# Meta-analysis of genome-wide association studies for circulating phylloquinone concentrations[1–5]

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## ABSTRACT

**Background:** Poor vitamin K status is linked to greater risk of several chronic diseases. Age, sex, and diet are determinants of circulating vitamin K; however, there is still large unexplained interindividual variability in vitamin K status. Although a strong genetic component has been hypothesized, this has yet to be examined by a genome-wide association (GWA) study.

**Objective:** The objective was to identify common genetic variants associated with concentrations of circulating phylloquinone, the primary circulating form of vitamin K.

**Design:** We conducted a 2-stage GWA meta-analysis of circulating phylloquinone in 2 populations of European descent from the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium Nutrition Working Group. Circulating phylloquinone was measured by using reversed-phase high-performance liquid chromatography. Results from adjusted cohort-specific discovery GWA analyses were meta-analyzed with inverse variance weights ($n = 2138$). Associations with circulating phylloquinone at $P < 1 \times 10^{-6}$ were then evaluated in a second-stage analysis consisting of one independent cohort ($n = 265$).

**Results:** No significant association was observed for circulating phylloquinone at the genome-wide significance level of $5 \times 10^{-8}$. However, from the discovery GWA, there were 11 single-nucleotide polymorphism (SNP) associations with circulating phylloquinone at $P < 1 \times 10^{-6}$, including a functional variant previously associated with warfarin dose and altered phylloquinone metabolism. These SNPs are on 5 independent loci on 11q23.3, 8q24.3, 5q22.3, 2p12, and 19p13.12, and they fall within or near the candidate genes *APOA1/C3/A4/A5* cluster (involved in lipoprotein metabolism), *COL22A1, CDO1, CTNAA2,* and *CYP4F2* (a phylloquinone oxidase), respectively. Second-stage analysis in an independent cohort further suggests the association of the 5q22.3 locus with circulating phylloquinone ($P < 0.05$).

**Conclusions:** Multiple candidate genes related to lipoprotein and vitamin K metabolism were identified as potential determinants of circulating phylloquinone. Further investigation with a larger sample is warranted to verify our initial findings and identify other loci contributing to circulating phylloquinone. Trials related to this study were registered at clinicaltrials.gov as NCT00005121 (Framingham Offspring Study) and NCT00005487 (Multi-Ethnic Study of Atherosclerosis). *Am J Clin Nutr* 2014;100:1462–9.

**Keywords** GWAS, phylloquinone, vitamin K, CYP4F2 , genetics

## INTRODUCTION

Vitamin K is an enzymatic cofactor for the posttranslational carboxylation of vitamin K–dependent proteins. Although the most commonly known vitamin K–dependent proteins are involved in coagulation, multiple vitamin K–dependent proteins are found in nonhepatic tissues, including cartilage, bone, and vascular tissue (1). Vitamin K insufficiency recently has been linked to a higher risk of several chronic diseases, including low bone mineral density (2, 3), hip fractures (4, 5), osteoarthritis (6), insulin resistance (7), and coronary calcium progression (8). Phylloquinone (also known as vitamin $K_1$) is the primary circulating form of vitamin K and reflects intake of the primary plant-based form in the diet. Circulating phylloquinone concentrations change as dietary intake changes, suggesting the measure of serum phylloquinone is a useful indicator of overall vitamin K status (9, 10). Nutritional biomarkers, which are not subject to the same bias as self-reported dietary measures and limitations in food composition databases, are considered more objective measures of nutrient status and are commonly used in nutritional epidemiologic studies (11).

A large interindividual variation in circulating phylloquinone has been identified in population-based cohorts (12, 13). The inter- and intraindividual variability for circulating phylloquinone is markedly greater than reported for other fat-soluble

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vitamins (14), and the bioavailability of phylloquinone from the ingestion of purified phylloquinone labeled with stable isotopes ranges from 2% to 80% (15). Serum phylloquinone also was found to vary according to race/ethnicity, and accordingly, we hypothesize a role for genetics in determining circulating phylloquinone concentrations (12).

Very few studies have assessed the genetic component influencing vitamin K status—specifically, circulating phylloquinone concentrations. Furthermore, these studies were often limited due to small sample sizes and inclusion of individuals with varying dietary intakes, geographical locations, age, and health status (16–19). However, it still remains unclear which loci, if any, can explain the wide interindividual variability in circulating phylloquinone concentrations, because to our knowledge, no genome-wide association (GWA)[6] investigation of circulating phylloquinone has been reported.

To identify common genetic variants associated with serum phylloquinone, we conducted a meta-analysis of GWA analyses of circulating phylloquinone by using data from 2 discovery cohorts ($n$ = 2138) from the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium Nutrition Working Group. The strongest allelic associations ($P < 1 \times 10^{-6}$) from this analysis were evaluated in a second-stage analysis consisting of one independent cohort ($n$ = 265).

## SUBJECTS AND METHODS

### GWA discovery and second-stage cohorts

Analysis was limited to individuals of European ancestry. Users of warfarin, a vitamin K antagonist, were also excluded. GWA was conducted in 2138 participants in whom genome-wide scans and circulating phylloquinone concentrations were available. Data from 2 independent CHARGE Consortium Nutrition Working Group cohorts were analyzed in the discovery stage: the Framingham Offspring Study (FOS) and the Health, Aging, and Body Composition (Health ABC) study. Second-stage analysis was carried out in a third CHARGE cohort: the Multi-Ethnic Study of Atherosclerosis (MESA). The analytic protocol for the present analysis was reviewed by the institutional review board (IRB) at Tufts Medical Center and Tufts University Health Sciences Campus.

#### *FOS*

The FOS is a community-based longitudinal study designed to examine cardiovascular disease risk in the offspring and their spouses of the Framingham Heart Study original cohort participants (20). In 1971, a total of 5124 individuals were enrolled in the study; since then, the cohort has been examined every 3–4 y. Vitamin K biochemical measures were made on samples collected between January 1995 and December 1998, during the sixth and seventh examination cycles (21). For the purpose of the present study, a total of 1607 adults who had available genetic data and provided consent to share genetic data, plasma phylloquinone, and valid dietary information were eligible (22). This study was approved by the IRB for Human Research at Boston University Medical Center.

#### *Health ABC study*

The Health ABC study is a prospective cohort study investigating the associations between body composition, weight-related health conditions, and functional decline in older adults (23). At the time of enrollment in Health ABC, all participants were well-functioning, community-dwelling adults aged 70–79 y. Participants were recruited from a random sample of white and black Medicare-eligible residents in the Pittsburgh, Pennsylvania, and Memphis, Tennessee, metropolitan areas. Participants have undergone annual examinations and semiannual phone interviews since study inception in 1997–1998. In 2012–2013, plasma phylloquinone was measured in 1110 participants of the Health ABC knee osteoarthritis substudy from samples obtained at the year 2 clinic visit (1998–1999). This substudy included participants with qualifying knee pain and randomly chosen participants without knee pain (24). Of the 1110 total participants, 531 met the criteria of European ancestry and no warfarin use and therefore were eligible for the present analysis. All participants provided informed consent before participating; consent forms

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[4] Supplemental Figures 1 and 2 and Supplemental Table 1 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[6] Abbreviations used: CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; FFQ, food-frequency questionnaire; FOS, Framingham Offspring Study; GWA, genome-wide association; Health ABC, Health, Aging and Body Composition; IRB, institutional review board; MAF, minor allele frequency; MESA, Multi-Ethnic Study of Atherosclerosis; SNP, single-nucleotide polymorphism; TRL, triglyceride-rich lipoprotein

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and study protocols were approved by the IRBs at each field center.

*MESA*

The MESA is an ongoing, observational study examining the prevalence and determinants of subclinical cardiovascular disease in a multiethnic cohort that began in 2000–2002 (25). The MESA cohort (n = 6814) was recruited from 6 U.S. communities, including Forsyth County, North Carolina; northern Manhattan and the Bronx, New York; Baltimore County, Maryland; St. Paul, Minnesota; Chicago, Illinois; and Los Angeles County, California. The study design and methods of MESA have been described in detail (25). MESA was approved by the IRBs at all 6 study sites (Wake Forest University, Columbia University, Johns Hopkins University, University of Minnesota, Northwestern University, and University of California at Los Angeles), and the present protocol also was approved by the IRB at Wake Forest University. All participants gave written informed consent. A randomly chosen subgroup of 780 MESA participants who were not users of warfarin and had serum phylloquinone measured from samples obtained at the baseline visit were considered for inclusion in the present analyses. Among these, we analyzed 265 individuals of European ancestry who had valid serum phylloquinone measurements; had information on dietary intake, demographics, and clinical covariates; and gave consent to share genetic data.

*Circulating phylloquinone*

Circulating phylloquinone was analyzed in all cohorts at the Vitamin K Laboratory at the USDA Human Nutrition Research Center on Aging at Tufts University in Boston, Massachusetts. Fasting plasma or serum was collected and frozen at −70°C. Plasma or serum phylloquinone was measured in thawed samples by using reverse-phase HPLC followed by fluorometric detection (26). The Vitamin K Laboratory currently participates in the international vitamin K external quality assurance scheme (27). The lower limit of detection for circulating phylloquinone by using the sample volumes available was 0.1 nmol/L. Samples with phylloquinone concentrations below the lower limit of detection were entered as 0.05 nmol/L (21, 26). Low and high control specimens had average values of 0.56 and 3.15 nmol/L, with a total (intra-assay plus interassay) CV of 15.2% and 10.9%, respectively (21).

*Dietary intake*

Usual dietary intakes of foods and beverages were assessed by using the self-administered 126-item Harvard food-frequency questionnaire (FFQ) in FOS, a 108-item interviewer-administered FFQ (Block Dietary Data Systems) in Health ABC, and a 120-item, self-administered, modified-Block FFQ in MESA. These FFQs have been described in detail (28–32). Vegetable intake included red, orange, and yellow vegetables and green leafy vegetables, excluding legumes and potatoes.

### Genotyping and GWA analysis

Genome-wide genotyping was conducted by using the Affymetrix platform for FOS and MESA and the Illumina platform for Health ABC as described previously (33–35). Each study performed quality control for genotyped single-nucleotide polymorphisms (SNPs) on the basis of minor allele frequency (MAF), call rate, and departure from Hardy-Weinberg equilibrium (**Supplemental Table 1**). Phased haplotypes from HapMap II CEU were used to impute ~2.5 million autosomal SNPs by using a hidden Markov model algorithm implemented in MACH for FOS and Health ABC and IMPUTE2 for MESA (36). SNPs with a low MAF (<1%) or a low imputation quality (MACH: $R^2 < 0.3$; IMPUTE: proper information <0.4) were removed.

Study-specific GWA analyses were conducted for circulating phylloquinone with a linear model adjusted for covariates to relate the outcome to genotyped and imputed SNP dosages by assuming an additive genetic model. To achieve normality for statistical testing with continuous outcomes, we applied natural logarithmic transformations to circulating phylloquinone concentrations before analysis. The basic model (model 1) included age, sex, and study-specific covariates, including population stratification by principal component analysis and clinical site, where applicable. Model 2 was adjusted as for model 1, with the addition of circulating triglyceride concentrations, because circulating phylloquinone is carried predominantly in triglyceride-rich lipoproteins (TRLs) (19). Model 3, the final model, was adjusted as for model 2 and further adjusted for vegetable intake to account for a healthful diet (37).

Results from cohort-specific GWA in FOS and Health ABC were combined in a fixed-effects meta-analysis with inverse variance weights by using METAL software (University of Michigan, Center for Statistical Genetics) (38). To account for population stratification, the association results from individual studies as well as meta-analyses were adjusted for genomic control. Genome-wide significance was considered at the Bonferroni-corrected threshold of $P < 5 \times 10^{-8}$.

Furthermore, we estimated total variance explained by variants with the lowest $P$ values at $P < 1 \times 10^{-4}$ by implementing the genome-wide complex trait analysis tool in FOS and adjusting for age, sex, and circulating triglyceride concentrations (39).

Associations with the lowest $P$ values at $P < 1 \times 10^{-6}$ were then evaluated in an independent cohort, MESA ($n$ = 265), by using the model in which a given SNP had the strongest level of significance in the discovery GWA analysis. Because 25% of participants in this cohort had circulating phylloquinone concentrations <0.1 nmol/L, the bimodal natural logarithmic-transformed distributions of circulating phylloquinone were inappropriate for linear regression and meta-analysis in combination with the discovery cohorts. Instead, serum phylloquinone was dichotomized based on median concentrations to low (≤1.0 nmol/L) and high (>1.0 nmol/L) groups, as has been previously conducted in this cohort for this phenotype (8, 12). The multivariable logistic regression model was used to determine the OR and 95% CIs for having low or high serum phylloquinone. Significance was considered at the Bonferroni-corrected threshold of $P < 0.01$ for 5 independent loci (0.05/5).

## RESULTS

Characteristics of the 2 discovery and second-stage cohorts are summarized in **Table 1**. No genome-wide significant association was observed for circulating concentrations of phylloquinone from the meta-analysis of the 2 discovery cohorts at the genome-wide significance level of $5 \times 10^{-8}$ (**Supplemental Figure 1**). However, we report 11 SNP associations with $P < 1 \times 10^{-6}$ on

**TABLE 1**
Characteristics of CHARGE participating cohorts[1]

| Participant characteristics | Discovery cohorts | | Second-stage cohort: |
|---|---|---|---|
| | FOS ($n$ = 1607) | Health ABC ($n$ = 531) | MESA ($n$ = 265) |
| Age, y | 56.7 ± 10.9[2] | 74.8 ± 2.9 | 61.9 ± 9.9 |
| Sex, M/F, $n$ | 760/847 | 242/289 | 120/145 |
| BMI, kg/m$^2$ | 28.2 ± 5.3 | 26.8 ± 4.2 | 27.7 ± 4.9 |
| Waist circumference, cm | 99.4 ± 5.5 | 99.8 ± 12.1[3] | 97.7 ± 14.9 |
| Circulating phylloquinone, nmol/L | 0.9 ± 2.5 | 1.1 ± 1.5 | 1.3 ± 1.4 |
| Circulating triglycerides, mg/dL | 134.7 ± 89.3 | 158.9 ± 96.5 | 132.6 ± 80.8 |
| Total cholesterol, mg/dL | 200.1 ± 35.8 | 208.7 ± 38.3 | 196.3 ± 35.2 |
| HDL cholesterol, mg/dL | 53.5 ± 16.6 | 52.6 ± 16.5 | 53.6 ± 15.9 |
| Fasting glucose, mg/dL | 102.8 ± 24.7 | 98.7 ± 23.6 | 90.2 ± 18.6 |
| Dietary intake | | | |
| Total energy, kcal/d | 1824 ± 607 | 1796 ± 660 | 1482 ± 661 |
| Total proteins, g/d | 78.3 ± 26.8 | 64.6 ± 25.7 | 57.8 ± 28.2 |
| Total carbohydrates, g/d | 228.5 ± 84.4 | 237.6 ± 86.9 | 183.5 ± 82.3 |
| Total fats, g/d | 62.5 ± 27.5 | 67.5 ± 33.5 | 53.66 ± 28.7 |
| Vegetables,[4] 1/2 cup, servings/d | 1.9 ± 1.1 | 3.1 ± 1.7 | 2.3 ± 1.2 |
| Vitamin K, $\mu$g/d | 156 ± 108 | NA | 137 ± 130 |

[1]CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; FOS, Framingham Offspring Study; Health ABC, Health, Aging, and Body Composition; MESA, Multi-Ethnic Study of Atherosclerosis; NA, not available.
[2]Mean; 95% CI in parentheses (all such values).
[3]Abdominal obesity.
[4]Vegetable intake included red, orange, and yellow vegetables and green leafy vegetables, excluding legumes and potatoes.

5 independent loci on 11q23.3, 8q24.3, 5q22.3, 2p12, and 19p13.12 (**Table 2**). Among the 11 SNPs, linkage disequilibrium at $r^2 > 0.91$ is evident among rs4645543, rs2199565, and rs7018214 in the 8q24.3 locus; rs2108622 and rs12609820 in the 19p13.12 locus; rs2192574 and rs4852146 in the 2p12 locus; and rs6862909, rs6862071, and rs4122275 in the 5q22.3 locus. The genomic control values for the meta-analysis of circulating phylloquinone were 1.008 for the age- and sex-adjusted model (model 1), 1.005 for the triglyceride-adjusted model (model 2), and 1.006 for the vegetable intake–adjusted model (model 3) (**Supplemental Figure 2**).

## Discovery analysis

In the basic model, associations at $P < 5 \times 10^{-7}$ were observed on 11q23.3 and 8q24.3. We observed an association on 11q23.3 for rs964184 ($\beta \pm$ SE: 0.23 ± 0.04 ln-nmol/L; $P = 5.91 \times 10^{-8}$) (**Figure 1**A). This variant resides closest to *ZNF259* and also near the *APOA1/C3/A4/A5* gene cluster, which includes several apolipoprotein genes, including *APOA1*, *APOC3*, *APOA4*, and *APOA5*. The association was strongly attenuated when adjusted for triglycerides in model 2 ($\beta \pm$ SE: 0.14 ± 0.04 ln-nmol/L; $P = 0.0011$). On 8q24.3, we observed associations for 3 SNPs in linkage disequilibrium ($r^2 > 0.91$), of which rs4645543 was the strongest signal ($\beta \pm$ SE: −0.42 ± 0.08 ln-nmol/L; $P = 2.00 \times 10^{-7}$). This region lies near *KCNK9*, a potassium channel protein, and further upstream of *COL22A1*, a collagen gene. The observed associations on 11q23.3 and 8q24.3 were both attenuated when adjusted for triglycerides in model 2 and after subsequent adjustment for vegetable intake (model 3) (Table 2).

In the triglyceride-adjusted model (model 2), we observed additional associations at $P < 5 \times 10^{-7}$ on 5q22.3, 2p12, and 19p13.12. The strongest signal on 5q22.3 was for rs6862909 ($\beta \pm$ SE: −0.96 ± 0.18 ln-nmol/L; $P = 1.24 \times 10^{-7}$) (Figure 1B). This variant lies near *CDO1*, a cysteine dioxygenase gene. The strongest signal in 2p12 was for rs4852146 ($\beta \pm$ SE: 0.19 ± 0.04 ln-nmol/L; $P = 1.42 \times 10^{-7}$) (Figure 1C). This variant resides near the catenin gene, *CTNNA2*. Finally, the strongest signal on 19p13.12 was for rs2108622 ($\beta \pm$ SE: 0.16 ± 0.03 ln-nmol/L; $P = 2.90 \times 10^{-7}$) (Figure 1D). This variant lies near *CYP4F2*, a gene encoding phylloquinone oxidase. These observed associations were attenuated after subsequent adjustment for vegetable intake (model 3) (Table 2).

Total variance in circulating phylloquinone concentrations explained by 355 SNPs reported with associations of $P < 1 \times 10^{-4}$ was determined by using genome-wide complex trait analysis. The proportion of phenotypic variability that can be explained by the additive SNP effects ($V_G/V_P$) was nonsignificant ($P > 0.05$) in FOS when adjusting for age, sex, and circulating triglyceride concentrations.

## Second-stage analysis

Associations at the 5 independent loci were further examined in a second-stage analysis consisting of one independent cohort. Results from the analysis are reported in Table 2. From the second-stage analysis, we observed nominal associations on 5q22.3 at $P < 0.05$ in the triglyceride-adjusted model (model 2), with the strongest signal for rs6862071 (OR: 63.1; 95% CI: 1.48, 2687; $P = 0.03$), a variant with a low MAF of 0.01.

## DISCUSSION

To our knowledge, this is the first meta-analysis of GWA to identify common genetic variants that determine circulating phylloquinone concentrations in humans. Despite the small number of population-based cohorts with available vitamin K biochemical measurements, the CHARGE consortium facilitated collaboration among various cohort studies, thereby achieving

**TABLE 2**
Summary of GWA discovery meta-analysis of associations at $P < 1 \times 10^{-6}$ and second-stage analysis[1]

| | | | | | Discovery GWA ($n$ = 2138) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Model 1[2] | | Model 2[3] | | Model 3[4] | | Second-stage analysis ($n$ = 265)[5] | |
| SNP | Chr | Nearest gene | Allele (effect/ noneffect) | Allele frequency (effect) | $\beta \pm$ SE | $P$ value | $\beta \pm$ SE | $P$ value | $\beta \pm$ SE | $P$ value | OR (95% CI) | $P$ value |
| rs964184 | 11 | *ZNF259* | G/C | 0.15 (0.002) | 0.23 ± 0.04 | $5.91 \times 10^{-8}$ | 0.14 ± 0.04 | 0.001 | 0.13 ± 0.04 | 0.002 | 1.06 (0.63, 1.75) | 0.83 |
| rs4645543 | 8 | *KCNK9* | T/C | 0.04 (0.003) | −0.42 ± 0.08 | $2.00 \times 10^{-7}$ | −0.35 ± 0.08 | $5.69 \times 10^{-6}$ | −0.35 ± 0.08 | $1.60 \times 10^{-5}$ | 0.98 (0.42, 2.30) | 0.97 |
| rs2199565 | 8 | *KCNK9* | T/G | 0.04 (0.003) | −0.42 ± 0.08 | $2.04 \times 10^{-7}$ | −0.35 ± 0.08 | $5.65 \times 10^{-6}$ | −0.35 ± 0.08 | $1.59 \times 10^{-5}$ | 0.99 (0.43, 2.30) | 0.99 |
| rs7018214 | 8 | *KCNK9* | C/T | 0.04 (0.002) | −0.39 ± 0.08 | $6.44 \times 10^{-7}$ | −0.33 ± 0.07 | $1.14 \times 10^{-5}$ | −0.33 ± 0.08 | $2.54 \times 10^{-5}$ | 1.05 (0.43, 2.53) | 0.92 |
| rs2108622 | 19 | *CYP4F2* | T/C | 0.3 (0.001) | 0.16 ± 0.03 | $8.78 \times 10^{-7}$ | 0.16 ± 0.03 | $2.90 \times 10^{-7}$ | 0.16 ± 0.03 | $9.68 \times 10^{-7}$ | 1.17 (0.75, 1.84) | 0.49 |
| rs12609820 | 19 | *CYP4F2* | C/T | 0.31 (0.004) | 0.16 ± 0.03 | $1.00 \times 10^{-6}$ | 0.16 ± 0.03 | $3.06 \times 10^{-7}$ | 0.16 ± 0.03 | $2.10 \times 10^{-6}$ | 1.20 (0.76, 1.90) | 0.44 |
| rs2192574 | 2 | *CTNAA2* | C/T | 0.11 (0.001) | 0.28 ± 0.06 | $1.82 \times 10^{-6}$ | 0.28 ± 0.06 | $1.49 \times 10^{-6}$ | 0.29 ± 0.06 | $8.23 \times 10^{-7}$ | 0.85 (0.46, 1.55) | 0.59 |
| rs4852146 | 2 | *CTNAA2* | C/T | 0.33 (0.007) | 0.18 ± 0.04 | $2.08 \times 10^{-6}$ | 0.19 ± 0.04 | $1.42 \times 10^{-7}$ | 0.18 ± 0.04 | $3.23 \times 10^{-6}$ | 0.97 (0.62, 1.51) | 0.89 |
| rs6862909 | 5 | *CDO1* | T/G | 0.01 (0.0002) | −0.81 ± 0.19 | $1.41 \times 10^{-5}$ | −0.96 ± 0.18 | $1.24 \times 10^{-7}$ | −0.94 ± 0.19 | $7.69 \times 10^{-7}$ | 41.3 (1.29, 1322) | 0.03 |
| rs6862071 | 5 | *CDO1* | T/A | 0.01 (0.0001) | −0.94 ± 0.22 | $2.29 \times 10^{-5}$ | −1.14 ± 0.22 | $1.35 \times 10^{-7}$ | −1.09 ± 0.23 | $1.73 \times 10^{-6}$ | 63.1 (1.48, 2687) | 0.03 |
| rs4122275 | 5 | *CDO1* | A/G | 0.02 (0.001) | −0.68 ± 0.17 | $4.76 \times 10^{-5}$ | −0.81 ± 0.16 | $4.31 \times 10^{-7}$ | −0.78 ± 0.17 | $3.90 \times 10^{-6}$ | 7.50 (0.98, 57.4) | 0.05 |

[1]Additive allele mode. Chr, chromosome; GWA, genome-wide association; SNP, single-nucleotide polymorphism.

[2]Analyses adjusted for age, sex, and study-specific covariates (e.g., study site, population stratification by principal components, when applicable) ($P < 10^{-6}$). Association coefficients are shown as $\beta$s ± SEs; $\beta$ represents the change in circulating phylloquinone (ln-nmol/L) per each additional copy of the effect allele.

[3]Analyses adjusted for age, sex, circulating triglycerides, and study-specific covariates (e.g., study site, population stratification by principal components, when applicable). Association coefficients are shown as $\beta$s ± SEs; $\beta$ represents the change in circulating phylloquinone (ln-nmol/L) per each additional copy of the effect allele.

[4]Analyses adjusted for age, sex, circulating triglycerides, vegetable intake, and study-specific covariates (e.g., study site, population stratification by principal components, when applicable). Association coefficients are shown as $\beta$s ± SEs; $\beta$ represents the change in circulating phylloquinone (ln-nmol/L) per each additional copy of the effect allele.

[5]Second-stage covariates determined by the model with the strongest $P$ value from discovery GWA. Association coefficients are shown as ORs and 95% CIs for having high (>1.0 nmol/L) circulating phylloquinone concentrations, per each additional copy of the effect allele.

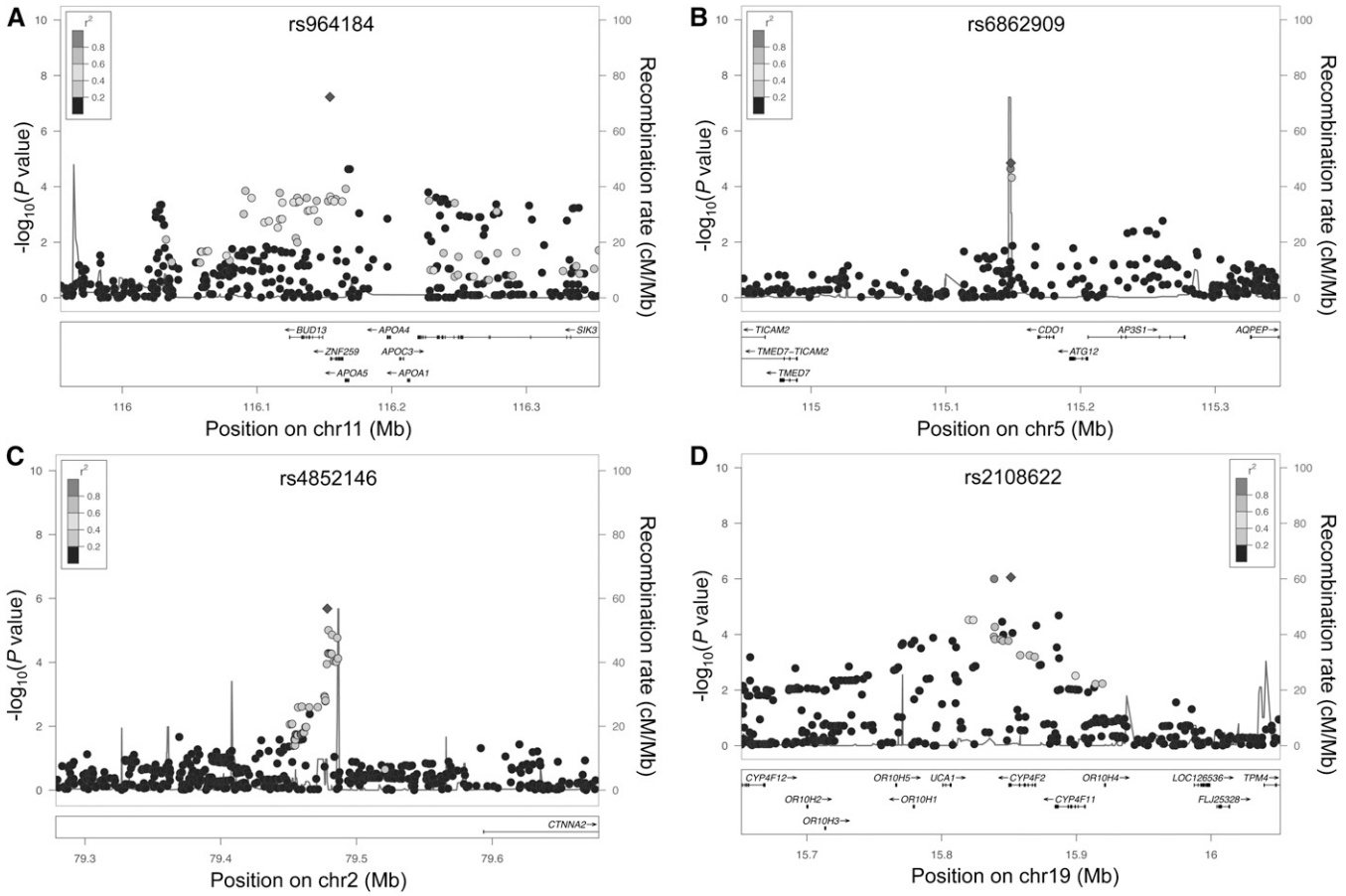


**FIGURE 1** Regional association plots for SNPs at 11q23.3 (A), 5q22.3 (B), 2p12 (C), and 19p13.12 (D). The panels show $-\log_{10} P$ values for SNPs from the discovery meta-analysis. Analyses were adjusted for age, sex, and study-specific covariates (e.g., study site and population stratification by principal components, when applicable) for 11q23.3 (A) and further adjusted for circulating triglycerides for 5q22.3, 2p12, and 19p13.12 (B–D). The SNPs shown are those within 200 kb of the index SNP: rs964184 on chromosome 11 (A), rs6862909 on chromosome 5 (B), rs4852146 on chromosome 2 (C), and rs2108622 on chromosome 19 (D). LD is indicated in grayscale in relation to the highlighted marker. The scheme is dark gray for strong LD ($r^2 \geq 0.8$), fading gray for lower LD, and black for no LD. chr, chromosome; cM, centimorgan; LD, linkage disequilibrium; Mb, mega base pair; SNP, single-nucleotide polymorphism.

larger sample sizes for a GWA investigation. None of the associations achieved the genome-wide significance threshold at $P < 5 \times 10^{-8}$, but we identified 5 top associations at $P < 1 \times 10^{-6}$ from the discovery GWA meta-analysis in modulating circulating phylloquinone, corresponding to 5 independent genomic regions. Although we observed stronger associations after the adjustment for circulating triglycerides, further adjustment for usual vegetable intake resulted in slightly attenuated *P* values.

Circulating phylloquinone concentrations were associated on 11q23.3 for rs964184, which flanks the apolipoprotein *APOA1/C3/A4/A5* gene cluster. This association was attenuated after adjustment for circulating triglycerides, indicating that the association was most likely mediated by phylloquinone transport on TRLs. Previous studies have reported associations between the rs964184 variant and lower circulating HDL cholesterol and higher triglyceride concentrations, supporting the hypothesis that this association is attributable to lipid transport (40, 41). In addition, this variant was identified in GWA investigations for circulating vitamin E concentrations when unadjusted for triglycerides (42). Because fat-soluble vitamins, including vitamin K, are transported on TRLs, this suggests shared common genetic determinants among all fat-soluble vitamins. Overall, the evidence highlights the importance of loci specific to circulating triglycerides in determining circulating phylloquinone concentrations and provides further rationale for adjusting for triglycerides in future analyses attempting to elucidate variants independent of lipid transport (19).

When we adjusted for triglycerides, we found associations for circulating phylloquinone on 19p13.12 for rs2108622, which is of particular interest because of its role in vitamin K metabolism. The rs2108622 is a nonsynonymous variant, resulting in an amino acid change at position 433 (V433M polymorphism) in the *CYP4F2* gene. The protein encoded by *CYP4F2* provides a nonlipid function for vitamin K and vitamin E metabolism. *CYP4F2* functions as a phylloquinone oxidase (43) and also plays a role in the hydroxylation reaction of vitamin E (42), indicating another shared common genetic variant influencing circulating concentrations of these 2 fat-soluble vitamins.

The rs2108622 variant has previously been associated with warfarin dose and altered phylloquinone metabolism (44). In vitro experiments indicate that carriers of the minor T allele at rs2108622 have reduced capacity to metabolize phylloquinone, suggesting elevated hepatic phylloquinone concentrations among individuals with this variant. As a result, these minor T allele carriers require a higher warfarin dose to elicit the same anticoagulant response as noncarriers (44). Our results also suggest that carriers of the minor T allele at rs2108622 have elevated

circulating concentrations of phylloquinone. It is also worth noting that the MAF for rs2108622 varies across ethnicities, ranging from a low frequency of 0.06 for Africans to higher frequencies of 0.23 and 0.25 for Europeans and Asians, respectively. The higher prevalence of the rs2108622 variant in Europeans and Asians could partly explain earlier reports of variability in serum phylloquinone concentrations among races and ethnicities (12). Our associations at the *CYP4F2* gene further suggest the importance of genes playing a role in vitamin K metabolism in determining circulatory concentrations of phylloquinone.

We also identified associations for circulating phylloquinone on the 2p12 locus for rs4852146 in the triglyceride-adjusted model. The rs4852146 SNP lies upstream of the *CTNNA2* gene, which encodes a cadherin-associated protein, suggesting that the SNP may play a potential role in gene regulation. A GWA study for bone mineral density at the forearm reported a genome-wide significant association at the *CTNNA2* locus (45), whereas a 2-year randomized controlled trial identified significant increases in bone mineral concentration at the ultradistal radius of women supplemented with combined vitamin K and vitamin D plus calcium (46). The overlap between our GWA findings and findings from the bone mineral density GWA study suggests a putative role of *CTNNA2* in determining circulating phylloquinone. *COL22A1*, a collagen precursor and a cell adhesion ligand for skin epithelial cells and fibroblasts (47), was also identified as a candidate gene for the associations on 8q24.3.

Among the selected variants evaluated at the second-stage analysis, we observed the strongest associations on 5q22.3 in the triglyceride-adjusted model, with the strongest signal for rs6862071. Whereas the *P* value indicates a significant association for rs6862071, the OR and 95% CI for the association should be considered with caution because of the low frequency of the variant. This variant lies near *CDO1*, a tumor suppressor gene silenced by promoter methylation in multiple human cancers (48). The mechanism by which this gene influences concentrations of circulating phylloquinone is not clear.

One of the major strengths of our study is its reduction in phenotypic measurement errors, owing to circulating phylloquinone from all participating cohorts being measured in the same laboratory by using a single assay with established methods of quality control. Early successes in GWA investigations were often attributed to a clear definition of phenotypes (49). Our use of the same laboratory and single assay may have improved our ability to detect genetic associations at $P < 1 \times 10^{-6}$. However, power was a considerable limitation in our investigation, given the very modest number of studies with available phylloquinone data. As suggested by the nonsignificant proportion of phenotypic variability attributable to the identified top hits, targeting larger sample sizes for future GWA investigations is necessary. In addition, the bimodal distribution of the MESA cohort impeded our ability to include the cohort in the discovery GWA meta-analysis to achieve greater power. Other limitations to this investigation are the inclusion of participants of single ancestry, which limits the generalizability of these results. Despite the hypothesized roles of the identified SNPs in affecting circulating phylloquinone, there is little evidence to establish causal relationships between most identified SNPs and circulating phylloquinone. It is also possible that the SNPs identified here may be proxies for other causal SNPs.

In conclusion, this meta-analysis of GWA for circulating phylloquinone concentrations from 2 cohorts and a second-stage analysis in one additional independent cohort has implicated several genes involved in various biological pathways in determining the circulating concentrations of phylloquinone. Our data may provide insight into the large interindividual variability in response to phylloquinone dietary intake and supplementation and potentially warfarin therapy. Further investigation with a larger, racially diverse sample is warranted to verify our initial findings and identify other loci contributing to circulating phylloquinone. In addition, circulating phylloquinone is an important biomarker that reflects "healthy" lifestyles and, at lower concentrations, is associated with an increased risk for various chronic diseases. However, it is not commonly measured in epidemiologic studies. Thus, we recommend that future studies collect data on circulating phylloquinone to study both the genetic and physiologic effects on human health.

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The authors' responsibilities were as follows—HSD, CES, TJW, EJB, JMO, and SLB: designed the study; HSD, MKS, TT, KR, MAN, XG, YL, JY, and DL: contributed to the statistical analyses; HSD, MKS, TT, JMO, and SLB: interpreted the data; HSD, MKS, CES, TT, AH, KR, WCJ, EJB, SBK, DSS, JMO, and SLB: wrote the manuscript; and all authors: read and approved the final version of the manuscript. None of the authors reported a conflict of interest related to the study.

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