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Vitamin K dependent protein activity and incident ischemic cardiovascular disease: the Multi-Ethnic Study of Atherosclerosis (MESA)

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Abstract

Objective—Vitamin K-dependent proteins (VKDPs), which require post-translational modification to achieve biologic activity, appear to contribute to thrombus formation, vascular calcification and vessel stiffness. Whether VKDP activity is prospectively associated with incident cardiovascular disease has not been studied.

Approach and Results—VKDP activity was determined by measuring circulating Des-gamma-carboxy Prothrombin (DCP) concentrations in a random sample of 709 multi-ethnic adults free of cardiovascular disease drawn from the Multi-Ethnic Study of Atherosclerosis. Lower DCP concentrations reflect greater VKDP activity. Subjects were followed for risk of ischemic cardiovascular disease (coronary heart disease, stroke, and fatal cardiovascular disease) over 11.0 years of follow up. A total of 75 first ischemic CVD events occurred during follow up. The incidence of ischemic cardiovascular disease increased progressively across DCP quartiles, with event rates of 5.9 and 11.7 per 1000 person-years in the lowest and highest quartiles. In analyses adjusted for traditional cardiovascular risk factors and measures of vitamin K intake, a doubling of DCP concentration was associated with a 1.53 (95% confidence interval, 1.09-2.13; p=0.008) higher risk of incident ischemic cardiovascular disease. The association was consistent across strata of participants with diabetes, hypertension, renal impairment, and low vitamin K nutritional intake.

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Conclusions—In this sample of middle-aged and older adults, VKDP activity was associated with incident ischemic cardiovascular events. Further studies to understand the role of this large class of proteins in cardiovascular disease is warranted.

Keywords

Vitamin K; prothrombin; phylloquinone; cardiovascular disease

Introduction

Vitamin K-dependent proteins (VKDPs) are a large class of proteins unified by their reliance on post-translational modification to achieve biologic activity. To date, nineteen VKDPs have been described, with important roles in coagulation, platelet function, and vascular biology. Prothrombin, the most well described VKDP, is produced within the liver, and circulates systemically until stimulation by a platelet plug, facilitating thrombus formation. Matrix Gla protein (MGP) [1] is produced within vascular smooth muscle cells, and inhibits vascular calcification by binding extracellular calcium[2]. Growth arrest specific factor-6 (Gas-6) is produced within platelets and the vascular wall and affects thrombus formation and cell survival[3]. Periostin[4], expressed in the cardiac ventricle, has a role in ventricular hypertrophy[5], valvular function and atherosclerosis[6].

Produced in an inactive form, all VKDPS obtain biologic activity through the conversion of a glutamic acid residue into glutamate, a complex process requiring vitamin K hydroquinone as a cofactor and regulated by γ-carboxylase enzyme within the endoplasmic reticulum. In this process, Vitamin K hydroquinone is converted to vitamin K epoxide, which in turn is recycled back to vitamin K hydroquinone by vitamin K epoxide reductase (VKOR). In addition to adequate vitamin K, enzyme activity and polymorphisms affect the carboxylation process, suggesting that the post-translational modification of such proteins have multiple levels of control.

Given the pleiotropic biologic effects of this large class of proteins, and emerging evidence for their role in cardiovascular physiology, we investigated whether VKDP activity was associated with cardiovascular disease in a well-characterized sample of adults drawn from across the United States. Since prothrombin rapidly undergoes carboxylation before being secreted from the liver, we measured circulating Des-gamma-carboxy Prothrombin (DCP) concentrations to indicate lower VKDP activity.

Materials and Methods

Materials and Methods are available in the online-only Data Supplement.

Results

Baseline characteristics stratified by DCP quartiles are presented in table I. Participants with higher DCP concentrations (i.e., lower VKDP activity) tended to be older, with higher BMI and cholesterol medication usage, with lower renal function and less overall physical activity. The rates of diabetes and hypertension were similar, but hs-CRP concentrations

increased across the DCP quartiles. As expected, increased phylloquinone concentrations, reflecting greater dietary vitamin K intake, were associated with lower DCP concentrations, reflecting greater VKDP activity. The weighted correlation between DCP and phylloquinone concentrations was inverse, as expected, but modest (r=-0.11;p= 0.006). A total of 84% of the cohort participants had a DCP >2 ng/ml (considered the threshold for VKDP inactivity) while 52% had a phylloquinone concentration <1 nmol/L (considered the threshold for inadequate vitamin K intake). In keeping with previous reports suggesting higher dietary vitamin K intake in those of Chinese descent[7], the majority of Chinese participants (55%) were in the lowest DCP quartile, compared to only 15% of white participants.

We documented 75 first ischemic CVD events during a median of 11.0 years of follow up, 16 myocardial infarctions, 29 coronary revascularization procedures, 22 fatal and nonfatal strokes, and 8 other fatal CHD events. Unadjusted cumulative incidence rates per DCP quartile are presented in Figure 2. In general, ischemic CVD incidence rates were higher with greater concentration of DCP.

We next assessed the dose-response relationship of DCP with ischemic CVD (Figure 3), which suggested a log-linear association of DCP with CVD incidence. We subsequently performed analyses of log-transformed DCP concentrations with risk, adjusting sequentially for demographics, cardiovascular risk factors, and measures of vitamin K intake. In these analyses (table II), a doubling of circulating DCP concentration was associated approximately 50% higher risk of ischemic CVD. The magnitude of this association was little changed with adjustment for traditional risk factors or phylloquinone concentrations. Given the previously recommended threshold of 2 ng/ml in DCP concentrations to indicate VKDP inactivity, we examined this cutpoint in fully adjusted models, with an adjusted hazard ratio of 3.42 (95% confidence interval, 0.97-12.09; p=0.06).

The association of DCP concentrations and ischemic CVD appeared to be consistent across a broad range of subgroups. We found no significant interaction of DCP with diabetes, hypertension, vitamin K intake, or hs-CRP (all multiplicative interaction terms p > 0.5).

Finally, redefining ischemic CVD to include only cardiac related events, DCP concentrations were associated with a 1.46 (95%CI 1.00-1.06; p=0.047), 1.59(95%CI 1.03-2.44;p=0.035), and 1.53(95%CI 1.00-2.35;p=0.054) odds of incident disease using Model I, II, and III, respectively.

Discussion

In this population of adults followed for 11 years, baseline measures of VKDP activity were associated with incident ischemic cardiovascular disease. Our findings raise awareness of this important class of proteins as a potential contributor to cardiovascular disease.

A large body of evidence relates VKDPs to cardiovascular disease. Early animal studies suggested that warfarin administration, a potent inhibitor of VKOR recycling, induces widespread medial vascular calcification[8, 9], otherwise known as Monckeberg calcification. Medial calcification has been associated with vascular stiffness[10] and increased mortality[11, 12]and is prevalent among individuals with diabetes and chronic kidney disease.

MGP has received considerable attention as the candidate VKDP protein responsible for the vascular calcification phenotype[13]. MGP null mice have widespread vascular calcification[1], and the circulating form of uncarboxylated MGP has been associated with vascular calcification in clinical studies[14-17]. Since dietary vitamin K may reduce the inactive form of MGP[18], dietary vitamin K intake has been hypothesized as a determinant of vascular calcification. Animal models have shown regression of vascular calcification with high vitamin K diets[19], but clinical data have not been uniform, with some studies suggesting a potential benefit of dietary vitamin K[20, 21], not supported by others[22, 23]. A randomized trial of vitamin K for this indication is ongoing[24].

Given the pleiotropic biologic roles of VKDPs, it is plausible that VKDPs have a role in CVD independent of any potential effect on vascular calcification. Gas-6, widely expressed in vascular smooth muscle cells and monocytes, has received increasing attention for its role in platelet aggregation, vascular morphology, and atherosclerosis. Post translational change of the N terminus of Gas6 confers the protein the ability to bind to aninonic phospholipids exposed on injured cell surfaces[25], and through a family of TAM receptors, Gas6 has multiple downstream effects, including promoting cell survival[26], migration[27], and remodeling, and potentially reducing atherosclerotic plaque formation[28, 29]. In addition, Gas6 inhibits the adhesion of leukocytes to endothelial cells[30] and reduces plaque inflammation in some[31], but not all[32], studies. Gas6 deficient mice have defective platelet signaling, but also seem to have a paradoxical protection against thromboembolic disease[33]. Thus, it is plausible that a generalized state of VKDP inactivity, as measured by increasing DCP concentrations, might also reflect a state of Gas-6 inactivity[34], and a consequent pro-atherosclerotic state. Periostin[4] is a newly described VKDP, named due to its localization in cortical bone periosteum and the periodontal ligament, with a role in embryonic cardiac development[35] and cardiac remodeling[5]. Periostin knockout ($Pn^{-/-}$) mice have an increased rate of ventricular rupture after myocardial infarction[36], but increased periostin expression is also seen in ventricular hypertrophy and fibrosis[37]. In addition, prothrombin itself has been linked to CVD, associated with an increased risk of venothrombotic disease[38] but not arterial disease[39].

In addition to the potential role of VKDPs in thrombus formation and vessel morphology, data suggests an association between VKDPs and inflammation. Administration of high dose dietary vitamin K reduces inflammatory gene expression in animal models[40, 41]. In our cohort, hs-CRP concentrations increased with greater VKDP inactivity. Given data suggesting that prothrombin is an acute phase reactant, whether increasing DCP concentrations reflect prothrombin production or a carboxylation failure is uncertain, although the association of DCP with ischemic cardiovascular disease was consistent across strata of hs-CRP. In our cohort, the weighted correlation for hs-CRP and DCP was r=0.10, p=0.006.

Our findings add to clinical data linking VKDP activity and cardiovascular disease. In a community-based study of over 4800 subjects, dietary vitamin K intake was inversely associated with incident cardiovascular disease[42]. Since most American diets meet the recommended daily vitamin K allowances, there are likely dietary independent factors affecting VKDP activity. The function of the gamma carboxylase enzyme, responsible for

converting a glutamic acid to glutamate residue, is impaired in kidney disease[43] and diabetes[44], perhaps accounting for the unexpectedly high prevalence of VKDP inactivity in chronic kidney disease[45]. In our analysis, the overall correlation between serum phylloquinone and DCP concentrations was low, potentially supporting a role for dietary independent factors, although phylloquinone concentrations may also be more sensitive to very recent vitamin K intake than are DCP levels. Although high doses of dietary vitamin K can improve VKDP activity, whether such treatment will lead to an improvement of outcomes is speculative.

The strengths of our study included a well-characterized multi-ethnic population with adjudicated, prospectively-measured endpoints and detailed phenotyping of cardiovascular risk factors. In addition, measurement of serum phylloquinone concentrations allowed the effect of VKDP activity on cardiovascular disease to be adjusted for nutritional vitamin K. Our study also has important limitations. The correlation between the activity of circulating VKDPs, such as prothrombin, and organ-specific VKDPs, such as periostin or MGP, are not known, and the assumption that DCP concentrations reflect overall VKDP activity cannot be validated without further study. Although this is the largest prospective study of DCP and cardiovascular disease to be performed to our knowledge, we only measured DCP in a subcohort of MESA participants and had limited power to examine associations within subgroups or across components of our composite endpoint. Nevertheless, increasing DCP quartiles had incrementally higher risks of incident CVD, that when examined continuously, had sufficient power to detect a significant overall association between DCP and incident CVD. Further research to understand the role of VKDPs in cardiovascular disease is warranted, including a better understanding of the factors that affect post translational carboxylation of VKDPs, the association between hepatic, platelet, and vascular smooth muscle cell VKDPs, and potential mechanisms of ischemia.

In summary, our analysis suggests that VKDP activity is associated with incident ischemic cardiovascular disease. Our results raise the possibility that dietary or pharmacological improvement of VKDP activity can reduce the incidence of CVD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

VKDP	Vitamin K dependent protein
MESA	Multi Ethnic Study of Atherosclerosis
DCP	Des-gamma-carboxy prothrombin
MGP	Matrix Gla protein
Gas-6	Growth Arrest Specific factor 6
LDL	Low density lipoprotein
HDL	High density lipoprotein

Significance

- Vitamin K dependent protein activity, as measured by circulating Des-gamma-carboxy Prothrombin (DCP) concentrations, is associated with incident ischemic cardiovascular disease.

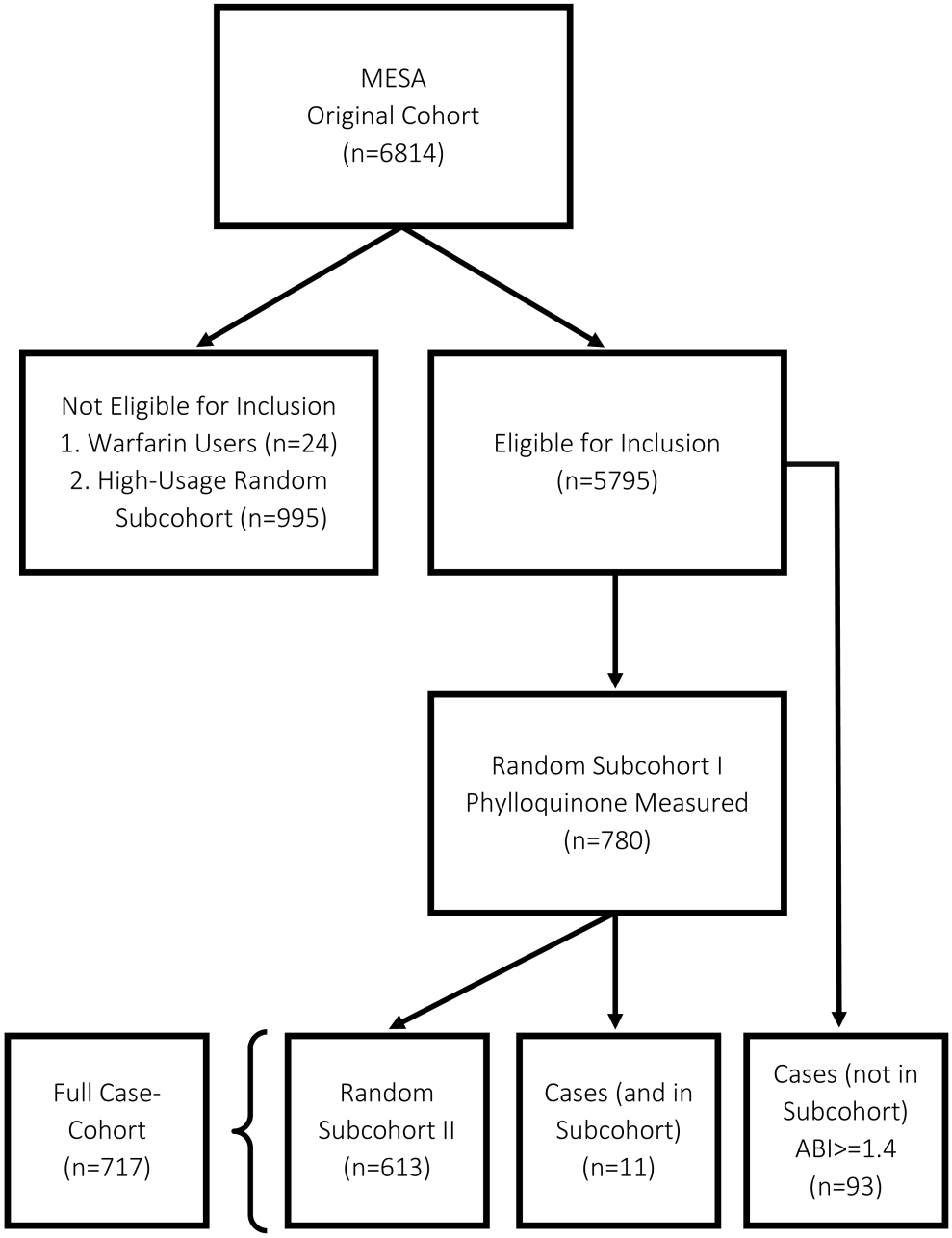
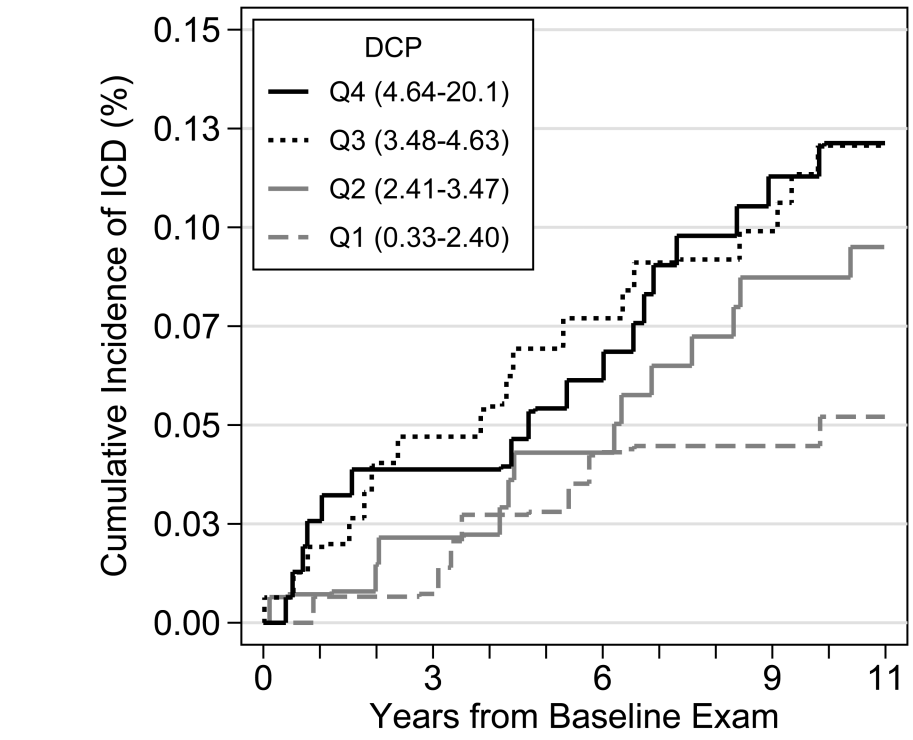


Figure 1. Participant selection for the MESA weighted cohort design



Number at risk

DCP Q4	1443	1319	1204	1092	684
DCP Q3	1455	1294	1216	1166	741
DCP Q2	1429	1332	1229	1108	679
DCP Q1	1427	1398	1238	1198	821

Figure 2. Cumulative Incidence of Ischemic Cardiovascular Disease according to quartiles of Des-gamma carboxy prothrombin (n=709)

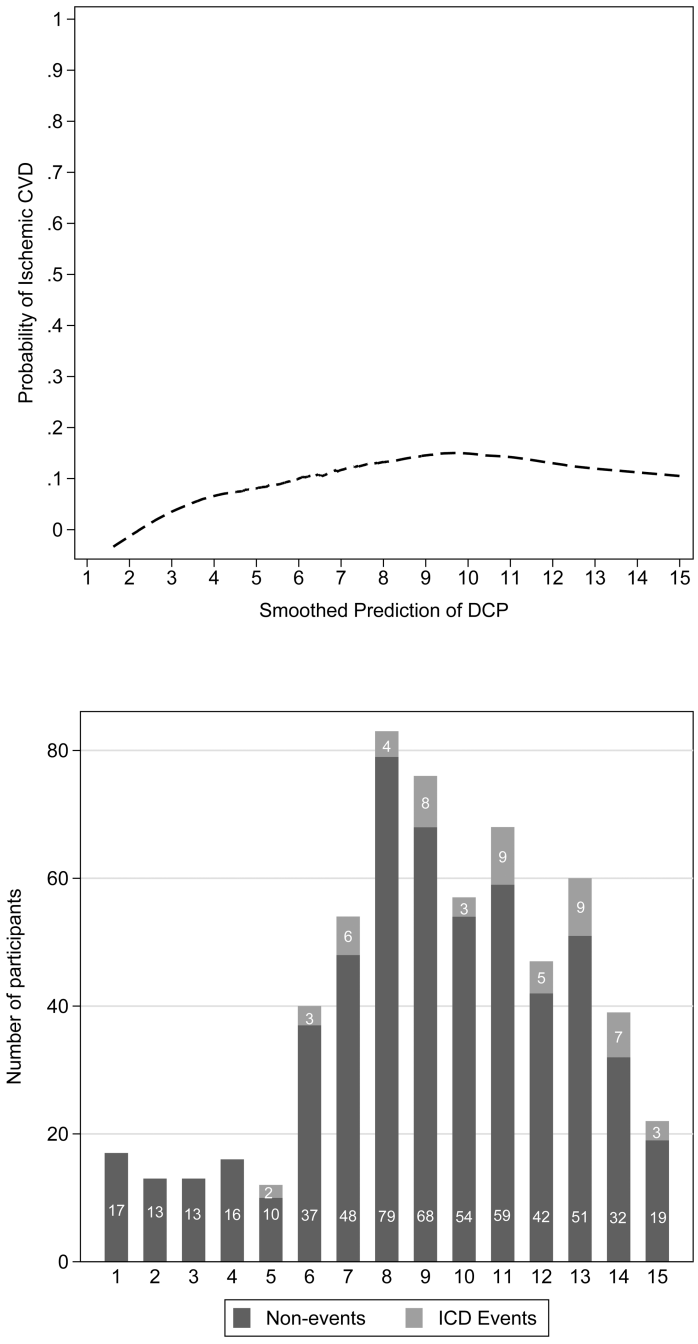


Figure 3. Locally weighted smoothed scatterplot and histogram of Des-gamma carboxy Prothrombin and Ischemic Cardiovascular Disease (n=617)

Table 1

Baseline characteristics of the case-cohort sample, stratified by DCP quartiles (n=709)

Variables	DCP Q1 (0.33-2.40) n=178	DCP Q2 (2.41-3.47) n=177	DCP Q3 (3.48-4.63) n=177	DCP Q4 (4.64-20.1) n=177
Age, years	61 (10)	63 (10)	62 (10)	63 (10)
Women, n (%)	92 (52)	92 (52)	92 (52)	81 (46)
Race/ethnicity, n (%)				
White	43 (24)	62 (35)	78 (44)	85 (48)
Chinese	42 (24)	17 (10)	10 (6)	8 (5)
Black	62 (35)	60 (34)	38 (21)	36 (20)
Hispanic	31 (17)	38 (21)	51 (29)	48 (27)
Body mass index	27 (6)	28 (6)	29 (6)	30 (5)
Cigarette smoking status, n (%)				
Never	97 (55)	94 (53)	84 (47)	85 (48)
Former	54 (31)	59 (33)	68 (38)	68 (38)
Current	24 (14)	24 (14)	25 (14)	24 (14)
Pack-years cigarette smoking	8 (18)	11 (19)	10 (18)	16 (29)
Intentional physical activity total (MET-min/day)	218 (310)	234 (379)	199 (364)	194 (238)
Current alcohol use, n (%)	96 (69)	96 (67)	116 (77)	93 (61)
High school graduate, n (%)	139 (79)	149 (84)	147 (83)	141 (80)
Diabetes	27 (15)	21 (12)	31 (18)	20 (11)
Antihypertension medication, n (%)	58 (33)	66 (37)	75 (42)	66 (37)
Systolic BP (mmHg)	124 (22)	128 (22)	126 (23)	130 (20)
Diastolic BP (mmHg)	72 (11)	72 (11)	71 (12)	73 (9)
LDL-C	108 (28)	116 (28)	119 (33)	119 (31)
HDL-C	54 (16)	53 (16)	50 (14)	46 (13)
Lipid-lowering medication, n(%)	23 (13)	24 (14)	34 (19)	42 (24)
Triglycerides, mg/dL, median (IQR)	94 (71-136)	103 (69-148)	108 (79-155)	134 (98-188)
Hs-CRP, median (IQR), mg/l	1.1 (0.6-3.3)	1.8 (0.9-3.7)	2.8 (1.1-6.6)	2.6 (1.3-5.0)
Estimated GFR	83 (18)	81 (20)	81 (18)	79 (19)
Urinary Albumin /Creatinine (mg/g)	14 (37)	20 (56)	39 (216)	40 (184)
Phylloquinone, (nmol/L)	1.8 (2.3)	1.5 (2.2)	1.2 (1.2)	1.2 (1.5)
Dihydrophylloquinone (nmol/L)	0.3 (1.1)	0.2 (0.5)	0.2 (0.6)	0.2 (0.8)

Mean (standard deviations) provided.

Abbreviation: BP = blood pressure; DCP = Des-gamma carboxy Prothrombin; eGFR = estimated glomerular filtration rate based on creatinine measurement; HDL = high-density lipoprotein cholesterol; Hs-CRP = high-sensitivity C-reactive protein; IQR = interquartile range; LDL-C = low-density lipoprotein cholesterol; MET = metabolic equivalent; Q = quartile.

Note: Sample n=709, except: Cigarette smoking n=706; Pack-years of smoking n=701; Intentional physical activity n=706; High school graduate n=706; Diabetes n=708; LDL-C n=701; HDL-C n=708; Triglycerides n=708; estimated GFR n=708; Urinary Albumin/Creatinine n=706; Phylloquinone n=630; Dihydrophylloquinone n=630. Descriptive statistics are not weighted for case-cohort design.

Table 2

Proportional Hazards Cox Regression Model Predicting the Likelihood of Ischemic Cardiovascular Disease by Des-gamma carboxy Prothrombin

		DCP (quartiles)				
	Events/N	**Q1 (0.33-2.40)**	**Q2 (2.41-3.47)**	**Q3 (3.48-4.63)**	**Q4 (4.64-<20.1)**	**DCP (continuous)**
Model 1	75/709	reference	1.38	1.83	1.71	1.46
			(0.62-3.06)	(0.81-4.12)	(0.79-3.70)	(1.08-1.97)
			p=0.428	p=0.143	p=0.173	p=0.013
Model 2	74/694	reference	1.48	1.96	2.03	1.58
			(0.62-3.52)	(0.83-4.66)	(0.82-5.04)	(1.13-2.22)
			p=0.378	p=0.126	p=0.125	0.008
Model 3	59/617	reference	1.46	1.82	1.87	1.53
			(0.61-3.50)	(0.78-4.23)	(0.76-4.58)	(1.09-2.13)
			p=0.39	p=0.163	p=0.171	0.013

Abbreviation: DCP = des-gamma carboxy Prothrombin; Q = quartile.

Note: Table shows hazard ratio (95% confidence interval). All models are weighted for case-cohort design and use robust standard errors. Model 1: Adjusted for age, gender, and race/ethnicity. Model 2: Adjusted for Model 1 + BMI, cigarette smoking (never, former, current), education (high school graduate), diabetes, systolic blood pressure, antihypertension medication use, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, high-sensitivity C-reactive protein, lipid-lowering medication, estimated glomular filtration rate, albumin and creatinine ratio, intentional physical activity, and current alcohol use. Model 3: Adjusted for Model 2 + phylloquinone, and dihydrophylloquinone.