentrations of fat-soluble vitamins in humans. J Nutr 1997;127:587–592
28. Edson KZ, Prasad B, Unadkat JD, et al. Cytochrome P450-dependent catabolism of vitamin K: ω-hydroxylation catalyzed by human CYP4F2 and CYP4F11. Biochemistry 2013;52:8276–8285
29. McDonald MG, Rieder MJ, Nakano M, Hsia CK, Rettie AE. CYP4F2 is a vitamin K1 oxidase: an explanation for altered warfarin dose in carriers of the V433M variant. Mol Pharmacol 2009;75:1337–1346
30. Xiao R, Boehnke M. Quantifying and correcting for the winner's curse in genetic association studies. Genet Epidemiol 2009;33:453–462
31. Major JM, Yu K, Wheeler W, et al. Genome-wide association study identifies common variants associated with circulating vitamin E levels. Hum Mol Genet 2011;20:3876–3883
32. Kataja-Tuomola M, Sundell JR, Männistö S, et al. Effect of α-tocopherol and β-carotene supplementation on the incidence of type 2 diabetes. Diabetologia 2008;51:47–53
33. Booth SL, Golly I, Sacheck JM, et al. Effect of vitamin E supplementation on vitamin K status in adults with normal coagulation status. Am J Clin Nutr 2004;80: 143–148