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Influence of genetic variation in the vitamin D pathway on plasma 25-hydroxyvitamin D_3 levels and survival among patients with metastatic colorectal cancer

Chen Yuan[1], **Lindsay Renfro**[2], **Pratibha B. Ambadwar**[2], **Fang-Shu Ou**[3], **Howard L. McLeod**[4], **Federico Innocenti**[5], **Jeffrey A. Meyerhardt**[1], **Brian M. Wolpin**[1], **Richard M. Goldberg**[6], **Axel Grothey**[7], **Charles S. Fuchs**[8], and **Kimmie Ng**[1]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute and Harvard Medical School, Boston, MA, USA

[2]Division of Biomedical Statistics and Informatics, Mayo Clinic, Rochester, MN, USA

[3]Alliance Statistics and Data Center, Mayo Clinic, Rochester, MN, USA

[4]DeBartolo Family Personalized Medicine Institute, Moffitt Cancer Center, Tampa, FL, USA

[5]Eshelman School of Pharmacy and Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

[6]West Virginia University Cancer Institute, Morgantown, WV, USA

[7]Division of Medical Oncology, Mayo Clinic, Rochester, MN, USA

[8]Yale Cancer Center, New Haven, CT, USA

Abstract

Purpose: The relationships of genetic variation in the vitamin D pathway with circulating 25-hydroxyvitamin D_3 [25(OH)D] levels and survival remain largely unknown for patients with metastatic colorectal cancer (mCRC).

Methods: Among 535 patients participating in a randomized trial of chemotherapy for mCRC, we prospectively measured baseline plasma 25(OH)D and examined 124 tagging single nucleotide polymorphisms (SNPs) within seven genes in the vitamin D pathway, including 5 SNPs associated with circulating 25(OH)D levels in previous genome-wide association studies (GWAS). We evaluated whether these SNPs were associated with plasma 25(OH)D levels and patient outcome

Corresponding Author: Chen Yuan, ScD, Dana-Farber Cancer Institute, 450 Brookline Ave, Boston, MA 02215. Phone: (617) 582-9983; Fax: (617) 632-5370; chen_yuan@dfci.harvard.edu.

Charles S. Fuchs declares consulting for Agios, Bain Capital, Bayer, Celgene, Dicerna, Eli Lilly, Entrinsic Health, Five Prime Therapeutics, Genentech, Gilead Sciences, KEW, Merck, Merrimack Pharmaceuticals, Pfizer, Sanofi, Taiho, and Unum Therapeutics. He also serves as a Director for CytomX Therapeutics and owns unexercised stock options for CytomX Therapeutics and Entrinsic Health. Other authors declare that they have no conflict of interest.

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(overall survival, time to progression, and tumor response), using linear, logistic, and Cox proportional hazards regression.

Results: We observed a significant association between 25(OH)D levels and an additive genetic risk score determined by the 5 GWAS-identified SNPs (P=0.0009). We did not observe any direct association between 25(OH)D-associated SNPs, individually or as a genetic risk score, and patient outcome. However, we found a significant interaction between 25(OH)D levels and rs12785878 genotype in *DHCR7* on overall survival ($P_{interaction}$=0.02).

Conclusion: Germline genetic variation in the vitamin D pathway informs baseline 25(OH)D levels among patients with mCRC. The association between 25(OH)D levels and overall survival may vary by *DHCR7* genotype.

Keywords

25-hydroxyvitamin D_3; single nucleotide polymorphisms; metastatic colorectal cancer; survival

Introduction

Vitamin D is hypothesized to play an important role in colorectal carcinogenesis. Vitamin D receptors (VDR) and 1-α-hydroxylase, which converts 25-hydroxyvitamin D_3 [25(OH)D] into 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2$D], are expressed in colon cancer cells (1–3). By binding to VDR, 1,25$(OH)_2$D induces differentiation and apoptosis (4–6), and inhibits proliferation (7), angiogenesis (8, 9), and metastasis (10) of colon cancer.

The major sources of vitamin D in humans are intake from foods and dietary supplements and exposure to ultraviolet B rays. In addition to environmental factors, twin and family studies indicate that genetics contributes substantially to variation in circulating 25(OH)D levels, with heritability estimated between 29% and 80% (11–14). Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNPs) that are associated with circulating 25(OH)D levels in or near four gene regions: (1) *CYP2R1* (cytochrome P450, family 2, subfamily R, member 1), encoding vitamin D 25-hydroxylase, which converts vitamin D_3 into 25(OH)D; (2) *CYP24A1*, encoding 24-hydroxylase, which degrades 25(OH)D and 1,25$(OH)_2$D; (3) *DHCR7*/*NADSYN1* (7-dehydrocholesterol reductase/nicotinamide adenine dinucleotide synthetase 1), which removes 7-dehydrocholesterol from the synthetic vitamin D pathway; and (4) *GC*, encoding vitamin D binding protein (15, 16). Among these SNPs, rs12785878 and rs3829251 in *DHCR7*/*NADSYN1* and rs2282679 in *GC* are located in introns, whereas rs10741657 and rs6013897 are proximal to *CYP2R1* and *CYP24A1*, respectively.

Because the GWAS data above were obtained from healthy individuals, the role of these vitamin D-related genetic variants is unknown for patients with metastatic colorectal cancer (mCRC), a population with a high prevalence of vitamin D deficiency (17). In this study, we investigated whether the SNPs above, as well as tagging SNPs from genes encoding factors with a biological role in vitamin D metabolism, were associated with baseline plasma 25(OH)D levels and treatment outcome among a cohort of patients with mCRC enrolled in a large, completed, phase III cooperative group clinical trial of palliative chemotherapy. In a

previous analysis of the same study cohort, we did not detect an overall association between 25(OH)D levels and patient outcome, though higher 25(OH)D levels were associated with improved overall survival among patients receiving infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX) (17).

Methods

Study population

Patients included in this study were drawn from the North Central Cancer Treatment Group (NCCTG) trial N9741, a phase III cooperative group trial of chemotherapy for patients with previously untreated mCRC. NCCTG is now a part of the Alliance for Clinical Trials in Oncology. Between October 1998 and April 2001, patients were enrolled and randomized to receive irinotecan, bolus fluorouracil, and leucovorin (IFL); FOLFOX; or irinotecan and oxaliplatin (IROX). Full details of the trial have been described elsewhere (18). Briefly, enrolled patients were required to have histologically proven unresectable colorectal adenocarcinoma, Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2, and adequate renal, liver, and bone marrow function. Exclusion criteria included previous treatment for advanced disease, symptomatic peripheral neuropathy or central nervous system metastases, uncontrolled or severe comorbid illnesses, and ≥ 3 loose stools per day. The protocol was approved by the institutional review board of each participating institution. Patients provided informed consent and were given the option of inclusion in a biomarker companion study for future research.

In total, 1,379 patients were enrolled in N9741 after the incorporation of the amendment to collect blood samples. Among those who provided blood samples, SNP data were available for 535 patients, of which 493 had 25(OH)D levels and 524 had survival information. Figure 1 illustrates the derivation of the final sample size. In a previous study, we did not observe any appreciable difference in baseline characteristics between the overall cohort of patients enrolled in N9741 and the subset participating in the biomarker companion study (19). Further, patients who did and did not provide blood samples had similar overall survival (median survival times: 18.1 and 17.0 months, respectively) (20).

Assessment of patient outcome

Death and disease progression were assessed among all patients, whereas objective response to chemotherapy was assessed among patients with evaluable disease. Overall survival was calculated from study entry to death or last contact. Time to progression (TTP) was calculated from study entry to disease progression or last disease assessment. Progression and response criteria have been described elsewhere (18).

Genotyping of SNPs in the vitamin D pathway

A total of 124 tagging SNPs were selected from seven gene regions in the vitamin D pathway: *CYP2R1*, *CYP24A1*, *CYP27B1*, *DHCR7/NADSYN1*, *GC*, *RXRA* (retinoid X receptor alpha), *VDR*. Tag SNPs were selected within each gene ± 2kb using data from the HapMap Project Phase I/II and III, with an r^2 cutoff of 0.8. Five SNPs previously identified as being associated with 25(OH)D levels (Tier 1 SNPs) were forced in: rs1993116 (r^2=1

with rs10741657), rs6013897, rs12785878, rs11234027 (r^2=1 with rs3829251), and rs2282679 (15, 16). SNP genotyping was performed using the Sequenom platform, with DNA from unrelated HapMap participants and CEPH (Centre d'Etude du Polymorphisme Humain) families serving as positive controls and Mendelian controls, respectively (21). Polymerase chain reaction (PCR) and extension primers were designed using Sequenom Assay Design software. PCR reactions, shrimp alkaline phosphatase digestion, and extension reactions were performed according to Sequenom's standard protocol with one exception: a linear adjustment to the PCR primer concentrations was made to standardize mass spectrometer peak heights. Any SNP or individual that had a success rate <85% was removed from further analysis. The Pedstats and MERLIN software packages were also used to identify and remove unlikely genotypes and genotypes that produced Mendelian errors. Invariant SNPs were also removed. The remaining SNPs were tested for Hardy −Weinberg equilibrium among unrelated individuals, using the software HWSIM. SNPs that passed this test were analyzed in Haploview, and several more SNPs were excluded as having been tagged by other genotyped SNPs.

Plasma 25(OH)D assessment

Blood samples were collected at study entry and sent to the Mayo Central Laboratory for Clinical Trials (Rochester, MN). To measure 25(OH)D, plasma samples were sent by overnight delivery to Heartland Assays (Ames, IA) for radioimmunoassay (22). Masked quality control samples were interspersed among the samples, and all laboratory personnel were blinded to patient outcome. The mean intra-assay coefficient of variation was 8%.

Statistical Analyses

We estimated the association between tagging SNPs and 25(OH)D levels, using linear regression either unadjusted or adjusted for age, sex, race/ethnicity, and season of blood collection. Genotypes were coded as 0, 1, or 2 to reflect the number of copies of the allele (additive models). We considered the possibility that a combination of Tier 1 SNPs may be associated with patient outcome. Therefore, we calculated an additive genetic risk score by summing the number of risk alleles (i.e., associated with lower 25(OH)D levels in GWAS) across the 5 Tier 1 SNPs, yielding a possible range of 0–10 alleles. In sensitivity analyses, we calculated a weighted genetic risk score that weighed each risk allele using the coefficient from unadjusted linear regression, and the findings remained unchanged (data not shown). A P value of <0.05 was considered statistically significant for Tier 1 SNPs and the genetic risk score. For the other tagging SNPs (Tier 2 discovery SNPs; Supplementary Table 1), a false discovery rate (FDR) of <0.05 was considered statistically significant to correct for multiple comparisons (23).

Cox proportional hazards regression (24) was used to examine the association of Tier 1 SNPs, the genetic risk score, and any significant Tier 2 SNP with overall survival and TTP. Logistic regression was used to estimate odds ratios (ORs) for tumor response. In multivariable models, we adjusted for age, sex, race/ethnicity, ECOG performance status, number of metastatic sites, and treatment arm. Tests of interaction between 25(OH)D levels and 25(OH)D-associated SNPs on overall survival were performed by entering their product in the model, evaluated by a likelihood ratio test. Our previous data demonstrated that higher

25(OH)D levels were associated with improved overall survival among patients receiving FOLFOX (17). Thus, we repeated the analyses above among patients receiving FOLFOX. All analyses were based on the study database frozen on 6/25/2013 and were performed with SAS software, version 9.4 (SAS Institute, Cary, NC), and all *P* values are two sided. Data collection and statistical analyses were conducted by the Alliance Statistics and Data Center. Data quality was ensured by review of data by the Alliance Statistics and Data Center following Alliance policies.

Results

Patient characteristics

Baseline patient characteristics of 524 patients are presented in Table 1. The mean age was 60 years (standard deviation, 11 years), with 59% males and 41% females. The mean plasma 25(OH)D level was 21.0 ng/mL (standard deviation, 10.2 ng/mL). Fifty percent of patients were vitamin D deficient (<20 ng/mL) and 32% were vitamin D insufficient (20 − <30 ng/mL). Only 11% of patients had 25(OH)D levels ≥33 ng/mL, the threshold previously shown to be associated with a potential beneficial effect on both colorectal cancer (CRC) incidence and survival (25,26). The median follow-up time among living patients was 9.2 years (90th percentiles: 10.7 years), until final data lock. During the follow-up, 85% of patients had progressed and 95% had died. Among 490 patients evaluable for response, 50% had a confirmed tumor response.

Association between genetic variation in the vitamin D pathway and plasma 25(OH)D levels

We examined the association between SNPs, individually or as a genetic risk score, and plasma 25(OH)D levels. In unadjusted models, four Tier 1 SNPs (rs1993116, rs12785878, rs11234027, rs6013897) and the genetic risk score were associated with 25(OH)D levels (all $P<0.05$; Table 2). After adjustment for covariates, rs1993116 in *CYP2R1* and the genetic risk score remained associated with 25(OH)D levels ($P=0.005$ and 0.0009, respectively). In the multivariable model, each one-allele increase in the genetic risk score was associated with a decrease of 0.93 ng/mL in 25(OH)D level. The score remained associated with 25(OH)D levels among patients receiving FOLFOX ($P=0.003$; data not shown). Three Tier 2 SNPs tended to be associated with 25(OH)D levels with an FDR <0.15 in either unadjusted or multivariable model (Table 3), but none of them reached the predefined FDR for statistical significance, so we did not explore them in further analyses.

Association between 25(OH)D-associated SNPs and patient outcome

We did not detect any direct association between individual Tier 1 SNPs or the genetic risk score and overall survival, TTP, or tumor response overall or among patients receiving FOLFOX (Table 4 and Supplementary Table 2). Overall, for each one-allele increase in the genetic risk score, the multivariable hazard ratios (HRs) were 1.01 [95% confidence interval (CI), 0.95 to 1.07; $P=0.79$] for death and 0.99 (95% CI, 0.93 to 1.05; $P=0.76$) for disease progression, and the multivariable OR for tumor response was 1.03 (95% CI, 0.92 to 1.16; $P=0.57$) (Table 4).

Association between plasma 25(OH)D levels and patient outcome, stratified by 25(OH)D-associated SNPs

We examined the association between plasma 25(OH)D levels and overall survival, stratified by Tier 1 SNPs and the genetic risk score. We found that the association varied by rs12785878 genotype in *DHCR7* (Table 5). For each 1-ng/mL increase in 25(OH)D level, the multivariable HR for death was 0.99 (95% CI, 0.97–1.00), 1.01 (95% CI, 0.99–1.02), and 1.02 (95% CI, 0.98–1.07) among patients with the TT, TG, and GG genotype at rs12785878, respectively ($P_{interaction}$=0.02).

Discussion

In this cohort of patients with previously untreated mCRC, baseline plasma 25(OH)D levels were associated with rs1993116 in *CYP2R1* as well as with an additive genetic risk score determined by the 5 SNPs associated with 25(OH)D levels in previous GWAS. We did not observe any direct association between 25(OH)D-associated SNPs and patient outcome, including overall survival, TTP, and tumor response, individually or as a genetic risk score. However, we detected an interaction between 25(OH)D levels and rs12785878 genotype in *DHCR7* on overall survival.

We observed an association between 25(OH)D levels and rs1993116 in *CYP2R1*. CYP2R1 is a microsomal enzyme that catalyzes C-25 hydroxylation of vitamin D_3 into 25(OH)D in the liver and other organs (27). A previous study suggested that rs12794714 in *CYP2R1* (r^2=0.46 with rs1993116) could affect CRC susceptibility in African Americans (28). Although the other Tier 1 SNPs were not individually associated with 25(OH)D levels, the additive genetic risk score was associated with the levels. One potential explanation is that our study had limited statistical power to detect a small effect of individual alleles on 25(OH)D levels but was able to see an effect from the combined contribution of the 5 Tier 1 SNPs. Another potential explanation is that the SNPs identified in GWAS of healthy participants may not contribute substantially to 25(OH)D levels among patients with mCRC, a population with a high prevalence of vitamin D deficiency (17).

Our findings do not support any direct association between 25(OH)D-associated SNPs and patient outcome. The null association could be due to limited statistical power, yet alternative explanations are possible. First, vitamin D may have limited impact on patient outcome once CRC has metastasized, although this is disputed by a recent large analysis of plasma 25(OH)D levels and survival among patients with mCRC enrolled in a phase III trial, CALGB/SWOG 80405 (29). However, some preclinical data suggest that VDR expression is decreased in less-differentiated colon cancer cell lines (30), which may result in loss of response of colon tumor cells to vitamin D actions. Second, the GWAS-identified SNPs collectively explain only ~5% of the variance in 25(OH)D levels (31). Third, our genetic risk score assumes that each included risk allele would be associated with worse patient outcome given their association with lower 25(OH)D levels. If this assumption is invalid, summing the number of risk alleles would underestimate the true association.

Many prospective epidemiological studies have investigated the association between vitamin D status and CRC survival, most of which found that higher vitamin D levels were

associated with improved CRC survival (32). However, knowledge gaps exist with respect to the interactions between vitamin D and genetic variation (33). A prospective cohort study in Scotland reported an interaction between plasma 25(OH)D levels and *VDR* genotype in relation to survival of non-metastatic CRC (34). In the current study, the association between 25(OH)D levels and overall survival varied according to rs12785878 genotype in *DHCR7*, which encodes the ultimate enzyme that converts 7-dehydrocholesterol into cholesterol. The enzyme is an important regulatory switch between cholesterol and vitamin D synthesis, since both processes require 7-dehydrocholesterol as the substrate (35). The SNP rs12785878 is located in an intron and does not modify the amino acid sequence of the protein. However, rs12800438, a SNP in high linkage disequilibrium (r^2=0.96) with rs12785878, has been found to be associated with DHCR7 mRNA levels in the liver (36), where cholesterol and 25(OH)D are mainly produced. Therefore, the differential association between 25(OH)D levels and overall survival by rs12785878 genotype can potentially be explained by the differential expression of DHCR7 in the liver. These data support the biological role of vitamin D in CRC survival and may inform which patients will benefit, or potentially experience harm, from vitamin D supplementation. To confirm this finding, functional studies are warranted to examine *DHCR7* as a regulator of vitamin D activity in relation to CRC survival.

A prospective study nested within a randomized chemotherapy trial, such as that in our analysis, has several advantages. First, all patients had histologically proven mCRC at study entry, limiting the impact of heterogeneity by disease stage. Second, patient treatment and follow-up were standardized, with regular examinations to prospectively record the date and nature of cancer progression. Detailed information on prognostic factors was prospectively collected at study entry. In addition, using germline genetic variants as proxies for vitamin D status limits bias due to non-genetic confounding and reverse causation, because these variants cannot be influenced by environmental, lifestyle or disease-related factors operating later in life.

Several limitations should be considered. First, our study participants are predominantly individuals of European descent, limiting the generalizability of our findings. However, the GWAS that identified 25(OH)D-associated SNPs were conducted in populations of European descent, so the underlying genetic association may not hold in other populations. Moreover, a racially homogeneous population is advantageous as it reduces the potential for confounding by population substructure. Second, if these SNPs are correlated with other loci that affect patient outcome, our results would be confounded (37). Third, we did not examine rs8018720 in *SEC23A* and rs10745742 in *AMDHD1*, which were associated with serum 25(OH)D levels in a recent European GWAS (38), but we will include them in future studies.

In conclusion, germline genetic variation in the vitamin D pathway informs baseline 25(OH)D levels among patients with mCRC. Our findings do not support any direct association between 25(OH)D-associated SNPs and patient outcome, though one SNP in *DHCR7* modifies the association between 25(OH)D levels and overall survival. This may be due to the fact that our study had a relatively small sample size, and/or that these SNPs account for only a small proportion of the variance in 25(OH)D levels. Future studies

examining the association between vitamin D-related genetic variants and patient outcome in larger populations are currently underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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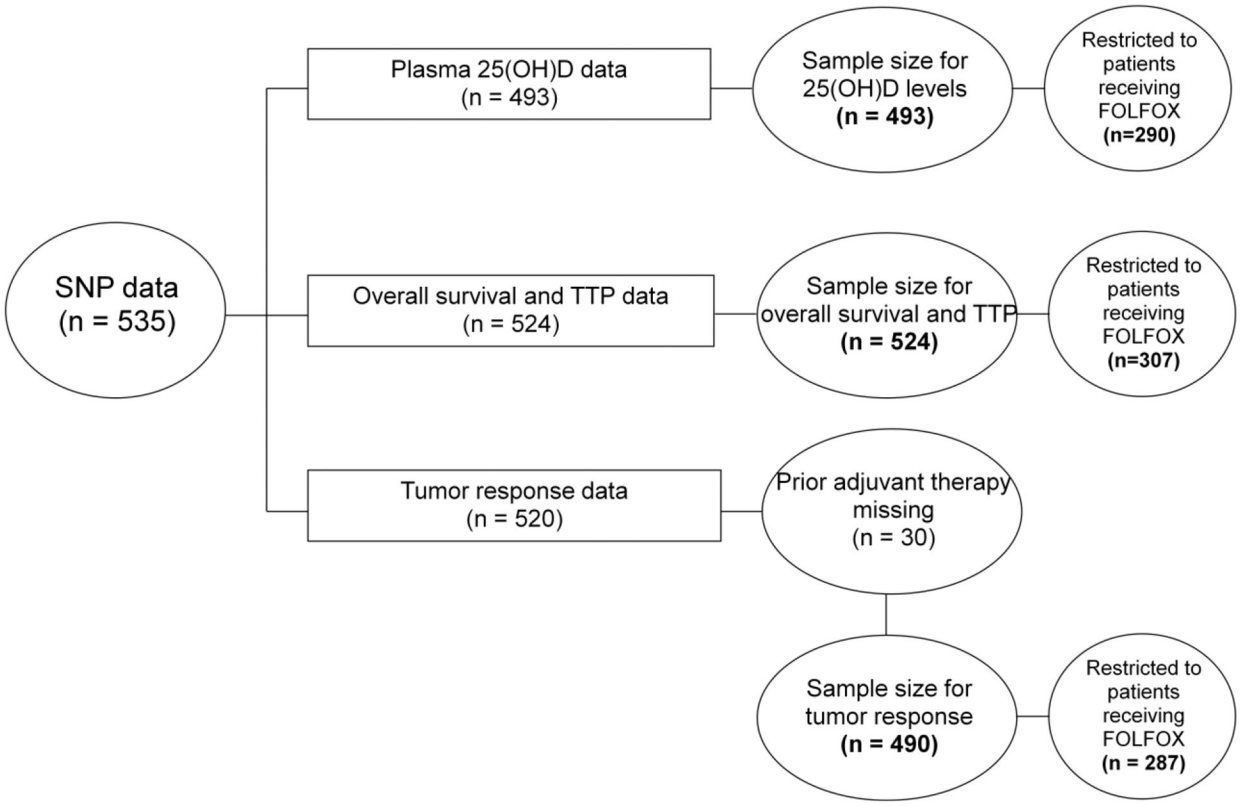

Figure 1. Derivation of sample size

25(OH)D 25-hydroxyvitamin D_3, *FOLFOX* fluorouracil, leucovorin, oxaliplatin, *SNP* single-nucleotide polymorphism, *TTP* time to progression

Table 1.

Baseline characteristics of patients with metastatic colorectal cancer[a]

Characteristic	No.	%
25(OH)D, ng/mL, mean (SD)	21.0(10.2)	
Age, years, mean (SD)	60(11)	
Sex		
Female	215	41.0
Male	309	59.0
Race/ethnicity		
White	450	85.9
Black	38	7.3
Other	31	5.9
Unknown/missing	5	0.9
ECOG performance status[b]		
0–1	500	95.4
2	24	4.6
No. of metastatic sites, median (range)	2(1–4)	
Liver-only metastasis		
Yes	127	24.2
No	397	75.8
One metastatic site, not including liver		
Yes	38	7.3
No	486	92.7
Multiple metastatic sites, not including liver		
Yes	62	11.8
No	462	88.2
Prior adjuvant therapy		
Yes	78	14.9
No	415	79.2
Missing	31	5.9
Treatment arm		
IFL	114	21.8
FOLFOX	307	58.6
IROX	103	19.7
Season of blood collection		
Summer (June, July, August)	135	27.4
Fall (September, October, November)	78	15.8
Winter (December, January, February)	133	27.0
Spring (March, April, May)	147	29.8

Characteristic	No.	%
Missing	31	5.9
Geographic region of registering site[c]		
Midwestern US	228	43.5
Northeastern US and Canada	114	21.8
Southern US and Puerto Rico	85	16.2
Western US	56	10.7
Missing	41	7.8

25(OH)D 25-hydroxyvitamin D3, *ECOG* Eastern Cooperative Oncology Group, *FOLFOX* fluorouracil, leucovorin, oxaliplatin, *IFL* irinotecan, bolus fluorouracil, leucovorin, *IROX* irinotecan, oxaliplatin, *SD* standard deviation

[a] Baseline information available for 524 patients

[b] Grade 0 = fully active, able to carry on all pre-disease performance without restriction; grade 1 = restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work; grade 2 = ambulatory and capable of all selfcare but unable to carry out any work activities, up and about more than 50% of waking hours

[c] Defined according to the US Census Bureau: Midwest = IL, IN, IA, KS, MI, MN, MO, NE, ND, OH, SD, WI; Northeast = CT, ME, MA, NH, NJ, NY, PA, RI, VT; South = AL, DE, FL, GA, KY, LA, MD, NC, OK, SC, TN, TX, VA, WV; West = AZ, CA, CO, HI, ID, MT, NV, NM, OR, UT, WA, WY

Table 2.

Association between 25(OH)D-associated SNPs in GWAS (Tier 1 SNPs), an additive genetic risk score, and plasma 25(OH)D levels among patients with metastatic colorectal cancer

Genetic variant[a]	Position (nearest gene)	Major/minor allele	MAF	Risk allele	Unadjusted		Multivariable[b]	
					Coefficient	*P*	Coefficient	*P*
rsl993116	*CYP2R1*	C/T	0.38	C	−2.31	0.0004	−1.76	0.005
rs6013897	*CYP24A1*	T/A	0.23	A	−1.89	0.02	−1.39	0.06
rsl2785878	*DHCR7*	T/G	0.32	G	−1.38	0.04	−0.38	0.58
rsl1234027	*NADSYN1*	G/A	0.19	A	−1.76	0.03	−1.12	0.15
rs2282679	*GC*	A/C	0.26	C	−0.96	0.19	−1.13	0.11
Genetic risk score	-	-	-	-	−1.31	<0.0001	−0.93	0.0009

25(OH)D 25-hydroxyvitamin D3, *GWAS* genome-wide association studies, *MAF* minor allele frequency, *SNP* single-nucleotide polymorphism

[a] Additive effect of a risk allele (i.e., associated with lower 25(OH)D levels) on 25(OH)D levels (continuous in ng/mL)

[b] Adjusted for age (continuous), sex (female, male), race/ethnicity (white, black, other, unknown/missing), and season of blood collection (summer, fall, winter, spring)

Table 3.

Three tagging SNPs in the vitamin D pathway (Tier 2 discovery SNPs) marginally associated with plasma 25(OH)D levels among patients with metastatic colorectal cancer[a]

SNP[b]	Position (nearest gene)	Major/minor allele	MAF	Unadjusted		Multivariable[c]	
				Coefficient	FDR	Coefficient	FDR
rs7041	*GC*	G/T	0.46	−1.91	0.13	−1.32	0.40
rsl045570	*RXRA*	G/T	0.17	2.66	0.13	2.59	0.07
rs4842196	*RXRA*	*A/C*	0.30	0.98	0.61	2.29	0.07

25(OH)D 25-hydroxyvitamin D3, *FDR* false discovery rate, *MAF* minor allele frequency, *SNP* single-nucleotide polymorphism

[a]SNPs associated with 25(OH)D levels with an FDR <0.15 in either unadjusted or multivariable model, none of which reached the predefined FDR for statistical significance

[b]Additive effect of a minor allele on 25(OH)D levels (continuous in ng/mL)

[c]Adjusted for age (continuous), sex (female, male), race/ethnicity (white, black, other, unknown/missing), and season of blood collection (summer, fall, winter, spring)

Table 4.

Association between 25(OH)D-associated SNPs in GWAS (Tier 1 SNPs), an additive genetic risk score, and survival among patients with metastatic colorectal cancer

Outcome[a]	Unadjusted		Multivariable[b]	
	HR or OR (95% CI)	*P*	HR or OR (95% CI)	*P*
Overall survival				
rsl993116	1.03 (0.91–1.17)	0.64	0.99 (0.87–1.14)	0.94
rs6013897	0.96 (0.83–1.12)	0.64	0.94 (0.80–1.11)	0.47
rsl2785878	1.05 (0.92–1.20)	0.46	1.00 (0.86–1.16)	0.99
rsl1234027	1.05 (0.89–1.23)	0.57	1.05 (0.89–1.25)	0.54
rs2282679	1.07 (0.92–1.24)	0.36	1.06 ((Ⅲ–1.24)	0.42
Genetic risk score	1.03 (0.97–1.08)	0.36	1.01 (0.95–1.07)	0.79
Time to progression				
rsl993116	0.91 (0.80–1.04)	0.16	0.89(0.77–1.02)	0.11
rs6013897	0.92 (0.78–1.09)	0.36	0.90 (0.75–1.07)	0.23
rsl2785878	1.11 (0.97–1.28)	0.14	1.09 (0.93–1.27)	0.27
rsl1234027	1.06 (0.90–1.25)	0.47	1.03 (0.87–1.23)	0.73
rs2282679	1.06 (0.91–1.23)	0.47	1.05 (0.89–1.23)	0.56
Genetic risk score	1.01 (0.95–1.07)	0.82	0.99 (0.93–1.05)	0.76
Tumor response				
rsl993116	1.05 (0.82–1.35)	0.68	1.04 (0.80–1.35)	0.78
rs6013897	1.04 (0.78–1.39)	0.78	1.09 (0.80–1.50)	0.58
rsl2785878	0.95 (0.73–1.22)	0.68	1.09 (0.82–1.44)	0.56
rsl1234027	0.94 (0.69–1.28)	0.70	0.98 (0.70–1.36)	0.90
rs2282679	1.11 (0.84–1.46)	0.45	1.04 (0.78–1.40)	0.77
Genetic risk score	1.01 (0.91–1.13)	0.83	1.03 (0.92–1.16)	0.57

25(OH)D 25-hydroxyvitamin D3, *CI* confidence interval, *ECOG* Eastern Cooperative Oncology Group, *FOLFOX* fluorouracil, leucovorin, oxaliplatin, *GWAS* genome-wide association studies, *HR* hazard ratio, *IFL* irinotecan, bolus fluorouracil, leucovorin, *IROX* irinotecan, oxaliplatin, *OR* odds ratio, *SNP* single-nucleotide polymorphism

[a] Additive effect of a risk allele (i.e., associated with lower 25(OH)D levels) on patient outcome

[b] Adjusted for age (continuous), sex (female, male), race/ethnicity (white, black, other, unknown/missing), ECOG performance status (0–1,2), number of metastatic sites (continuous), and treatment arm (IFL, FOLFOX, IROX)

Table 5.

Association between plasma 25(OH)D levels and overall survival among patients with metastatic colorectal cancer, stratified by rs12785878 genotype in *DHCR7*

rsl2785878	No. patients	Multivariable hazard ratio (95% confidence interval)[a]	$p_{interaction}$
TT	232	0.99 (0.97–1.00)	
TG	205	1.01 (0.99–1.02)	0.02
GG	55	1.02 (0.98–1.07)	

25(OH)D 25-hydroxyvitamin D3, *CI* confidence interval, *ECOG* Eastern Cooperative Oncology Group, *FOLFOX* fluorouracil, leucovorin, oxaliplatin, *IFL* irinotecan, bolus fluorouracil, leucovorin, *IROX* irinotecan, oxaliplatin

[a]Effect of each 1-ng/mL increase in 25(OH)D level on overall survival, adjusted for age (continuous), sex (female, male), race/ethnicity (white, black, other, unknown/missing), ECOG performance status (0–1, 2), number of metastatic sites (continuous), treatment arm (IFL, FOLFOX, IROX), and season of blood collection (summer, autumn, winter, spring)