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A Prospective Study of Plasma Vitamin D Metabolites, Vitamin D Receptor Gene Polymorphisms, and Risk of Hypertension in Men

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Abstract

Purpose—Laboratory studies have suggested that vitamin D inadequacy may be implicated in development of hypertension. Evidence from epidemiologic studies remains limited. We aim to examine the prospective associations of circulating vitamin D metabolites, vitamin D receptor (VDR) gene polymorphisms, and their interaction with risk of hypertension.

Methods—We conducted prospective analyses among 1,211 US men that were free of baseline hypertension and had baseline plasma 25hydroxy-vitamin D (25(OH)D) or 1,25dihydroxy-vitamin D (1,25(OH)₂D) measured and VDR *BsmI* or *FokI* polymorphisms genotyped.

Results—During 15.3-year follow-up, 695 men developed incident hypertension. After multivariable adjustment, the hazard ratios (HRs) and 95% CIs for hypertension across increasing quartiles of circulating vitamin D metabolites were 1.00 (ref), 0.94 (0.69–1.27), 0.69 (0.50–0.96), and 0.82 (0.60–1.13) for 25(OH)D (p, trend: 0.43), and 1.00, 0.92 (0.66–1.27), 1.12 (0.82–1.54), and 1.19 (0.86–1.63) for 1,25(OH)₂D (p, trend: 0.16). Compared with carriers of VDR *BsmI* bb, carriers of bB or BB had a HR of 1.25 (1.04–1.51) for hypertension. For VDR *FokI* polymorphism, compared with carriers of FF and Ff combined, carriers of ff had a HR of 1.32 (1.03–1.70). The relation between plasma 25(OH)D and risk of hypertension did not differ by VDR *BsmI* and *FokI* polymorphisms.

Conclusions—In a prospective cohort of men, we found suggestive evidence for an inverse association between plasma 25(OH)D and risk of hypertension. We also found associations between VDR *BsmI* and *FokI* polymorphisms with hypertension risk. More research is needed to further determine the role of vitamin D in hypertension prevention.

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Keywords

vitamin D; polymorphisms; prospective study; hypertension; men

Introduction

Recent research has linked inadequate vitamin D status to many non-skeletal chronic diseases including cardiovascular disease and hypertension. Vitamin D can down-regulate the renin-angiotensin system (RAS), a key regulatory cascade controlling blood pressure (BP). Treatment of cells with active vitamin D directly reduced the promoter activity on the renin gene. [1] In vitamin D receptor (VDR) knockout mouse, renin expression and plasma angiotensin II concentrations dramatically increased and BP elevated.[1] In individuals with insufficient vitamin D, plasma concentrations of renin[2] and angiotensin II[2, 3] were higher, and renal plasma flow response to infused angiotensin II was blunted.[3]

Despite these intriguing laboratory findings, evidence from population studies regarding the relation of vitamin D metabolites, including 25hydroxy-vitamin D (25(OH)D, the indicator of vitamin D status) and 1,25dihydroxy-vitamin D (1,25(OH)₂D, the biologically active form of vitamin D), with BP remains limited. Some cross-sectional[4] and case-control[5, 6] studies showed significant associations between blood concentrations of 25(OH)D or 1,25(OH)₂D and BP levels or hypertension status. In prospective studies, two health professional cohorts reported an inverse relation between baseline plasma 25(OH)D and risk of incident hypertension,[7, 8] while other population-based studies did not find associations between baseline 25(OH)D and longitudinal BP change[9, 10] or risk of future hypertension. [9] In addition, although variations in the VDR gene would presumably modify the effect of circulating vitamin D metabolites, these potential interactions have not been well examined with respect to hypertension risk. The objective of our study is to further evaluate vitamin D inadequacy as a novel risk factor for hypertension. We examined the prospective associations of plasma vitamin D metabolites, VDR gene polymorphisms, and their interaction with risk of developing hypertension in a cohort of middle-aged and older US male physicians.

Methods

Study Population

The Physicians' Health Study (PHS) was a randomized, double-blind, placebo-controlled trial of low-dose aspirin and β -carotene in the primary prevention of cardiovascular disease and cancer. The design and methods have been described previously.[11] In 1982, 22,071 US male physicians, aged 40–84 years and free of cardiovascular disease, cancer (except non-melanoma skin cancer), and other major illnesses were randomized into the PHS. The study was approved by the Brigham and Women's Hospital institutional review board. All participants provided written informed consent.

Nested case-control studies were previously conducted in the PHS for prostate cancer[12] and colon cancer.[13] In these studies, plasma 25(OH)D and 1,25(OH)₂D were measured, plus VDR *BsmI* and *FoXI* polymorphisms were genotyped in stored baseline blood samples. The current study utilizes controls in these previously conducted cancer case-control studies. In a total of 2,046 control subjects with measurements of either plasma vitamin D metabolites or VDR polymorphisms, we excluded those who had baseline hypertension (n=533) or missing incident hypertension status during follow-up (n=302), leaving 1,211 initially normotensive men for a prospective analysis. Among them, 660 men had measurement of plasma 25(OH)D, 651 had measurement of plasma 1,25(OH)₂D, 898 had

genotype of VDR *BsmI* polymorphism, 885 had genotype of VDR *FokI* polymorphism, and 356 had both measurements of vitamin D metabolites and VDR gene polymorphisms.

Assessment of Vitamin D Metabolites and VDR Gene Polymorphisms

Plasma concentrations of 25(OH)D and 1,25(OH)₂D were determined by radioimmunosorbant assay in two separate batches in the laboratory of Dr. Bruce Hollis (Medical University of South Carolina, Charleston, SC, USA). The assay methods have been described previously.[12, 13] Mean within-person coefficients of variation for blinded duplicate quality control samples ranged from 7.9% to 8.1% for the two metabolites over two batches. The VDR *BsmI* and *FokI* polymorphisms were determined by PCR amplification followed by restriction enzyme digestion at the Dana Farber/Harvard Cancer Center Genotyping Core, using DNA extracted from baseline blood specimens.

Ascertainment of Hypertension

Hypertension was identified from self-reports on the baseline and follow-up questionnaires, meeting at least one of three JNC 7 criteria: systolic BP (SBP) ≥ 140 mmHg, diastolic BP (DBP) ≥ 90 mmHg, or use of anti-hypertensive medication. Incident hypertension was defined as newly developed hypertension among those free of baseline hypertension. Participants reported the date for diagnosis of hypertension. If the diagnosis date was missing, it was randomly assigned between the annual questionnaires without and with hypertension. Men who developed cancer or cardiovascular disease, for which the management may impact BP levels, prior to the development of hypertension were censored on the date of cancer or cardiovascular disease diagnosis. Among physicians, self-reported BP has been shown to correlate well with measured SBP ($r=0.72$) and DBP ($r=0.60$).[14]

Baseline Covariates

On the PHS baseline questionnaires, men provided self-reports of age (in years), height and weight (used for calculation of body mass index [BMI] in kg/m²), cigarette smoking (never, past, current), alcohol use (none, monthly, weekly, daily), vigorous exercise (none, 1–3 times / month, 1 time / week, 2–4 times / week, 5–6 times / week, daily), multivitamin use (never, past, current), history of diagnosed diabetes (no, yes), and history of hyperlipidemia (treatment, diagnosis, or total cholesterol ≥ 240 mg/dL).

Data Analyses

All statistical analyses were conducted using SAS software (SAS Institute, Cary, NC, USA) version 9, with a two-sided significance level of 0.05. We first compared hypertension risk factors between men who developed incident hypertension versus those who did not. We then divided plasma vitamin D metabolites into quartiles. Because the concentrations of vitamin D metabolites differed by time of blood collection and assay batch, we used season- (summer/fall vs. winter/spring) and batch- specific cutpoints for 25(OH)D and batch-specific cutpoints for 1,25(OH)₂D. We used Cox regression models to estimate hazard ratios (HRs) for incident hypertension across quartiles of vitamin D metabolites, using the lowest quartile as the reference. We also repeated analyses for 25(OH)D using pre-determined clinical cutpoints. Multivariable models assessed the association after adjusting for known hypertension risk factors. To examine the association of VDR gene polymorphisms with hypertension risk, we compared the *BsmI* and *FokI* genotype frequencies by incident hypertension status, and used Cox regression models to calculate HRs for each genotype. We finally compared 25(OH)D and hypertension relation by VDR gene polymorphisms, and used likelihood ratio test to evaluate the statistical significance of interaction.

Results

Among 1211 men free of hypertension at baseline, 695 developed incident hypertension over a mean of 15.2 (maximum: 27.4) years follow-up. The baseline characteristics of these participants are shown in Table 1. Those who developed hypertension tended to have higher BMI and greater alcohol consumption compared to those who remained normotensive. Other lifestyle factors and clinical profiles did not significantly differ by participants' incident hypertension status. Presumably due to variations in sun exposure, mean concentration of 25(OH)D was significantly higher in blood samples collected in summer/fall (76.1 ± 27.2 nmol/L) than in those collected in winter/spring (56.8 ± 21.8 nmol/L). Plasma 25(OH)D and 1,25(OH)₂D were positively correlated in both summer/fall (Pearson $r = 0.21$, $p < 0.0001$) and winter/spring ($r = 0.29$, $p = 0.002$). In univariate analyses, baseline 25(OH)D was similar among men with and without incident hypertension regardless of the season of blood collection; unexpectedly, baseline 1,25(OH)₂D collected in summer/fall was higher among men who developed incident hypertension. When the VDR gene polymorphisms were compared, the minor allele frequency (MAF) of BsmI, but not FokI, polymorphism was significantly higher in those who developed incident hypertension.

Table 2 shows the risk of incident hypertension across increasing quartiles of vitamin D metabolites. The risk of hypertension was significantly lower in the third quartile of baseline plasma 25(OH)D compared with the lowest quartile. The association was strengthened after adjusting for lifestyle factors, but did not change further after adjusting for clinical factors. In the fully adjusted model (multivariable model 2), the HRs (95% CIs) of hypertension across increasing quartiles of 25(OH)D were 1.00 (reference), 0.94 (0.69–1.27), 0.69 (0.50–0.96), and 0.82 (0.60–1.13) (p , trend: 0.43). The test for a curvilinear trend of association was marginally significant ($p = 0.08$). Using clinical cutpoints, the multivariable model 2 HRs of hypertension across baseline plasma 25(OH)D of 50–<75, 75–<100, and 100 nmol/L were 1.03 (0.75–1.42), 0.79 (0.56–1.11), and 0.94 (0.62–1.40), respectively, compared with 25(OH)D <50 nmol/L. For plasma 1,25(OH)₂D, the corresponding HRs (95% CI) of hypertension across increasing quartiles were 1.00, 0.92 (0.66–1.27), 1.12 (0.82–1.54), and 1.19 (0.86–1.63), respectively (p , trend: 0.16).

In the current study, the VDR *BsmI* and *FokI* genotype frequencies were in Hardy-Weinberg equilibrium (both $p > 0.05$). *BsmI* minor allele B was significantly associated with increased risk of hypertension. Compared with carriers of *BsmI* bb, carriers of bB and BB had a multivariable model 2 HRs of 1.27 (95%CI: 1.04–1.55) and 1.19 (95%CI: 0.90–1.56), respectively, for incident hypertension.(Table 3) When the bB and BB genotypes were combined, the HR was 1.25 (95%CI: 1.04–1.51). For *FokI* polymorphism, an association with risk of hypertension was found only in the recessive model. Compared with carriers of FF and Ff genotypes combined, carriers of ff had a multivariable model 2 HR of 1.32 (95%CI: 1.03–1.70) for incident hypertension.(Table 3)

When the joint association of circulating vitamin D metabolites and VDR gene polymorphisms was examined, the association between plasma 25(OH)D and risk of hypertension was similar regardless of the selected VDR polymorphisms.(Figure) The multivariable model 2 HRs of hypertension across increasing 25(OH)D quartiles ranged from 1.0 (reference) to 0.77 among carriers of *BsmI* bB or BB genotypes and from 0.80 to 0.60 among carriers of *BsmI* bb genotype. For *FokI* polymorphism, the corresponding HRs ranged from 1.0 to 0.51 among carriers of ff genotype and from 0.54 to 0.44 among carriers of Ff or FF genotypes. The interactions were not statistically significant.

Discussion

In this prospective cohort of middle-aged and older US male physicians, we found suggestive evidence that men with higher concentration of baseline plasma 25(OH)D had a lower risk of developing hypertension. In addition, we found that bb or BB genotype of VDR *BsmI* polymorphism and ff genotype of VDR *FokI* polymorphism were associated with an increased risk of hypertension. There was no significant interaction between plasma 25(OH)D and VDR *BsmI* and *FokI* polymorphisms in association with risk of hypertension.

Early studies have shown that residence at higher latitudes,[15] dark skin,[16] and winter season,[17] all characterized with lower vitamin D photosynthesis from sun exposure, are associated with higher BP. Subsequently, some,[4] but not all,[18, 19] cross-sectional studies found inverse association between plasma 25(OH)D or 1,25(OH)₂D with BP levels. In case-control studies comparing hypertensive patients with normotensive controls, higher,[5] lower,[6] and similar[20] blood concentrations of vitamin D metabolites have all been reported. Prospective studies of this association are few. In a subsample of the Health Professionals Follow-up Study and Nurses' Health Study (NHS), comparing participants who had baseline plasma 25(OH)D <37.5 nmol/L versus ≥75 nmol/L, the multivariable relative risk of incident hypertension was 6.13 for men and 2.67 for women.[7] A later study among 1,500 younger women in the NHS II reported an odds ratio (OR) of 1.66 for incident hypertension in the lowest versus highest quartile of baseline 25(OH)D.[8] Among 559 women aged 24–44 years in the population-based longitudinal Michigan Bone Health and Metabolism Study, vitamin D insufficiency defined as 25(OH)D <80 nmol/L was associated with increased risk of systolic hypertension with an OR of 3.0 (95% CI: 1.01–8.77).[21] However, several other studies of general populations found no significant associations between baseline 25(OH)D and longitudinal change in BP[9, 10] or risk of future hypertension.[9]

A major hypothesized mechanism through which vitamin D may impact BP is the RAS.[1] Vitamin D is a potent hormone that down-regulates the renin gene expression in the kidney.[22] Vitamin D may also regulate BP through effects on calcium homeostasis,[23] vascular smooth muscle cell[24] and endothelial cell[25] function, inflammation,[26] and insulin sensitivity.[27] In our study of male physicians, compared with men in the lowest quartile of baseline 25(OH)D, those in the third quartile had a significantly lower risk of incident hypertension. Men in the highest quartile of baseline 25(OH)D did not have further reduced risk of hypertension. A recent study of 4,863 postmenopausal women enrolled in the Women's Health Initiative (WHI) showed similar finding that the lower risk of incident hypertension in the upper 3 quartiles of baseline 25(OH)D was statistically significant only in the 3rd quartile.[10] Such non-linear association has also been noted in studies of 25(OH)D with other cardiovascular outcomes.[28] The reasons for the non-linearity of associations are unclear. We cannot rule out the possibility of a finding by chance. Future research to determine the optimal concentration of 25(OH)D for cardiovascular health remains a high priority.

Our study found no clear association between baseline 1,25(OH)₂D and risk of hypertension. Using blood samples collected in summer/fall, we found an unexpected higher baseline 1,25(OH)₂D in men who later developed incident hypertension than those who did not. However, such association was not found using samples collected in winter/spring and was no longer significant after multivariable adjustment, suggesting that the observed association may be explained by season-related confounding factors such as physical activity. As the biologically active metabolite of vitamin D, 1,25(OH)₂D is under homeostatic control that tends to maintain the circulating level within a narrow range. In

contrast, 25(OH)D is the major circulating metabolite of vitamin D and reflects overall availability of vitamin D for functional activities.

The main activity of vitamin D is mediated by VDR, a nuclear transcription factor. The gene that encodes VDR is located on chromosome 12q13.1. Among the known VDR gene polymorphisms, *BsmI* is the only one that has been linked to BP. In a study of healthy Spanish subjects, SBP was higher with *BsmI* bb genotype than bB or BB genotypes in men, but not in women.[18] On the contrary, in a Koreans study, *BsmI* B allele carriers had higher SBP (2.7–3.7 mmHg) and DBP (1.9–2.5 mmHg) and a higher prevalence of hypertension (OR=2.1) than bb carriers.[29] Our study found a frequency distribution of *BsmI* genotype similar to the Spanish study, but the associations with hypertension consistent with the Korean study. Because *BsmI* is not located in exons or at exon-intron boundaries, it is unlikely to change the structure of VDR or produce splicing errors. Alternatively, strong linkage disequilibrium (LD) was observed between *BsmI* polymorphisms and polyA variable number of tandem repeat (VNTR) in the 3' untranslated region. The polyA VNTR is thought to involve in post-transcriptional control of gene expression[30] and thus may explain the observed association. To our knowledge, the association of ff genotype of *FokI* polymorphism with an increased risk of hypertension has not been reported prior to our study. The *FokI* polymorphism defines two potential translation initiation sites and produces two versions of VDR protein, a long *F*-VDR and a short *f*-VDR. Compared with the *F*-VDR, the *f*-VDR has shown higher capacity as a transcription factor.[31]

Our study did not find significant interactions between circulating vitamin D metabolites and VDR gene polymorphisms in relation to the risk of hypertension. In the Spanish study, a strong positive correlation between serum 25(OH)D and SBP and DBP was found only in men with VDR *BsmI* BB genotype.[18] The authors postulated that the carriers of *BsmI* B allele might be more sensitive to the effect of vitamin D. In our study, the relation between plasma 25(OH)D and risk of hypertension appeared relatively stronger among men who carried VDR *FokI* ff genotype than Ff/FF genotypes. We therefore cannot rule out the possibility that the adverse effect of vitamin D insufficiency on development of hypertension is more manifest among men with higher genetic susceptibility.

Several limitations of the current study deserve consideration. First, because vitamin D metabolites were measured in baseline blood after long-term storage, measurement error is a concern. However, the relation of plasma 25(OH)D with known predictors is similar in serum frozen for 40 y versus 2 y,[32] supporting the use of long-term stored blood samples for assessment of vitamin D status. Second, we relied on self-reported information to ascertain hypertension, which is subject to misclassification. Whereas self-reported hypertension in health professionals has demonstrated high validity.[14] In a subsample of the PHS, the status of self-reported incident hypertension was confirmed in 92% of men through telephone interview. Third, despite comprehensive adjustment for hypertension risk factors in our analysis, residual confounding remains possible as in all observational studies. In particular, many circulating biomarkers related to physiologic function and metabolism of vitamin D such as parathyroid hormone, calcium, and phosphorous are unavailable for our study. Nevertheless, in the study that had measured and adjusted for these biomarkers as covariates, the association between low 25(OH)D and increased risk of hypertension remained significant,[8] which argues against substantial residual confounding by these factors. Fourth, due to the moderate sample size of our study, power may be limited to detect small difference in risk of hypertension as statistically significant. Finally, the PHS is comprised of male physicians who are predominantly Caucasians, thus our findings may not be generalizable to men of different ethnicity and socioeconomic status and women.

Small, short-term intervention trials have reported BP-lowering effects of vitamin D supplements in selected patients.[33, 34] However, the largest trial of vitamin D to date - the WHI - found no difference in BP change and incident hypertension between those randomized to calcium (1000 mg/d) plus vitamin D (400 IU/d) supplementation versus placebo among 36,282 postmenopausal women over 7 years of treatment.[35] One potential explanation for this null finding is that the dose of vitamin D used in the WHI was too low to sufficiently increase the 25(OH)D concentrations to the level that has documented health benefits.[36] In addition, low adherence to study regimen in the WHI may have blunted treatment effect.[36] A recent meta-analysis including 11 trials of vitamin D found a significant reduction in DBP (-3.1 mmHg, 95% CI: $-5.5, -0.6$), but not in SBP, with vitamin D treatment compared with placebo.[37] The effect was more pronounced in subjects with elevated BP at baseline than in normotensive subjects. Considering that vitamin D inadequacy is a highly prevalent health problem, more research is needed to determine if improving vitamin D status represents an effective strategy for hypertension prevention.

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Abbreviation

RAS	renin-angiotensin system
BP	blood pressure
SBP	systolic blood pressure
DBP	diastolic blood pressure
VDR	vitamin D receptor
25(OH)D	25hydroxy-vitamin D
1,25(OH)₂D	1,25dihydroxy-vitamin D
PHS	Physicians' Health Study
HR	hazard ratio
BMI	body mass index
NHS	Nurses' Health Study
WHI	Women's Health Initiative
LD	linkage disequilibrium
VNTR	variable number of tandem repeat

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FIGURE.

Multivariable model 2 in Table 2 and 3 was used. Plasma 25(OH)-vitamin D (25(OH)D) was divided to quartiles (Q1–Q4), vitamin D receptor (VDR) gene polymorphisms were categorized to dominant model for *BsmI* and recessive model for *FokI*, according to their respective relation with risk of hypertension. Interaction was tested using likelihood ratio test.

Joint association of plasma 25(OH)-vitamin D and vitamin D receptor gene polymorphisms with the risk of hypertension

TABLE 1

Characteristics	Men with plasma vitamin D metabolites measurements		Men with vitamin D receptor polymorphism genotypes			
	Incident hypertension		p ^(b)	Incident hypertension		
	Yes (N=367)	No (N=293)		Yes (N=537)	No (N=376)	P
Age, years ^(a)	56.6±7.4	57.6±7.6	0.10	57.1±7.5	57.5±8.2	0.38
Body mass index, kg/m ²	24.6±2.5	24.2±2.4	0.05	24.6±2.3	24.3±2.5	0.12
Cigarette smoking, %			0.97			0.23
Never	47.4	47.8		49.4	52.4	
Past	45.5	44.7		43.4	38.3	
Current	7.1	7.5		7.3	9.3	
Alcohol use, %			0.007			0.17
None	13.8	21.6		15.0	20.0	
Monthly	10.2	5.8		9.4	7.0	
Weekly	44.9	48.0		47.6	47.2	
Daily	31.1	24.7		28.1	25.9	
Exercise, %			0.12			0.51
None	9.9	12.0		12.5	11.0	
1–3 / month	18.1	11.0		16.1	12.3	
1 / week	15.9	17.9		16.5	16.4	
2–4 / week	38.4	44.3		37.2	42.4	
5–6 / week	9.6	7.9		10.5	9.9	
Daily	8.2	6.9		7.3	8.0	
Multivitamin use, %			0.73			0.94
Never	62.4	62.9		64.6	65.1	
Past	14.2	15.8		13.6	12.8	
Current	23.4	21.3		21.8	22.1	
History of diabetes, %	1.63	2.05	0.69	1.12	1.60	0.53
History of hypercholesterolemia, %	12.4	15.7	0.24	13.2	17.9	0.06
Systolic blood pressure, mmHg	124.1±7.6	119.7±8.6	<0.0001	123.9±8.0	119.0±8.5	<0.0001
Diastolic blood pressure, mmHg	77.8±5.7	74.7±6.6	<0.0001	77.7±5.8	74.7±6.3	<0.0001

Characteristics	Men with plasma vitamin D metabolites measurements			Men with vitamin D receptor polymorphism genotypes		
	Incident hypertension		P ^(b)	Incident hypertension		P
Plasma 25(OH)-vitamin D	Yes (N=367)			Yes (N=537)		
	No (N=293)			No (N=376)		
	Winter/Spring			Winter/Spring		
	N			N		
Concentration, nmol/L ^(c)	59			58		
	57.9±20.7			55.9±22.5		
	Summer/fall			Summer/fall		
	N			N		
Concentration, nmol/L	308			235		
	74.9±27.5			77.4±26.2		
	Plasma 1,25(OH) ₂ -vitamin D			Plasma 1,25(OH) ₂ -vitamin D		
	N			N		
Winter/Spring	57			57		
	83.5±17.7			83.7±20.0		
	Summer/fall			Summer/fall		
	N			N		
Concentration, pmol/L ^(d)	306			231		
	89.4±21.8			84.8±18.7		
	Vitamin D receptor polymorphisms			Vitamin D receptor polymorphisms		
	N			N		
BsmI	527			371		
	40.3			34.9		
	MAF, %			MAF, %		
	0.01			0.01		
FokI	522			363		
	38.2			37.2		
	MAF, %			MAF, %		
	0.20			0.20		

MAF, Minor Allele Frequency.

^(a)Mean ± SD are shown for continuous variables and percentages are shown for categorical variables.

^(b)P values were derived from *t*-test for continuous variables and χ^2 test for categorical variables.

^(c)To convert 25(OH)-vitamin D in nmol/L to ng/mL, divide by 2.496.

^(d)To convert 1,25(OH)₂-vitamin D in pmol/L to pg/mL, divide by 2.6.

TABLE 2
Hazard ratios and 95% confidence intervals of hypertension according to concentrations of plasma vitamin D metabolites

Concentrations of vitamin D metabolites					P, trend ^(a)
	1 st	2 nd	3 rd	4 th	
25(OH)-vitamin D					
Quartile ^(b)	1 st Quartile	2 nd Quartile	3 rd Quartile	4 th Quartile	
N of cases / total	97 / 164	97 / 164	79 / 167	94 / 165	
Age and race adjusted model	1.00 (reference)	1.02 (0.77–1.36)	0.74 (0.55–0.99)	0.91 (0.68–1.20)	0.47
Multivariable model 1 ^(c)	1.00 (reference)	0.97 (0.73–1.29)	0.70 (0.51–0.94)	0.78 (0.58–1.07)	0.15
Multivariable model 2 ^(d)	1.00 (reference)	0.94 (0.69–1.27)	0.69 (0.50–0.96)	0.82 (0.60–1.13)	0.43
Clinical cutpoint, nmol/L ^(e)	<50	50–<75	75–<100	100	
N of cases / total	73 / 136	144 / 244	93 / 178	57 / 102	
Age and race adjusted model	1.00 (reference)	1.15 (0.86–1.54)	0.86 (0.63–1.18)	1.01 (0.70–1.44)	0.42
Multivariable model 1	1.00 (reference)	1.12 (0.83–1.50)	0.78 (0.57–1.09)	0.89 (0.61–1.32)	0.13
Multivariable model 2	1.00 (reference)	1.03 (0.75–1.42)	0.79 (0.56–1.11)	0.94 (0.62–1.40)	0.32
1,25(OH) ₂ -vitamin D					
Quartile ^(f)	1 st Quartile	2 nd Quartile	3 rd Quartile	4 th Quartile	
N of cases / total	87 / 162	80 / 162	95 / 165	101 / 162	
Age and race adjusted model	1.00 (reference)	0.84 (0.62–1.14)	1.07 (0.80–1.44)	1.20 (0.90–1.60)	0.08
Multivariable model 1	1.00 (reference)	0.85 (0.63–1.17)	1.03 (0.77–1.39)	1.13 (0.84–1.52)	0.23
Multivariable model 2	1.00 (reference)	0.92 (0.66–1.27)	1.12 (0.82–1.54)	1.19 (0.86–1.63)	0.16

^{a)}Linear trend across increasing concentrations of 25(OH)-vitamin D was tested using the median value of each category as an

^(a) Linear trend across increasing concentrations of 25(OH)-vitamin D was tested using the median value of each category as an ordinal variable.

^(b) Range for quartiles of 25(OH)-vitamin D: Batch 1, Winter / Spring: 13.0 – 39.9, 40.0 – 61.9, 62.0 – 76.5, 76.6 – 125.3 nmol/L; Batch 1, Summer / Fall: 18.0 – 54.4, 54.5 – 70.2, 70.3 – 89.9, 90.0 – 167.2 nmol/L; Batch 2, Winter / Spring: 24.7 – 36.9, 37.0 – 48.5, 48.6 – 68.7, 68.8 – 88.1 nmol/L; Batch 2, Summer / Fall: 20.7 – 57.8, 57.9 – 74.9, 75.0 – 93.5, 93.6 – 149 nmol/L. To convert 25(OH)-vitamin D in nmol/L to ng/mL, divide by 2.496.

^(c) Multivariable model 1 additionally adjusted for cigarette smoking status (never, past, current), alcohol consumption (none, monthly, weekly, daily), exercise (none, 1–3/m, 1/w, 2–4/w, 5–6/w, daily), and multivitamin use (never, past, current).

^(d) Multivariable model 2 adjusted for all covariates in model 1 plus body mass index (continuous), history of diabetes (yes, no), and history of hypercholesterolemia (yes, no).

^(e) Models using clinical cutpoints also adjusted for season of blood collection (winter/spring or summer/fall).

^(f)Range for quartiles of 1,25(OH)₂-vitamin D: Batch 1: 36.9 – 76.3, 76.4 – 88.2, 88.3 – 101.8, 101.9 – 177.6 pmol/L; Batch 2: 29.9 – 67.9, 68.0 – 80.7, 80.8 – 93.9, 94.0 – 155.5 pmol/L. To convert 1,25(OH)₂-vitamin D in pmol/L to pg/mL, divide by 2.6.

TABLE 3
Hazard ratios and 95% confidence intervals of hypertension according to vitamin D receptor gene polymorphisms

	<i>BsmI</i> polymorphism			<i>FokI</i> polymorphism			P
	bb	bB	BB	FF	ff	ff	
N of cases / total	182 / 346	265 / 420	80 / 132	208 / 347	229 / 407	85 / 131	
Age and race adjusted model	1.00 (reference)	1.36 (1.12–1.64)	1.27 (0.98–1.65)	1.00 (reference)	0.94 (0.78–1.14)	1.23 (0.95–1.58)	0.30
	1.00 (reference)	1.34 (1.12–1.60)		1.00 (reference)		1.27 (1.00–1.60)	
Multivariable model 1 ^(b)	1.00 (reference)	1.31 (1.08–1.59)	1.25 (0.96–1.63)	1.00 (reference)	0.92 (0.76–1.12)	1.23 (0.95–1.59)	0.34
	1.00 (reference)	1.29 (1.08–1.55)		1.00 (reference)		1.28 (1.01–1.63)	
Multivariable model 2 ^(c)	1.00 (reference)	1.27 (1.04–1.55)	1.19 (0.90–1.56)	1.00 (reference)	0.87 (0.72–1.06)	1.23 (0.94–1.61)	0.50
	1.00 (reference)	1.25 (1.04–1.51)		1.00 (reference)		1.32 (1.03–1.70)	

^(a) P values were derived from additive effect model in which wild-type homozygotes, heterozygotes, and variant homozygotes were coded as 0, 1, and 2, respectively.

^(b) Multivariable model 1 additionally adjusted for cigarette smoking status (never, past, current), alcohol consumption (none, monthly, weekly, daily), exercise (none, 1–3/m, 1/w, 2–4/w, 5–6/w, daily), multivitamin use (never, past, current).

^(c) Multivariable model 2 adjusted for all covariates in model 1 plus body mass index (continuous), history of diabetes (yes, no), and history of hypercholesterolemia (yes, no).