Research Article

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Prediagnostic 25-Hydroxyvitamin D, *VDR* and *CASR* Polymorphisms, and Survival in Patients with Colorectal Cancer in Western European Populations

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Abstract

Background: Individuals with higher blood 25-hydroxyvitamin D [25(OH)D] levels have a lower risk of developing colorectal cancer (CRC), but the influence of 25(OH)D on mortality after CRC diagnosis is unknown.

Methods: The association between prediagnostic 25(OH)D levels and CRC-specific ($N = 444$) and overall mortality ($N = 541$) was prospectively examined among 1,202 participants diagnosed with CRC between 1992 and 2003 in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort. Multivariable Cox proportional hazards models were used to calculate HRs and corresponding 95% CIs according to 25(OH)D quintiles and genetic variation within the *VDR* and *CASR* genes. Potential dietary, lifestyle, and metabolic effect modifiers were also investigated.

Results: There were 541 deaths, 444 (82%) due to CRC. Mean follow-up was 73 months. In multivariable analysis, higher 25(OH)D levels were associated with a statistically significant reduction in CRC-specific ($P_{trend} = 0.04$) and overall mortality ($P_{trend} = 0.01$). Participants with 25(OH)D levels in the highest quintile had an adjusted HR of 0.69 (95% CI: 0.50–0.93) for CRC-specific mortality and 0.67 (95% CI: 0.50–0.88) for overall mortality, compared with the lowest quintile. Except for a possible interaction by prediagnostic dietary calcium intake ($P_{interaction} = 0.01$), no other potential modifying factors related to CRC survival were noted. The *VDR* (*FokI* and *BsmI*) and *CASR* (rs1801725) genotypes were not associated with survival.

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Conclusions: High prediagnostic 25(OH)D levels are associated with improved survival of patients with CRC.

Impact: Our findings may stimulate further research directed at investigating the effects of blood vitamin D levels before, at, and after CRC diagnosis on outcomes in CRC patients. *Cancer Epidemiol Biomarkers Prev; 21(4); 582–93. ©2012 AACR.*

Introduction

Evidence supporting a decreased risk of colorectal cancer (CRC) by higher circulating 25-hydroxyvitamin D [25(OH)D] levels is strong, particularly from prospective cohort studies in American and European populations (1–8). However, despite very promising findings from cell culture/animal models (9–13), very few observational studies have to date investigated an effect of vitamin D on survival after CRC diagnosis (14–16). Limited existing evidence shows an improvement in CRC-specific and overall survival with higher vitamin D levels, but can be criticized for limited sample size (14, 16) or use of predicted, not actual, postdiagnosis circulating 25(OHD)D levels (15). Some studies have also shown vitamin D levels or genetic variation within the vitamin D pathway to influence survival in other cancers (17–19). However, nothing is known about potential roles of vitamin D–related genes [e.g., vitamin D receptor (*VDR*) or calcium sensing receptor (CASR)] or other possible effect modifiers in relation to any association of vitamin D with CRC survival. In addition, it has been proposed that high-dose calcitriol, the hormonally active form of vitamin D, might restore sensitivity to chemotherapy (20); hence, in addition to calcitriol treatment, high vitamin D status before diagnosis may provide survival benefits.

Currently, little is known about the effects of pre- and postdiagnostic dietary or lifestyle factors on CRC survival. This is an important point, especially because habits and exposures before cancer diagnosis may affect postdiagnostic lifestyle, and because cancer patients may have a strong interest in making appropriate changes in their diet and lifestyle (21) and could potentially benefit from recommendations on healthy cancer recurrence–preventing diets, supplement use (including vitamin D), and lifestyle factors as part of their treatment, posttreatment recovery, and cancer counseling. Such recommendations should be based on carefully conducted randomized clinical trials (RCT). In this regard, findings from large observational studies can provide guidance for the conduct of future RCTs.

In consideration of these points, we investigated whether 25(OH)D levels determined prediagnostically are associated with CRC-specific and overall mortality in patients with CRC diagnosed within the context of a very large European prospective cohort study. We also explored potential modifying factors and whether select genetic polymorphisms in the *VDR* and *CASR* genes may influence CRC outcomes.

Methods

Study population and collection of data

CRC cases in this analysis were participants in a nested case–control study within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort, a large prospective study with over 520,000 participants enrolled in 23 centers in Denmark, France, Germany, Greece, Italy, the Netherlands, Norway, Spain, Sweden, and United Kingdom. The methods of the EPIC study have been described in detail elsewhere (22, 23). Between 1992 and 1998, standardized lifestyle and personal history information, anthropometric data, and blood samples were collected from most participants at recruitment. Diet over the previous 1 year was measured at baseline by validated country-specific dietary questionnaires developed to ensure high compliance and better measures of local dietary habits (22). Blood samples are stored at the International Agency for Research on Cancer (IARC; Lyon, France) in −196°C liquid nitrogen for all countries except Denmark (−150°C, nitrogen vapor) and Sweden (−80°C, freezers) where samples are stored locally.

Cancer incidence follow-up

Cancer incidence was determined through record linkage with regional cancer registries (Denmark, Italy, the Netherlands, Norway, Spain, Sweden, and United Kingdom; complete up to June 2003) or via a combination of methods, including the use of health insurance records, contacts with cancer and pathology registries, and active follow-up through study subjects, and their next-of-kin (France, Germany, Naples, and Greece; complete up to June 2002).

Vital status follow-up

Vital status follow-up (98.5% complete) was collected by record linkage with regional and/or national mortality registries in all countries except France, Germany, and Greece where data are collected through an active follow-up. Censoring dates for complete follow-up were between December 2006 and December 2008 in Denmark, the Netherlands, Spain, the United Kingdom, Sweden, Norway, and Italy. In Germany, Greece, and France, follow-up was based on a combination of methods, including health insurance records, cancer and pathology registries, and active follow-up through study subjects and their next-of-kin. In these centers, the end of follow-up was defined as the last known date of contact or the date of death whichever came first. The last update of endpoint

information occurred between December 2006 and June 2010.

Mortality data were coded according to the 10th revision of the International Classification of Diseases, Injuries and Causes of Death (ICD-10). Up to 6 qualifiers of the cause of death were reviewed. The outcome of interest was assigned based on the underlying cause of death.

Case ascertainment and selection

The detailed description of case selection was previously published (1). Briefly, CRC cases were selected among participants (men and women) who developed colon (C18.0–C18.7, according to the ICD-10), rectum (C19–C20), and overlapping/unspecified origin tumors (C18.8 and C18.9). Cancers of the anus were excluded. CRC is defined as the combination of the colon and rectal cancers.

Case exclusions included 21 nonadenocarcinoma, 87 due to missing 25(OH)D measurements, and 25 because death certificate and/or autopsy report was the primary source of information for cancer diagnosis giving a final sample size of 1,202 CRC cases (759 colon and 443 rectum). Cases from EPIC collaborating centers in Norway and the Malmo center in Sweden were not included into this analysis because either very few CRC cases were diagnosed after blood donation (Norway) or blood samples were not available for biomarker analyses (Malmo, Sweden; ref. 1). The number of cases for analyses of genetic variation was 1,095 for *VDR FokI*; 1,103 for *VDR BsmI*; and 1,137 for *CASR* (Table 1) due to incomplete genotyping data. For analyses of dietary calcium, additional 2 CRC cases were excluded due to missing nutrient intake values.

The EPIC study was approved by the Ethical Review Board of the IARC and the Institutional Review Board of each participating EPIC center. Written consent was obtained from EPIC participants at enrolment into the study.

Blood 25(OH)D measurements

Details of 25(OH)D measurements have been published previously (1). Briefly, vitamin D status was quantitatively determined by measuring 25(OH)D in 25 μL of serum (heparin plasma for Swedish samples) using a commercially available enzyme immunoassay kit (OCTEIA 25 (OH)D Kit, Immuno Diagnostic Systems; Boldon, United Kingdom) at the Laboratory for Health Protection Research, National Institute for Public Health and the Environment, the Netherlands. The interassay coefficient of variation as determined with 2 kit control samples was 5.9% at the level of 20.3 nmol/L and 5.4% at the level of 77.4 nmol/L. No significant between-day drift, time shifts, or other trends were observed, and the percentage of variance attributable to batch to batch differences was 4.5%.

Genotyping

Genotyping procedures were described previously (24). Briefly, the *VDR* (*BsmI*: rs1544410, 60890G>A; *FokI*: rs2228570, 27823T>C) and *CASR* (A986S; rs1801725, G>T) polymorphisms were genotyped by Taqman methodology in 384-well plates read with the Sequence Detection Software on an ABI-Prism7900 instrument, according to the manufacturer's instructions (Applied Biosystems). Primers and probes were supplied by Applied Biosystems (Assays-by-Design). Each plate included a negative control. Positive controls were duplicated on a separate plate. For all genotypes, the assay success rate was >97% and the internal study duplicate rate was >99%. Failed genotypes were not repeated.

Covariates

Prognostic factors, known and hypothesized to have an effect on CRC survival, were extracted from the medical records [age at diagnosis, year of diagnosis, tumor stage, grade of differentiation (well/moderately/poorly differentiated, unknown), and location (proximal/distal colon, colon, rectum)] and from baseline questionnaires [age at recruitment, sex, body mass index (BMI), physical activity (METs-hours), dietary calcium intake, smoking status (never, former, and current smokers)]. They were considered to be potential confounding variables. Several criteria were used to assess confounding factors: (i) biological plausibility, (ii) whether the variable of interest was associated with the outcome and exposure, and (iii) whether the HR of the primary exposure variable substantially changed (by >10%) after adding the potential confounding variable in the model.

Statistical analyses

Death from CRC was the primary endpoint. Death from any cause was used as a secondary endpoint. Adjusted cumulative incidence curves were used to assess the influence of 25(OH)D on CRC-specific mortality accounting for competing risks (deaths from other causes; ref. 25).

A Cox proportional hazards model stratified by country of cancer diagnosis with time since CRC diagnosis as the time variable was used to calculate HRs and 95% CI of CRC-specific and all-cause mortality, adjusted for age at diagnosis, sex, cancer stage, grade of tumor differentiation, location of tumor, smoking status, BMI, physical activity, and year of diagnosis. Because available information on tumor stage differed between EPIC centers, a harmonization procedure was used as follows: first, we assigned a broad category for tumor stage (I–IV) based on the TNM staging ($N = 464$), then, if available, based on Dukes classification ($N = 268$), and, finally, based on categories "localized/metastatic/metastatic regional/metastatic distant" provided by study centers ($N = 255$). If several tumor staging classifications were available, a cross-checking for discrepancies was carried out. Other covariates including dietary calcium intake, alcohol consumption, education were tested but not included into the final model because they did not meet the criteria for confounders (change in coefficient of interest by >10% after adding the potential confounder).

Table 1. Selected baseline characteristics of CRC cases ($N = 1,202$) according to quintile of blood 25(OH)D in the EPIC study

	Blood 25(OH)D				
Characteristic	**Quintile 1: <36.3 nmol/L ($N = 242$)**	**Quintile 2: 36.4–48.6 nmol/L ($N = 239$)**	**Quintile 3: 48.7–60.5 nmol/L ($N = 241$)**	**Quintile 4: 60.6–76.8 nmol/L ($N = 240$)**	**Quintile 5: >76.8 nmol/L ($N = 240$)**
25(OH)D, mean (SD), nmol/L	28.5 (6.0)	42.6 (3.7)	54.5 (3.5)	67.8 (4.4)	99.3 (25.5)
Age at diagnosis, mean (SD), y	62.1 (7.2)	62.0 (7.1)	62.5 (7.7)	62.1 (7.6)	62.0 (7.1)
Women, *N* (%)	144 (59.5)	128 (53.6)	125 (51.9)	109 (45.4)	100 (41.7)
Stage of disease, *N* (%)					
I	45 (18.6)	56 (23.4)	51 (21.2)	48 (20.0)	41 (17.1)
II	52 (21.5)	43 (18.0)	37 (15.4)	50 (20.8)	59 (24.6)
III	73 (30.2)	73 (30.5)	83 (34.4)	71 (29.6)	67 (27.9)
IV	33 (13.6)	29 (12.1)	23 (9.5)	19 (7.9)	24 (10.0)
Unknown	39 (16.1)	38 (15.9)	47 (19.5)	52 (21.7)	49 (20.4)
Grade of differentiation, *N* (%)					
Well differentiated	12 (5.0)	15 (6.3)	25 (10.4)	9 (3.8)	13 (5.4)
Moderately differentiated	75 (31.0)	76 (31.8)	58 (24.1)	76 (31.7)	74 (30.8)
Poorly differentiated	14 (5.8)	16 (6.7)	23 (9.5)	15 (6.3)	13 (5.4)
Unknown	141 (58.3)	132 (55.2)	135 (56.0)	140 (58.3)	140 (58.3)
Location of primary tumor, *N* (%)					
Colon	160 (66.1)	145 (60.7)	166 (68.9)	144 (60.0)	144 (60.0)
Rectum	82 (33.9)	94 (39.3)	75 (31.1)	96 (40.0)	96 (40.0)
Smoking status, *N* (%)					
Never smoker	95 (39.3)	102 (42.7)	102 (42.3)	100 (41.7)	92 (38.3)
Former smoker	60 (24.8)	72 (30.1)	74 (30.7)	96 (40.0)	99 (41.3)
Current smoker	85 (35.1)	63 (26.4)	60 (24.9)	44 (18.3)	49 (20.4)
BMI, mean (SD), kg/m^2	27.4 (4.9)	26.9 (4.5)	26.8 (4.4)	26.7 (3.9)	25.8 (3.7)
Physical activity, mean (SD), METs	80.8 (52.8)	78.7 (45.2)	89.4 (57.6)	83.1 (48.5)	94.9 (58.0)
Dietary calcium, mean (SD), mg/d	964 (412)	973 (411)	988 (430)	1024 (470)	1051 (403)
Season of blood collection					
Winter	119 (49.2)	96 (40.2)	73 (30.3)	47 (19.6)	47 (19.6)
Spring	64 (26.4)	60 (25.1)	62 (25.7)	61 (25.4)	46 (19.2)
Summer	12 (5.0)	24 (10.0)	42 (17.7)	77 (32.1)	91 (37.9)
Autumn	47 (19.4)	59 (24.7)	64 (26.6)	55 (22.9)	56 (23.3)
VDR BsmI (rs1544410; 60890G>A), *N* (%)[a]					
bb (*GG*)	73 (33.2)	76 (34.9)	76 (34.4)	75 (33.0)	73 (33.6)
bB (*GA*)	114 (51.8)	103 (47.3)	104 (47.1)	111 (48.9)	116 (53.5)
BB (*AA*)	33 (15.0)	39 (17.9)	41 (18.6)	41 (18.1)	28 (12.9)
VDR FokI (rs2228570; 27823T>C), *N* (%)[a]					
FF (*CC*)	88 (39.6)	85 (39.7)	84 (38.7)	85 (37.4)	81 (37.7)
fF (*CT*)	109 (49.1)	99 (46.3)	96 (44.2)	104 (45.8)	91 (42.3)
ff (*TT*)	25 (11.3)	30 (14.0)	37 (17.1)	38 (16.7)	43 (20.0)
CASR (rs1801725, G>T; A986S), *N* (%)[a]					
GG	168 (73.0)	167 (74.9)	162 (71.4)	177 (76.6)	171 (75.7)
GT	56 (24.4)	51 (22.9)	62 (27.3)	49 (21.2)	51 (22.6)
TT	6 (2.6)	5 (2.2)	3 (1.3)	5 (2.2)	4 (1.8)

NOTE: Missing values were not excluded from percentage calculations, thus the sum of percentages across subgroups may not add up to 100%.

[a]The number of cases for analyses of genetic variation was 1,095 for *VDR FokI*; 1,103 for *VDR BsmI*; and 1,137 for *CASR* due to incomplete genotyping data.

The proportional hazards assumption was met as assessed by finding the correlation between the Schoenfeld residuals and including a time-dependent covariate in the Cox model. The following exposures of interest were examined: (i) 25(OH)D concentrations [as quintiles, as predefined categories (<24, 25–49, 50–74, 75–99, ≥100 nmol/L; ref. 26), and per 24.96 nmol/L (equivalent to 10 ng/mL) increase], and (ii) polymorphisms in the *VDR* and *CASR* genes.

Subgroup analyses were conducted by categories of biologically plausible effect modifiers. Adjusted HRs and 95% CI were reported for a 24.96 nmol/L increment in 25(OH)D for CRC-specific and overall mortality. Tests of statistical multiplicative interaction between 25(OH)D and relevant factors were assessed by including in the model the cross product of 25(OH)D as a continuous variable and the covariate as a continuous or dichotomous variable, as appropriate.

The effects of the season or month of blood collection on 25(OH)D levels in relation to CRC were assessed by 2 approaches: (i) adjustment for season of blood collection; (ii) standardization of 25(OH)D levels [by the method of Munger and colleagues (27) and by adding the overall mean of 25(OH)D for all subjects to the residuals derived from a simple regression model fitted to 25(OH)D concentration by month of blood collection].

The effect of incomplete tumor stage information on effect estimates was investigated by several approaches (28): (i) combining all missing values for tumor stage into a single "missing" category (primary analysis); (ii) limiting to "complete" records; (iii) imputation of missing CRC stage values based on the available information for sex, age at cancer diagnosis, year of diagnosis, vital status, tumor location, and period between cancer diagnosis and death under the missing at random assumption (28) with SAS PROC MI procedure. All statistical tests were 2-sided with $P < 0.05$ considered statistically significant (SAS software, version 9.2; SAS Institute).

Results

Patient characteristics

Among 1,202 eligible CRC cases, there were 541 deaths (from CRC = 444, other neoplasms = 35, circulatory disease = 21, respiratory disease = 3, mental disorders = 2, infections = 1, anemia = 1, other causes = 8, and missing = 26). Mean follow-up was 73 months (SD = 49 months). Vitamin D concentrations were measured on average 46 months (SD = 26, range = 0.5–138 months) before CRC diagnosis. Selected baseline characteristics of study participants according to quintiles of blood vitamin D levels are listed in Table 1.

Vitamin D and survival

Higher prediagnostic 25(OH)D levels were associated with a statistically significant reduction in CRC-specific and overall mortality after adjusting for multiple prognostic factors (Table 2) and accounting for competing risks of death for CRC-specific mortality (Fig. 1). Subjects in the highest (>76.9 nmol/L) lowest 25(OH)D quintile had an adjusted HR of 0.69 (95% CI: 0.50–0.93; $P_{trend} = 0.04$) for CRC-specific mortality and 0.67 (95% CI: 0.50–0.88; $P_{trend} = 0.01$) for overall mortality. Similar results were obtained in analyses restricted to complete CRC stage records: HR_{Q5} versus $_{Q1} = 0.65$ (95% CI: 0.46–0.91; $P_{trend} = 0.03$) for CRC-specific and HR_{Q5} versus $_{Q1} = 0.65$ (95% CI: 0.48–0.89; $P_{trend} = 0.01$) for overall mortality. By tumor location, higher 25(OH)D was associated with reduced mortality for both colon ($P_{trend} = 0.61$ for CRC-specific and $P_{trend} = 0.16$ for total mortality) and rectal cancers ($P_{trend} < 0.01$ for CRC-specific and $P_{trend} = 0.01$ for overall mortality; Supplementary Table S1). Though the association was somewhat stronger for rectal cancer (HR_{Q5} versus $_{Q1} = 0.48$, 95% CI: 0.29–0.80 for CRC-specific and HR_{Q5} versus $_{Q1} = 0.55$, 95% CI: 0.35–0.88 for overall mortality) than for colon cancer (HR_{Q5} versus $_{Q1} = 0.79$, 95% CI: 0.53–1.19 for CRC-specific and HR_{Q5} versus $_{Q1} = 0.69$, 95% CI: 0.48–1.01 for overall mortality); no statistically significant heterogeneity by tumor location was observed ($P_{heterogeneity}$: CRC-specific = 0.28 and overall mortality = 0.53; Table 3). Considering predefined 25(OH)D categories, those with 25(OH)D levels of 100 nmol/L or more versus deficient (<25 nmol/L) had a multivariable adjusted HR of 0.55 (95% CI: 0.32–0.94; $P_{trend} = 0.04$) for CRC-specific mortality and 0.53 (95% CI: 0.33–0.87; $P_{trend} = 0.02$) for overall mortality (Supplementary Table S2).

Sensitivity analyses showed: (i) reduction of CRC-specific and overall mortality with increasing 25(OH)D levels after exclusion of cases diagnosed within 2, 3, and 5 years of blood collection; (ii) similar results for analyses restricted to complete CRC stage records or imputation of missing stage values (Table 3). The effect estimates were stronger among patients with stage I/II disease (HRs for CRC-specific mortality = 0.78, 95% CI: 0.61–1.00 and overall mortality = 0.80, 95% CI: 0.66–0.98; Table 3). Adjustment or standardization of 25(OH)D by month of blood collection did not substantially change the effect estimates (data not shown). No significant heterogeneity by geographic region (Northern/Central/Southern Europe) was observed ($P = 0.702$).

Interactions with other predictors of mortality

The examination of possible interactions across strata of other factors related to CRC survival and recurrence showed that the inverse association between 25(OH)D and CRC-specific and overall mortality remained unchanged across most subcategories. One potential interaction was observed with dietary calcium intake ($P = 0.01$), such that participants with high baseline dietary calcium intake (≥928 mg/d) had decreased CRC-specific and overall mortality with increasing levels of vitamin D, but not participants with low calcium intake (<928 mg/d; Table 3). In the analyses by predefined categories, participants with high dietary calcium intake (≥928 mg/d) and high prediagnosis vitamin D levels (>100 nmol/L) had an adjusted HR of 0.24 (95% CI: 0.11–0.54; $P_{trend} = 0.01$) for

Table 2. HRs and 95% CIs for CRC-specific and overall mortality according to quintiles of blood 25(OH)D in the EPIC study ($N = 1,202$)

25(OH)D category	Category range, nmol/L	No.	Event	HR (95% CI)	P_{trend}[a]
CRC-specific mortality					
Age adjusted[b]					
Quintile 1	<36.3	242	104	1.00 (ref.)	0.02
Quintile 2	36.4–48.6	239	85	0.75 (0.56–1.00)	
Quintile 3	48.7–60.5	241	95	0.90 (0.68–1.19)	
Quintile 4	60.6–76.8	240	78	0.68 (0.51–0.92)	
Quintile 5	>76.8	240	82	0.69 (0.52–0.92)	
Multivariable[c]					
Quintile 1	<36.3	242	104	1.00 (ref.)	0.04
Quintile 2	36.4–48.6	239	85	0.76 (0.56–1.02)	
Quintile 3	48.7–60.5	241	95	0.93 (0.69–1.24)	
Quintile 4	60.6–76.8	240	78	0.78 (0.58–1.06)	
Quintile 5	>76.8	240	82	0.69 (0.50–0.93)	
Overall mortality					
Age adjusted[b]					
Quintile 1	<36.3	242	128	1.00 (ref.)	<0.01
Quintile 2	36.4–48.6	239	108	0.79 (0.61–1.03)	
Quintile 3	48.7–60.5	241	117	0.89 (0.69–1.15)	
Quintile 4	60.6–76.8	240	95	0.68 (0.52–0.90)	
Quintile 5	>76.8	240	93	0.65 (0.49–0.84)	
Multivariable[c]					
Quintile 1	<36.3	242	128	1.00 (ref.)	<0.01
Quintile 2	36.4–48.6	239	108	0.82 (0.63–1.07)	
Quintile 3	48.7–60.5	241	117	0.91 (0.70–1.18)	
Quintile 4	60.6–76.8	240	95	0.78 (0.59–1.03)	
Quintile 5	>76.8	240	93	0.67 (0.50–0.88)	

Abbreviation: ref., referent category.

[a]P_{trend} was calculated with the median value of each 25(OH)D category as a continuous variable, adjusted for variables in the corresponding models.

[b]HRs, 95% CIs, and *P* values are adjusted for age at diagnosis (in years as a continuous variable) and stratified by country of residence.

[c]Multivariable HRs, 95% CIs, and *P* values are adjusted for age at diagnosis (in years as a continuous variable), sex (men or women), cancer stage (I–IV, unknown), grade of tumor differentiation (well differentiated, moderately differentiated, poorly differentiated, or unknown), location of primary tumor (colon or rectum), smoking status (current, former, never smoker, or unknown), BMI (in kg/m^2 as a continuous variable), physical activity (in METs as a continuous variable), season of blood collection (winter, spring, summer, and autumn), and year of diagnosis (as a continuous variable), and stratified by country of residence.

CRC-specific mortality and 0.26 (95% CI: 0.13–0.53; $P_{trend} < 0.01$) for overall mortality compared with participants with the lowest 25(OH)D levels (<25 nmol/L). Whereas among participants with low calcium intake, the corresponding HRs were 0.86 (95% CI: 0.41–1.82; $P_{trend} = 0.88$) for CRC-specific mortality and 0.92 (95% CI: 0.46–1.86; $P_{trend} = 0.73$) for overall mortality.

VDR and *CASR* genetic polymorphisms and survival

VDR BsmI or *FokI* polymorphisms were not associated with CRC-specific (Table 4) or overall mortality (data not shown). No survival difference was observed by *CASR* (rs1801725) genotype except for a potential protective effect of having at least 1 *T* allele (age-, sex-, and stage-adjusted HR for *GT/TT* vs. *GG* was 0.86; 95% CI: 0.68–1.08; $P = 0.19$). Similarly null results were obtained in analyses stratified by tumor location and median-dichotomized 25 (OH)D levels (data not shown).

Discussion

Among participants with CRC, higher prediagnostic blood 25(OH)D levels were associated with a significant reduction in CRC-specific and overall mortality. A potential interaction by prediagnostic dietary calcium intake was observed, which deserves further investigation. No statistically significant differences in survival were found by genetic variation in the *VDR* or *CASR* genes.

Several prospective epidemiologic studies (3–8, 29) including from this cohort (1) have consistently found an

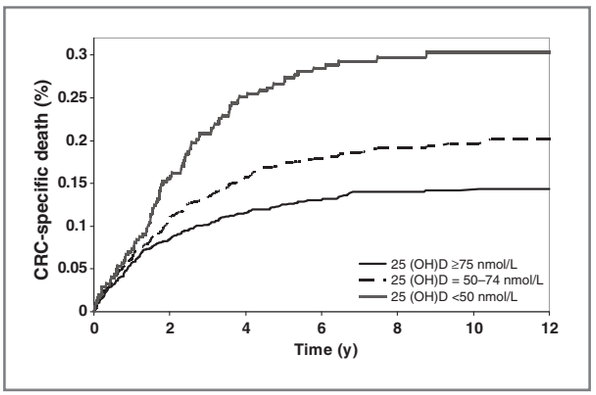

Figure 1. Adjusted cumulative incidence curve of CRC-specific mortality by predefined levels of prediagnostic 25(OH)D (<50, deficient; 50–74, insufficient; ≥50 nmol/L, sufficient vitamin D status, on the basis of proposed levels of vitamin D deficiency/insufficiency).

inverse association between higher prediagnostic 25(OH)D levels and CRC risk. Similar to the results for CRC incidence, higher vitamin D levels have been suggested to be inversely associated with CRC-specific and overall mortality among persons diagnosed with CRC in a small number of studies (14–16). Findings from the Nurses' Health Study and the Health Professionals Follow-up Study have shown an association between either higher prediagnostic 25(OH)D levels (14) or higher predicted postdiagnosis 25(OH)D score (15) and improvement in CRC-specific and overall survival. However, 1 study (14) was limited by its relatively small sample size and the other (15) by its use of predicted, not actual, postdiagnosis vitamin D levels. Another study from Japan has suggested that higher 25(OH)D levels at surgery are associated with a better survival (16), but it is also limited by small sample size. Our findings are in line with these results supporting the favorable influence of higher prediagnostic vitamin D status on the outcomes in patients with CRC.

There is strong experimental evidence supporting the protective effects of vitamin D against CRC development and progression. Proposed mechanisms for these effects involve bile acid catabolism, direct effects on cell proliferation, differentiation, apoptosis, growth factor signaling, immunomodulation, and reduction of invasiveness and angiogenesis (30, 31). Vitamin D seems to be able to

Table 3. Adjusted HRs and 95% CIs for an increment of 24.96 nmol/L (equivalent to 10 ng/mL) of 25(OH)D for CRC-specific and overall mortality across strata of potential effect modifiers

	CRC-specific mortality		Overall mortality	
Risk factor	**Multivariable[a] HR (95% CI)**	**$P_{interaction}$ or P_{trend}**	**Multivariable[a] HR (95% CI)**	**$P_{interaction}$ or P_{trend}**
All participants	0.92 (0.85–1.00)	0.07[b]	0.91 (0.84–0.99)	0.03[b]
Sensitivity analyses				
Participants with complete CRC stage data	0.93 (0.84–1.02)	0.11[b]	0.92 (0.84–1.01)	0.08[b]
All participants with imputed CRC stage data[c]	0.93 (0.85–1.02)[d]	0.15[d]	0.92 (0.84–1.00)[d]	0.05[d]
Follow-up[e]				
≥2 y	0.90 (0.81–1.00)	0.06[b]	0.89 (0.81–0.98)	0.02[b]
≥3 y	0.92 (0.81–1.04)	0.16[b]	0.92 (0.83–1.03)	0.15[b]
≥5 y	0.88 (0.73–1.06)	0.17[b]	0.90 (0.76–1.06)	0.21[b]
Sex				
Women	0.87 (0.76–1.00)	0.26	0.86 (0.76–0.99)	0.29
Men	0.97 (0.86–1.09)		0.94 (0.84–1.04)	
Age at diagnosis, y				
<62.4	0.86 (0.76–0.99)	0.38	0.89 (0.79–1.01)	0.80
≥62.4	0.96 (0.85–1.08)		0.90 (0.81–1.01)	
Anatomical site				
Colon	0.99 (0.88–1.11)	0.28	0.95 (0.86–1.06)	0.53
Rectum	0.81 (0.69–0.95)		0.85 (0.73–0.98)	
Colon subsite[f]				
Proximal	0.91 (0.77–1.09)	0.72	0.94 (0.80–1.10)	0.57
Distal	1.05 (0.86–1.29)		0.95 (0.79–1.15)	
Stage[g]				
I and II	0.78 (0.61–1.00)	0.25	0.80 (0.66–0.98)	0.23
III and IV	0.97 (0.87–1.08)		0.96 (0.87–1.06)	

(Continued on the following page)

Table 3. Adjusted HRs and 95% CIs for an increment of 24.96 nmol/L (equivalent to 10 ng/mL) of 25(OH)D for CRC-specific and overall mortality across strata of potential effect modifiers (Cont'd)

	CRC-specific mortality		Overall mortality	
Risk factor	**Multivariable[a] HR (95% CI)**	**$P_{interaction}$ or P_{trend}**	**Multivariable[a] HR (95% CI)**	**$P_{interaction}$ or P_{trend}**
Year of diagnosis				
1993–1999	0.89 (0.77–1.03)	0.53	0.84 (0.73–0.96)	0.24
1999–2004	0.95 (0.85–1.07)		0.96 (0.86–1.07)	
Season of blood collection[h]				
Summer/autumn	0.97 (0.84–1.11)	0.23	0.96 (0.85–1.09)	0.14
Winter/spring	0.89 (0.78–1.01)		0.87 (0.77–0.98)	
Season of diagnosis[h]				
Summer/autumn	0.92 (0.80–1.06)	0.71	0.89 (0.78–1.01)	0.44
Winter/spring	0.92 (0.82–1.04)		0.92 (0.82–1.03)	
Dietary calcium, mg/d[i]				
<928	1.00 (0.89–1.13)	0.01	0.99 (0.89–1.11)	0.01
≥928	0.85 (0.74–0.97)		0.84 (0.74–0.94)	
Smoking status				
Never smoker	0.86 (0.73–1.02)	0.13	0.87 (0.74–1.02)	0.24
Former smoker	0.99 (0.86–1.13)		0.97 (0.86–1.10)	
Current smoker	0.74 (0.61–0.91)		0.80 (0.67–0.95)	
BMI, kg/m^2				
<25	0.87 (0.74–1.01)	0.53	0.86 (0.75–0.99)	0.31
25–29.9	0.96 (0.85–1.09)		0.94 (0.84–1.06)	
≥30	0.96 (0.74–1.24)		1.00 (0.80–1.26)	

NOTE: Subgroup analyses were conducted by time between blood collection and cancer diagnosis, sex, age at diagnosis (median-dichotomized; <62, ≥62 years), location of tumor (colon vs. rectum; and within the colon, proximal vs. distal), cancer stage (I–II vs. III–IV), season of diagnosis (winter/spring vs. summer/autumn), year of diagnosis (median-dichotomized; <1999, ≥1999), prediagnostic BMI (WHO categories: <25, normal; 25–29, overweight; ≥30 kg/m^2, obese), smoking status (current, former, never), and dietary calcium intake (median-dichotomized; <928, ≥928 mg/d). In interaction analyses, adjusted HRs and 95% CI for an increment of 24.96 nmol/L of 25(OH)D levels for CRC-specific and overall mortality were reported. Tests of statistical interaction between 25(OH)D and relevant factors were assessed by including in the model the cross product of 25(OH)D levels as a continuous variable and the covariate as a continuous or dichotomous variable, as appropriate.

[a]Multivariable HRs, 95% CIs, and *P* values are adjusted for age at diagnosis (in years as a continuous variable), sex (men or women), cancer stage (I–IV, unknown), grade of tumor differentiation (well differentiated, moderately differentiated, poorly differentiated, or unknown), location of primary tumor (colon or rectum), smoking status (current, former, never smoker, or unknown), BMI (in kg/m^2 as a continuous variable), physical activity (in METs as a continuous variable), season of blood collection (winter, spring, summer, and autumn), and year of diagnosis (as a continuous variable), and stratified by country of residence. In the stratified models, the stratifying variable was not adjusted for. *P* value for interaction is presented unless otherwise indicated.

[b]P_{trend}.

[c]Missing stage data were imputed using the algorithm described in the Statistical analysis section.

[d]Combined HR estimate and 95% CI; *P* value from a *t* test for the hypothesis that the parameter is equal to its null value.

[e]Multivariable model including all CRC cases with time interval between blood collection and cancer diagnosis (follow-up) of more than 2, 3, and 5 years.

[f]Only colon tumors with known locations were included. Unspecified ($N = 63$) and overlapping lesion of colon ($N = 9$) tumors were excluded.

[g]Missing data were not included in the analyses.

[h]Summer/autumn period included June, July, August, September, October, and November; winter/spring period included December, January, February, March, April, and May.

[i]In the analyses by predefined categories, participants with high dietary calcium intake (≥928 mg/d) and high prediagnostic vitamin D levels (>100 nmol/L) had an adjusted HR of 0.24 (95% CI: 0.11–0.54; $P_{trend} = 0.012$) for CRC-specific mortality and 0.26 (95% CI: = 0.13–0.53; $P_{trend} = 0.002$) for overall mortality compared with participants with the lowest 25(OH)D levels (<25 nmol/L). Whereas among participants with low calcium intake, the corresponding HRs were 0.86 (95% CI: 0.41–1.82; $P_{trend} = 0.877$) for CRC-specific and 0.92 (95% CI: 0.46–1.86; $P_{trend} = 0.733$) for overall mortality.

Table 4. The association of *VDR* and *CASR* genotypes with CRC-specific mortality in the EPIC study

	No.	Event	Age adjusted[a] HR (95% CI)	Multivariable[b] HR (95% CI)
VDR BsmI (rs1544410)				
bb (GG)	373	132	1.00 (ref.)	1.00 (ref.)
bB (GA)	548	202	1.07 (0.86–1.33)	1.01 (0.81–1.27)
BB (AA)	182	62	0.94 (0.69–1.27)	1.18 (0.87–1.61)
P_{trend}[c]			0.76	0.42
VDR FokI (rs2228570)				
FF (CC)	423	162	1.00 (ref.)	1.00 (ref.)
fF (CT)	499	178	0.90 (0.73–1.12)	0.96 (0.77–1.19)
ff (TT)	173	59	0.81 (0.60–1.10)	0.94 (0.70–1.28)
P_{trend}[c]			0.27	0.90
CASR (rs1801725)				
GG	845	321	1.00 (ref.)	1.00 (ref.)
GT	269	90	0.88 (0.70–1.12)	0.93 (0.73–1.17)
TT	23	5	0.53 (0.22–1.28)	0.47 (0.19–1.14)
P_{trend}[c]			0.08	0.11

Abbreviation: ref., referent category.

[a]HRs, 95% CIs, and *P* values are adjusted for age at diagnosis (in years as a continuous variable) and stratified by country of residence.

[b]Multivariable HRs, 95% CIs, and *P* values are adjusted for age at diagnosis (in years as a continuous variable), sex (men or women), cancer stage (I–IV, unknown). Further adjustment for other covariates did not change estimates substantially.

[c]P_{trend} from the log-additive model.

act directly on the colorectal mucosa, which has been shown to express VDR and key vitamin D metabolizing enzymes (32–34), suggesting local production of the active vitamin D hormone [1,25-(OH)-vitamin D] from its main circulating form 25(OH)D. The latter is known to be the best indicator of vitamin D status integrating dietary and supplemental intakes and UV light exposure (35). It is biologically plausible that vitamin D actions may be modified by calcium intake because vitamin D is a major regulator of calcium homeostasis. Very few studies primarily of colorectal adenomas have shown that either both agents act together to reduce the risk or recurrence of adenomas (36–38) or that the inverse association with 25(OH)D is observed mostly among those with low calcium intake (39). Previous studies considering 25(OH)D-CRC survival association did not observe any interactions with dietary calcium intake (14, 15). However, in this study, we found a suggestion that the reduction in mortality with increasing levels of circulating 25(OH)D may be limited to participants with higher dietary calcium intake. If verified in other studies, calcium supplementation in combination with vitamin D may be potentially useful for improved survival in CRC patients.

Vitamin D acts at least in part through binding with the VDR resulting in an activation of more than 200 vitamin D–responsive genes that are involved in various signaling pathways (40). Though numerous single-nucleotide polymorphisms (SNP) have been discovered in the human *VDR* gene, only relatively few that are thought to be functionally important have been studied in relation to CRC and found to be only weakly associated with CRC risk (41–43). No relevant studies on CRC survival were published. As to survival after other cancer diagnosis, the *VDR* haplotype (*G-T-C,Cdx2-FokI-BsmI*) and SNPs related to the lowest *VDR* expression or function (*FokI*, *Cdx2*) were associated with worse survival in patients with advanced non–small cell lung (17, 18) and epithelial ovarian (44) cancers. These findings showed no association of the *FokI* and *BsmI* genotypes with survival after CRC diagnosis and did not highlight any interactions with circulating 25(OH)D levels. The *CASR A986S* polymorphism, which may affect the receptor's function, has been shown to be associated with variation in serum calcium (45). Nevertheless, this study did not observe any statistically significant associations of the *CASR A986S* genotypes with CRC survival. But, there was a suggestion that the *TT* genotype may be associated with a reduction in CRC-specific and overall mortality.

The strengths of our study include its prospective design (though not originally established to investigate cancer prognosis), large size, detailed data on potential confounders, high follow-up rate, geographically diverse European populations, and prediagnostic measurement of circulating 25(OH)D level, the best indicator of bodily vitamin D status integrating dietary and supplemental vitamin D intakes and vitamin D internally produced from UV B exposure to the skin. A key limitation is the use of a single measurement of 25(OH)D taken on average 46 months before cancer diagnosis as a marker of vitamin D exposure, which may not reflect vitamin D status at the

time of or after CRC diagnosis. To address this, we conducted additional analyses by the time period between blood collection and cancer diagnosis, showing little change in the association between 25(OH)D and mortality. In addition, we assessed any effects of the season or month of blood collection using various approaches that indicated no substantial influence of the seasonal variation in vitamin D status on our results. Another limitation is the unavailability of data on CRC treatment. An assumption may be that CRC treatment may not differ substantially by European country. Nevertheless, to account for this, our analyses were conducted stratified by country of CRC diagnosis and adjusted for year of diagnosis to account for possible changes in the CRC treatment during the period under study. To estimate the effect of missing CRC stage data, we have used several approaches (28), all of which have shown the robustness of effect estimates against uncertainties in CRC stage classification. Though the study was the largest compared with other prospective studies on the same topic, it may still be limited for investigation of possible 25(OH)D-diet/lifestyle/gene interactions. It is also possible that higher 25(OH)D levels are acting as a proxy for healthy lifestyle (e.g., high physical activity, lower BMI, and healthier diet), which may independently influence CRC survival. However, our results were adjusted for prediagnostic BMI and physical activity, though the latter might have been a subject to the measurement error and misclassification in the EPIC study (46). An additional potential limitation which is inherent to all observational studies is the presence of possible residual confounding. However, in our multivariate models a large number of potentially important for CRC survival confounding variables were considered. It is also important to note that cancer survivors are very likely to make lifestyle changes including initiation of vitamin and mineral supplement use after diagnosis (21, 47). Thus, although studies using prediagnostic levels of vitamin D might be limited, they are still contributing to the overall evidence that vitamin D may improve survival after CRC diagnosis.

This large and comprehensive study, based on the EPIC cohort has shown that higher blood vitamin D levels before CRC diagnosis are associated with reduction in CRC-specific and overall mortality. Further prognostic studies among cancer patients are needed to determine whether 25(OH)D levels at diagnosis and postdiagnosis correlate with those measured before diagnosis, and influence all-cause and disease-specific survival among CRC patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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