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Genetic Variants and Associations of 25-Hydroxyvitamin D Concentrations With Major Clinical Outcomes

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Abstract

Context—Lower serum 25-hydroxyvitamin D concentrations are associated with greater risks of many chronic diseases across large, prospective community-based studies. Substrate 25-hydroxyvitamin D must be converted to 1,25-dihydroxyvitamin D for full biological activity, and complex metabolic pathways suggest that interindividual variability in vitamin D metabolism may alter the clinical consequences of measured serum 25-hydroxyvitamin D.

Objective—To investigate whether common variation within genes encoding the vitamin D–binding protein, megalin, cubilin, *CYP27B1*, *CYP24A1*, and the vitamin D receptor (*VDR*) modify associations of low 25-hydroxyvitamin D with major clinical outcomes.

Design, Setting, and Participants—Examination of 141 single-nucleotide polymorphisms in a discovery cohort of 1514 white participants (who were recruited from 4 US regions) from the community-based Cardiovascular Health Study. Participants had serum 25-hydroxyvitamin D measurements in 1992–1993 and were followed up for a median of 11 years (through 2006). Replication meta-analyses were conducted across the independent, community-based US Health, Aging, and Body Composition (n=922; follow-up: 1998–1999 through 2005), Italian Invecchiare in Chianti (n=835; follow-up: 1998–2000 through 2006), and Swedish Uppsala Longitudinal Study of Adult Men (n = 970; follow-up: 1991–1995 through 2008) cohort studies.

Main Outcome Measure—Composite outcome of incident hip facture, myocardial infarction, cancer, and mortality over long-term follow-up.

Results—Interactions between 5 single-nucleotide polymorphisms and low 25-hydroxyvitamin D concentration were identified in the discovery phase and 1 involving a variant in the *VDR* gene replicated in independent meta-analysis. Among Cardiovascular Health Study participants, low 25-hydroxyvitamin D concentration was associated with hazard ratios for risk of the composite outcome of 1.40 (95% CI, 1.12–1.74) for those who had 1 minor allele at rs7968585 and 1.82 (95% CI, 1.31–2.54) for those with 2 minor alleles at rs7968585. In contrast, there was no evidence of an association (estimated hazard ratio, 0.93 [95% CI, 0.70–1.24]) among participants who had 0 minor alleles at this single-nucleotide polymorphism.

Conclusion—Known associations of low 25-hydroxyvitamin D with major health outcomes may vary according to common genetic differences in the vitamin D receptor.

Vitamin D status is defined by the circulating concentration of 25-hydroxyvitamin D. [1,2] In large, prospective cohort studies, lower serum 25-hydroxyvitamin D concentrations are associated with greater risks of hip fracture, myocardial infarction (MI), cancer, and death.[3–10] In experimental models, disruption of the vitamin D–endocrine axis stimulates inflammatory cytokines, activates the renin-angiotensin system, and impairs skeletal mineralization.[11–13] The totality of these findings suggests that low 25-hydroxyvitamin D concentration may be a modifiable risk factor for many chronic diseases, which has motivated ongoing clinical trials to test whether vitamin D supplementation can reduce the risk of disease development.[14] Laboratory testing for serum 25-hydroxyvitamin D and empirical therapy with vitamin D supplements have increased dramatically worldwide, with substantial associated costs.

Substrate 25-hydroxyvitamin D must be converted to 1,25-dihydroxyvitamin D, which is the potent hormonal form of vitamin D, for full biological activity. Conversion requires transportation in the blood by the vitamin D–binding protein, internalization via cell surface proteins megalin and cubilin, and metabolism by the 1-α hydroxylase enzyme. Activated 1,25-dihydroxyvitamin D then binds to the vitamin D receptor and regulates expression of a diverse array of vitamin D responsive genes. Elimination of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D is primarily catalyzed by the 24-α hydroxylase enzyme.[1,2] These complex metabolic pathways suggest that interindividual variability in vitamin D metabolism may alter the clinical consequences of measured serum 25-hydroxyvitamin D.

We hypothesized that known serum 25-hydroxyvitamin D disease relationships would differ according to common variation in 25-hydroxyvitamin D metabolism genes. We investigated whether single-nucleotide polymorphisms (SNPs) within genes encoding proteins that reside downstream from 25-hydroxyvitamin D modified associations of low serum 25-hydroxyvitamin D concentration with a composite outcome of incident hip fracture, MI, cancer, and mortality over long-term follow-up.

METHODS

Study Populations

The Cardiovascular Health Study (CHS) is a cohort study of cardiovascular disease among 5888 ambulatory adults aged 65 years or older living in 1 of 4 US communities.[15] We measured serum 25-hydroxyvitamin D concentrations during 1992–1993 in 2312 CHS participants without prevalent cardiovascular disease.[16] The Health, Aging, and Body Composition (Health ABC) study is a cohort study of changes in body composition among 3075 community-dwelling adults aged 70 to 79 years.[17] Serum 25-hydroxyvitamin D concentrations were measured in 2998 participants during 1998–1999. The Invecchiare in Chianti (InCHIANTI) study is a population-based cohort study of 1453 primarily older persons living in the Chianti region of Tuscany, Italy, with 25-hydroxyvitamin D concentration ascertainment during 1998–2000.[18] The Uppsala Longitudinal Study of Adult Men (ULSAM) is a cohort study of 2322 initially 50-year-old Swedish men aimed at identifying metabolic risk factors for cardiovascular disease.[19] Concentrations of 25-hydroxyvitamin D were measured during 1991–1995 in 1221 men. We excluded participants from these 4 study cohorts with self-reported nonwhite race (to reduce potential confounding effects of population stratification), as well as those who had prevalent disease (hip fracture, MI, or cardiovascular disease, depending on the cohort, or cancer), unsuccessful genotyping, or failed 25-hydroxyvitamin D ascertainment. All participants provided informed consent, and institutional review boards reviewed and approved the procedures at all sites.

Concentrations of 25-Hydroxyvitamin D and Genotype Ascertainment

Circulating serum 25-hydroxyvitamin D concentrations were measured using mass spectrometry (CHS and ULSAM) or radioimmunoassay (DiaSorin RIA[20]; Health ABC and InCHIANTI). All inter-assay coefficients of variation were less than 10.2% (eTable 1 at http://www.jama.com). We evaluated 25-hydroxyvitamin D as a dichotomous variable because we and others[3,4,6,8,10,21] have observed threshold associations of 25-hydroxyvitamin D with disease risks. We used season-specific cut points because of the known seasonal variability in 25-hydroxyvitamin D concentrations,[22–25] and its associated impact on modeling.[26] We defined low vitamin D concentration as the lowest season-specific quintile to ensure a similar definition across the study cohorts, which used different methods to measure serum 25-hydroxyvitamin D.

Genotyping was performed using Illumina platforms and software (eTable 1). Genome-wide association data for the CHS was drawn from the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium,[27] and SNPs were excluded for a call rate of less than 97% or a Hardy-Weinberg equilibrium *P* value of less than 10^{-5}. We identified the following 6 genes based on their known role in metabolic pathways downstream from 25-hydroxyvitamin D: vitamin D–binding protein (*GC*; NCBI Entrez Gene NG_012837.2), megalin (*LRP2*; NCBI Entrez Gene NG_012634.1), cubilin (*CUBN*; NCBI Entrez Gene NG_008967.1), 1-α hydroxylase (*CYP27B1*; NCBI Entrez Gene NG_007076.1), 24-α hydroxylase (*CYP24A1*; NCBI Entrez Gene NG_008334.1), and the vitamin D receptor (*VDR*; NCBI Entrez Gene NG_008731.1). We studied 141 SNPs located within 20 kb of these genes that were successfully genotyped and had a minor allele frequency in the CHS of at least 5%.

Composite Outcome

The study outcome was the time from 25-hydroxyvitamin D measurement to the first occurrence of incident hip fracture, incident MI, incident cancer (excluding non–melanoma skin cancer), or death from any cause. This composite outcome was selected prior to the analyses to capture previously described associations of 25-hydroxyvitamin D with clinically important disease outcomes and to maximize statistical power to detect potential interactions. In the CHS (follow-up until 2005 or 2006), an events committee adjudicated MI cases.[28] Hip fracture was defined by the *International Classification of Diseases, Ninth Revision* codes, and incident cancer cases were identified by linking the CHS records with population-based cancer registries. InCHIANTI investigators ascertained outcome information by examining and interviewing participants every 3 years (through 2006), and mortality data was obtained by the Tuscany Regional Health Authority. In the Health ABC study (follow-up until December 31, 2005), participants or their proxies reported any hospitalizations, outpatient cancer, fracture, or angioplasty events. Incident hip fracture, MI, and cancer events were adjudicated using medical records and other supporting documents. Incident events in the ULSAM (through December 31, 2008) were identified using the Swedish National Patient Register and the Swedish Cause of Death Register.

Statistical Analyses

All survival analyses used Cox proportional hazards models with robust standard errors, adjusting for age and sex. Additional variables such as physical activity, hypertension, diabetes, and body mass index were not included in the models because the candidate genes (selected specifically on the basis of their role in the downstream metabolism of 25-hydroxyvitamin D) are unlikely to alter the impact of these potential confounding factors on the composite disease outcome through mechanisms outside of the vitamin D pathway. We graphically displayed a penalized spline[29] to describe the functional form of the association between season-specific 25-hydroxyvitamin D concentration and incident events in the CHS.

We estimated the multiplicative interaction between low vitamin D concentration and each candidate SNP on risk of the composite outcome, and computed interaction *P* values using Wald tests. In these models, an exponential coefficient provides an estimate of the relative difference in the association of low 25-hydroxyvitamin D with risk of the composite outcome corresponding to the presence of 1 additional copy of the minor allele (ie, an estimated hazard ratio ratio [HRR]). To account for multiple statistical testing in the discovery phase, false discovery rate *q* values were calculated.[30] We used a false discovery rate threshold of .25, meaning that we expected up to 25% of our declared discoveries to be false. It is common to use a threshold such as .20 or .25 in candidate gene studies, which use

prior knowledge to select candidate genes and which conduct replication in independent cohorts.[31,32]

Genetic variants with interaction *q* values below the .25 threshold in the CHS discovery cohort were tested in the Health ABC, InCHIANTI, and ULSAM cohorts for replication. We calculated summary HRRs and 95% confidence intervals using Mantel-Haenszel methods and a fixed-effects meta-analysis model. Given that estimates from the discovery phase are likely biased away from the null,[33] we used the pooled results from the independent cohorts alone to determine successful replication at the .05 significance level. We performed tests for cohort-level heterogeneity of the interaction estimates using the Woolf method.[34] We carried out additional analyses in the CHS cohort to explore the nature of any replicated interactions. Within strata defined by each genetic variant, we computed Kaplan-Meier estimates and unadjusted incidence rates of the composite outcome according to 25-hydroxyvitamin D status, as well as adjusted HRs comparing participants with normal and low 25-hydroxyvitamin D concentrations. We jointly modeled interactions between the 2 *VDR* SNPs and 25-hydroxyvitamin D concentration to examine whether both variants independently modified the association of low 25-hydroxyvitamin D with risk of the composite outcome.

We report 95% confidence intervals and 2-sided *P* values. No adjustments for multiple comparisons in the replication phase were conducted. Statistical analyses were completed using R version 2.11.0 (R Project for Statistical Computing), Stata versions 10.1 and 11.2 (Stata-Corp), and SAS version 9.1 (SAS Institute Inc).

RESULTS

Baseline Characteristics

In the CHS cohort, we excluded 341 participants who had prevalent hip fracture or cancer, 309 participants of non-white race, and 148 participants who were unable to be genotyped, resulting in a discovery study population of 1514 (eFigure 1). We excluded Health ABC participants who had prevalent cardiovascular disease (n = 771), cancer (n = 414), or hip fracture (n = 24), missing (n=102) or high 25-hydroxyvitamin D concentrations (>100 ng/mL; n = 2), nonwhite race (n = 690), or unsuccessful genotyping (n = 73), creating a cohort of 922 participants. In the InCHIANTI study, we excluded participants who did not return for future visits or have outcomes adjudicated (n = 277), and those with missing 25-hydroxyvitamin D concentrations (n = 66), unsuccessful genotyping (n=114), or prevalent hip fracture, MI, or cancer (n = 161), leaving 835 participants. We excluded ULSAM participants with missing kidney function measurement (n = 65), and those with a history of MI (n = 94), hip fracture (n = 14), or cancer (n = 78), creating a replication cohort of 970 men.

The CHS discovery cohort was 70% female, with a mean age of 74 years and mean serum 25-hydroxyvitamin D concentration of 26.7 ng/mL. Participants who had low 25-hydroxyvitamin D concentrations were more likely to be female, had a higher mean body mass index, and were more likely to have diabetes or hypertension vs those who had normal 25-hydroxyvitamin D concentrations. Demographic and comorbidity profiles of participants from the Health ABC, InCHIANTI, and ULSAM replication cohorts were generally similar to those of the CHS discovery cohort, with a few notable exceptions. The Health ABC population had a considerably higher prevalence of male participants and vitamin D supplement use, the InCHIANTI participants were younger and more likely to be male, and the ULSAM cohort was composed entirely of men. Mean 25-hydroxyvitamin D concentration was lower among the InCHIANTI participants, and season-specific thresholds defining low vitamin D concentration varied by cohort (Table 1).

Analyses of the CHS Discovery Cohort

In the CHS cohort, 948 participants (63%) experienced an event contained within the composite outcome over a median (maximum) follow-up period of 11 (15) years. Consistent with previous studies, there was evidence of a threshold association between serum 25-hydroxyvitamin D concentration and risk of the composite outcome (eFigure 2). With adjustment for age and sex, low 25-hydroxyvitamin D concentration was associated with an HR of 1.32 (95% CI, 1.13–1.54; P=.001).

Of the 141 SNPs tested for discovery in the CHS cohort, 6 met the false-discovery rate threshold of 0.25. Two are located (±20 kb) within *VDR*, 2 in *CUBN*, and 2 in *CYP27B1*. The 2 identified *CYP27B1* variants are in near-perfect linkage disequilibrium[35]; we carried only rs703842 forward to the replication phase because direct information on rs2069502 was not ascertained in the ULSAM. Each additional copy of the minor allele in the 5 remaining identified SNPs was associated with a 30% to 40% difference in the HR (P value range: .002–.008). The magnitudes and directions of each interaction were consistent across the individual events that comprised the composite outcome (Table 2). An additional 5 SNPs (all in *VDR* or *CUBN*) had P values of less than .05, but q values of greater than .25. This included the well-known *VDR* variant BsmI (rs1544410; HRR, 0.76, P = .02).

Replication Analyses

In the Health ABC, ULSAM, and In-CHIANTI cohorts, a total of 1216 participants experienced the composite outcome. Estimates of association between low 25-hydroxyvitamin D concentration and risk of the composite outcome were in the same direction in all 4 cohorts, but statistical significance was attained only in the Health ABC cohort (eTable 2). Of the 5 variants identified in the CHS cohort, only the *VDR* SNP rs7968585 reached statistical significance for modifying the association between low 25-hydroxyvitamin D concentration and risk of the composite outcome in independent replication meta-analyses. An additional *VDR* SNP (rs2239179) attained statistical significance in a meta-analysis that included CHS results (Table 3). Each additional copy of the minor allele at rs7968585 was associated with an HRR of 1.22 (95% CI, 1.09–1.36) and each additional copy of the minor allele at rs2239179 was associated with an HRR of 0.85 (95% CI, 0.76–0.97). The HRRs for these 2 interactions were reasonably consistent across the independent cohorts (eFigure 3), and there was no statistical evidence of heterogeneity. The meta-analysis interactions also demonstrated similar magnitudes and directions across the individual events that comprised the composite outcome (eTable 3). These 2 *VDR* SNPs are in moderate linkage disequilibrium (R^2= 0.55).[35]

The nature of the observed interactions can be seen by comparing estimates of disease-free survival, as well as incidence rates of the composite outcome, across groups defined by each variant and by 25-hydroxyvitamin D concentration status (CHS data; Table 4 and eFigure 4). Without accounting for 25-hydroxyvitamin D concentration, there was no evidence in the CHS cohort of main effect associations between either rs7968585 (HR per additional minor allele, 1.00 [95% CI, 0.91–1.06]; P = .93) or rs2239179 (HR, 0.93 [95% CI, 0.85–1.02]; P = .13) and risk of the composite outcome. In an analysis jointly modeling both SNPs and their interactions with 25-hydroxyvitamin D concentration, there was insufficient statistical evidence to suggest that both rs7968585 (HRR, 1.26 [95% CI, 0.95–1.68]; P = .11) and rs2239179 (HRR, 0.86 [95% CI, 0.64–1.16]; P = .08) independently modified the association of low vitamin D concentration with risk of the composite outcome.

COMMENT

We found a SNP within the *VDR* gene that significantly modified associations of low serum 25-hydroxyvitamin D concentration with major health outcomes of hip fracture, MI, cancer,

and death over long-term follow-up. Findings were observed within a large community-based study of older adults in the United States and were consistent in magnitude and direction across individual disease outcomes, and replicated in a meta-analysis of 3 large independent cohorts. An additional *VDR* SNP significantly modified the low 25-hydroxyvitamin D–disease association in a meta-analysis that included results from the discovery and replication cohorts. The discovered SNPs, which are common in European populations, identified subsets of individuals for whom associations between low 25-hydroxyvitamin D concentration and disease outcomes were either strongly positive vs null. These results suggest that individuals with specific 25-hydroxyvitamin D metabolism genotypes may be particularly susceptible to, or protected from, the potential adverse health effects of low vitamin D.

The 2 identified *VDR* variants rs7968585 and rs2239179 are common SNPs, with minor allele frequencies of 0.48 and 0.42, respectively. The SNPs have moderate linkage (R^2=0.55) and exploratory analyses provided insufficient evidence (P=.08) of independent interactions between each of these variants and low 25-hydroxyvitamin D concentration on risk of the composite outcome. These results make it unclear whether the interactions between the 2 observed SNPs and 25-hydroxyvitamin D concentration represent unique signals.

The rs7968585 and rs2239179 SNPs are not located in a coding region or GT-AG splice site.[36] However, the 2 variants are in linkage disequilibrium with a cluster of common SNPs (BsmI, *Taq*I, and ApaI) at the 3′ end of the*n VDR* gene that are associated with clinical outcomes in other studies. The rs7968585 SNP is intergenic, located 3.2 kb downstream from *VDR*, and in moderate to high linkage disequilibrium with ApaI (rs7975232, R^2= 0.87), *Taq*I (rs731236, R^2 = 0.65), and BsmI (rs1544410, R^2= 0.65).[35] One recent study in postmenopausal women with osteoporosis found *Taq*I to be associated with differential response to calcitriol and calcium therapy.[37] Candidate gene studies and meta-analyses link the ApaI and BsmI variants with the development of several types of cancer.[38–41] Specific ApaI, *Taq*I, and BsmI genotypes may also be related to decreased bone mineral density and elevated fracture risk.[42–44] The ApaI and *Taq*I variants were not genotyped in the CHS and thus were not candidates in the discovery phase. The SNP BsmI was genotyped in the CHS, and its interaction with low 25-hydroxyvitamin D concentration was of borderline statistical significance (HRR, 0.76; P = .02, q = 0.29). The other genetic variant identified in this study, rs2239179, is an intronic *VDR* SNP that may be related to the risk of renal cancer.[45]

There is biological plausibility that genetic variation within the vitamin D receptor could alter associations of 25-hydroxyvitamin D concentrations with disease outcomes. The *VDR*, a member of the steroid-receptor gene superfamily, plays a central role in mediating vitamin D signaling. The biologically active 1,25-dihydroxyvitamin D ligand binds to the *VDR*, which then forms a heterodimer with the retinoid X receptor. The *VDR* then enters the nucleus to bind vitamin D response elements in multiple target genes through a conserved DNA binding sequence.[46] Genetic variation within the *VDR* could alter the response to 25-hydroxyvitamin D stores. For example, greater *VDR* affinity for 1,25-dihydroxyvitamin D or greater *VDR* activity for a given amount of 25-hydroxyvitamin D could provide protection in situations of low 25-hydroxyvitamin D substrate, such as residence in communities at high latitudes. These conjectures remain speculative because the biological effect of the discovered genetic variants on *VDR* function remains unknown.

Strengths of our study include the use of large, community-based cohort studies that include long-term follow-up, excellent retention, and independent adjudication of major health events to identify potentially important interactions. The use of a composite outcome captures many of the suspected biological effects of low vitamin D across a wide range of

health processes. We performed a replication phase in 3 independent cohorts that included long-term follow-up for the same clinical disease events as the discovery cohort to confirm findings and obtain more reliable and precise estimates of the identified interactions.

Our study has several important limitations. First, there was some heterogeneity of the estimated interactions across the study cohorts, which could be explained by differences in the demographic, comorbidity, or 25-hydroxyvitamin D distributions at baseline, or by contrasting incidence rates of the composite outcome. Second, genetic information in our study was derived from SNPs measured in whole genome chip platforms, which incompletely characterize the target genes and cannot precisely identify potential causal variants. In addition, we lack functional data to explore why the identified *VDR* variants may modify 25-hydroxyvitamin D associations. With a single discovery cohort and candidate gene approach, we could have missed other important interactions between genotype and 25-hydroxyvitamin D concentration. Our assessment of low 25-hydroxyvitamin D concentrations is based on a single measurement that may not best reflect typical circulating concentrations over a longer period. Furthermore, the demographic group included in our study was primarily older white adults, and the findings may not be generalizable to other demographic or ethnic groups.

In summary, results of this candidate gene study indicate that known associations of low serum vitamin D concentration with clinical outcomes may vary according to genetic differences in the vitamin D receptor. These findings represent a first step toward identifying what may be clinically relevant effects of 25-hydroxyvitamin D metabolism genes and may contribute to a better understanding of the biological impact of genetic variation within the vitamin D receptor. Further studies are needed to confirm these observed associations and to enhance knowledge of how variation in vitamin D metabolism genes may stratify individuals as to their susceptibility to vitamin D deficiency. Evaluating the identified interactions in randomized clinical trials of vitamin D supplementation, when available, would help to assess the validity of our results and pave the way toward identifying individual patients who may benefit most from vitamin D interventions.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline Characteristics in the Cardiovascular Health Study, Health ABC, InCHIANTI, and ULSAM Cohorts

	No. (%) of Participants						
	Cardiovascular Health Study						
	25-Hydroxyvitamin D Level[b]						
	Normal (n = 1210)	Low (n = 304)	Overall (n = 1514)	Health ABC Overall (n = 922)	InCHIANTI Overall (n = 835)	ULSAM Overall (n = 970)	All Cohorts Combined (n = 4241)
Age, mean (SD), y	73.7 (4.5)	74.7 (4.8)	73.9 (4.6)	74.5 (2.8)	66.0 (15.7)	71.0 (0.6)	71.8 (8.2)
Male sex	408 (34)	53 (17)	461 (30)	435 (47)	393 (47)	970 (100)	2259 (53)
Current smoker	103 (9)	39 (13)	142 (10)	67 (7)	173 (21)	202 (21)	584 (14)
Any alcohol intake	568 (47)	135 (44)	703 (46)	543 (59)	642 (77)	813 (84)	2701 (64)
Body mass index, mean (SD)[c]	26.3 (4.2)	26.9 (5.3)	26.4 (4.4)	26.4 (4.0)	27.1 (4.1)	26.2 (3.4)	26.5 (4.1)
Diabetes[d]	108 (9)	34 (11)	142 (9)	118 (13)	73 (9)	101 (10)	434 (10)
Hypertension[e]	629 (52)	184 (61)	813 (54)	589 (64)	251 (30)	717 (74)	2370 (56)
Estimated GFR, mean (SD), mL/min/1.73m^2[f]	78 (18.9)	80.6 (21.3)	78.5 (19.4)	70.7 (13.7)	80.4 (17.5)	76.0 (12.5)	76.6 (16.8)
Vitamin D supplement use	5 (0.4)	0	5 (0.3)	132 (14)	NA	37 (4)	174 (4)
25-Hydroxyvitamin D							
Serum level, mean (SD), ng/mL	29.7 (9.2)	14.4 (4.1)	26.7 (10.4)	29.2 (10.2)	22.2 (14.3)	27.5 (7.5)	26.5 (10.9)
Season-specific 20th percentile, ng/mL							
Winter			14	18	10	17	15
Spring			18	19	13	16	16
Summer			22	23	15	17	19
Autumn			19	23	12	19	18

Abbreviations: ABC, Aging and Body Composition; GFR, glomerular filtration rate; InCHIANTI; Invecchiare in Chianti; NA, data not available; ULSAM, Uppsala Longitudinal Study of Adult Men.

[a] Unless otherwise indicated.

[b] Normal defined as above the season-specific 20th percentile of 25-hydroxyvitamin D concentrations and low as below the season-specific 20th percentile.

[c] Calculated as weight in kilograms divided by height in meters squared.

[d] Defined by the American Diabetes Association criteria: fasting glucose level of 126 mg/dL or higher or use of any hypoglycemic medication (in ULSAM: or by 120-minute postload oral glucose tolerance test 311.1 mmol/L).

[e] Defined by systolic blood pressure of 140 mm Hg or higher, diastolic blood pressure of 90 mm Hg or higher, or use of any antihypertensive medication.

[f] Defined by the Modification of Diet in Renal Disease study.

Table 2

Single-Nucleotide Polymorphisms (SNPs) That Modified the Association of Low Vitamin D Concentration With Risk of Clinical Outcomes Among 1514 Participants in the Cardiovascular Health Study Discovery Cohort

				Composite Outcome (n = 948)			HRR (95% CI)[c]			
SNP	**Gene[a]**	**Substitution[b]**	**MAF**	**HRR (95% CI)[c]**	***P* Value**	***q* Value**	**Death (n = 768)**	**MI (n = 214)**	**Hip Fracture (n = 178)**	**Cancer (n = 337)**
rs7968585	*VDR*	T → C	0.48	1.4 (1.1–1.7)	.002	0.18	1.3 (1.0–1.6)	1.2 (0.8–1.9)	1.2 (0.8–1.9)	1.5 (1.1–2.2)
rs2239179	*VDR*	G → A	0.42	0.7 (0.6–0.9)	.008	0.18	0.8 (0.7–1.0)	0.7 (0.5–1.1)	0.9 (0.6–1.5)	0.6 (0.4–0.9)
rs1801222	*CUBN*	C → T	0.32	1.4 (1.1–1.8)	.004	0.18	1.2 (0.9–1.5)	1.4 (0.9–2.2)	1.4 (0.8–2.4)	1.3 (0.8–1.9)
rs12766939	*CUBN*	A → G	0.28	0.7 (0.6–0.9)	.007	0.18	0.8 (0.6–1.1)	0.8 (0.5–1.3)	0.5 (0.3–0.9)	1.0 (0.6–1.5)
rs703842	*CYP27B1*	T → C	0.31	0.7 (0.6–0.9)	.007	0.18	0.8 (0.6–1.0)	0.8 (0.5–1.2)	0.4 (0.2–0.8)	0.8 (0.5–1.2)

Abbreviations: HRR, hazard ratio ratio; MAF, minor allele frequency; MI, myocardial infarction.

[a]The SNP is located within the gene ±20 kb.

[b]Indicates allele substitution, with minor allele listed second.

[c]The HRR (adjusted for age and sex) is the ratio for each additional minor allele of the hazard ratio describing the association between low 25-hydroxyvitamin D concentration and disease risk.

Table 3

Replication Results in the Health ABC, InCHIANTI, and ULSAM Cohorts for the Interactions Between Low Vitamin D Concentration and Single-Nucleotide Polymorphisms (SNPs) on Risk of the Composite Outcome That Were Identified in the Cardiovascular Health Study (CHS) Discovery Cohort

									Meta-analysis				
			Health ABC		InCHIANTI		ULSAM		Replication[c]		Full[d]		
SNP	Gene[a]	CHS HRR[b]	HRR (95% CI)[b]	*P* Value	HRR (95% CI)[b]	*P* Value	HRR (95% CI)[b]	*P* Value	HRR (95% CI)[b]	*P* Value	HRR (95% CI)[b]	*P* Value	*P* Value[e]
rs7968585	*VDR*	1.40	1.14 (0.97–1.34)	.12	1.59 (1.01–2.51)	.04	1.12 (0.88–1.42)	.37	1.16 (1.02–1.32)	.02	1.22 (1.09–1.36)	<.001	.24
rs2239179	*VDR*	0.74	0.95 (0.81–1.10)	.48	0.66 (0.41–1.05)	.08	NA	NA	0.91 (0.79–1.06)	.15	0.85 (0.76–0.97)	.01	.11
rs1801222	*CUBN*	1.39	0.91 (0.77–1.08)	.29	0.70 (0.42–1.15)	.15	1.05 (0.81–1.36)	.70	0.93 (0.81–1.07)	.30	1.03 (0.92–1.16)	.58	.01
rs12766939	*CUBN*	0.72	1.06 (0.88–1.28)	.52	1.26 (0.81–1.95)	.30	1.16 (0.87–1.54)	.32	1.11 (0.96–1.28)	.17	0.99 (0.87–1.12)	.84	.02
rs703842	*CYP27B1*	0.72	1.04 (0.89–1.21)	.65	0.99 (0.62–1.58)	.97	0.82 (0.62–1.08)	.16	0.98 (0.86–1.12)	.77	0.91 (0.81–1.02)	.12	.07

Abbreviations: ABC, Aging and Body Composition; HRR, hazard ratio ratio; InCHIANTI; Invecchiare in Chianti; NA, data not available; ULSAM, Uppsala Longitudinal Study of Adult Men.

[a] The SNP is located within the gene±20 kb.

[b] The HRR (adjusted for age and sex) is the ratio for each additional minor allele of the hazard ratio describing the association between low 25-hydroxyvitamin D concentration and disease risk.

[c] Estimates from replication cohorts only: Health ABC, InCHIANTI, and ULSAM (if data available).

[d] All estimates (includes CHS results).

[e] For heterogeneity.

Table 4

Joint Associations of *VDR* Single-Nucleotide Polymorphisms and Low 25-Hydroxyvitamin D Concentration on Risk of the Composite Outcome in the Cardiovascular Health Study

	No. of Events	Person-Years	Incidence Rate (95% CI)[a] Within Groups	Difference Between Groups[b]	Hazard Ratio (95% CI) for Low 25-Hydroxyvitamin D[c]
rs7968585					
No minor alleles					
Normal level of 25-hydroxyvitamin D[d]	193	2992	6.5 (5.6 to 7.4)	−0.4 (−2.3 to 1.4)	0.93 (0.70 to 1.24)
Low level of 25-hydroxyvitamin D[e]	54	895	6.0 (4.5 to 7.9)		
1 minor allele					
Normal level of 25-hydroxyvitamin D[d]	374	5657	6.6 (6.0 to 7.3)	2.3 (0.6 to 4.1)	1.40 (1.12 to 1.74)
Low level of 25-hydroxyvitamin D[e]	111	1239	9.0 (7.4 to 10.8)		
2 minor alleles					
Normal level of 25-hydroxyvitamin D[d]	167	2775	6.0 (5.1 to 7.0)	4.1 (1.2 to 7.1)	1.82 (1.31 to 2.54)
Low level of 25-hydroxyvitamin D[e]	49	482	10.2 (7.5 to 13.4)		
rs2239179					
No minor alleles					
Normal level of 25-hydroxyvitamin D[d]	250	3861	6.5 (5.7 to 7.3)	3.4 (0.9 to 5.9)	1.58 (1.19 to 2.11)
Low level of 25-hydroxyvitamin D[e]	66	669	9.9 (7.6 to 12.6)		
1 minor allele					
Normal level of 25-hydroxyvitamin D[d]	364	5547	6.6 (5.9 to 7.3)	2.3 (0.6 to 4.0)	1.44 (1.17 to 1.77)
Low level of 25-hydroxyvitamin D[e]	116	1309	8.9 (7.3 to 10.6)		
2 minor alleles					

	No. of Events	Person-Years	Incidence Rate (95% CI)[a] Within Groups	Difference Between Groups[b]	Hazard Ratio (95% CI) for Low 25-Hydroxyvitamin D[c]
Normal level of 25-hydroxyvitamin D[d]	120	2016	6.0 (4.9 to 7.1)	−0.9 (−3.0 to 1.1)	0.80 (0.55 to 1.18)
Low level of 25-hydroxyvitamin D[e]	32	638	5.0 (3.4 to 7.1)		

[a] These unadjusted rates are reported as the number of events per 100 person-years and the 95% CIs are Poisson-based.

[b] Computed as incidence rate in participants with low 25-hydroxyvitamin D levels minus the incidence rate in those with normal 25-hydroxyvitamin D levels.

[c] Risk of the composite outcome in participants with low 25-hydroxyvitamin D vs risk in those with normal 25-hydroxyvitamin D; the HRs were adjusted for age and sex.

[d] Defined as above the season-specific 20th percentile of 25-hydroxyvitamin D levels.

[e] Defined as below the season-specific 20th percentile of 25-hydroxyvitamin D levels.