Research Article

Cancer Epidemiology, Biomarkers & Prevention

Polymorphic Variation in the *GC* and *CASR* Genes and Associations with Vitamin D Metabolite Concentration and Metachronous Colorectal Neoplasia

Elizabeth A. Hibler[1,2], Chengcheng Hu[1], Peter W. Jurutka[3,4], Maria E. Martinez[1,2], and Elizabeth T. Jacobs[1,2]

Abstract

Background: Vitamin D levels and calcium intake have been associated with risk of colorectal neoplasia, and genetic variation in vitamin D pathway genes may affect circulating vitamin D metabolite concentrations and/or risk for colorectal lesions. This study evaluated associations between polymorphic variation in the Gc-globulin (*GC*) and calcium-sensing receptor (*CASR*) and odds for metachronous colorectal neoplasia and vitamin D metabolite concentrations.

Methods: Participants from the Ursodeoxycholic Acid (UDCA) and Wheat Bran Fiber (WBF) trials (n = 1,439) were analyzed using a single-nucleotide polymorphism (SNP) tagging approach, with a subset ($n = 404$) of UDCA trial participants for whom vitamin D metabolite concentrations were also available. A total of 25 *GC* and 35 *CASR* tagSNPs were evaluated using multiple statistical methods.

Results: Principal components analyses did not reveal gene-level associations between *GC* or *CASR* and colorectal neoplasia; however, a significant gene-level association between *GC* and 25(OH)D concentrations ($P < 0.01$) was observed. At the individual SNP level and following multiple comparisons adjustments, significant associations were observed between seven *GC* (rs7041, rs222035, rs842999, rs1155563, rs12512631, rs16846876, and rs1746825) polymorphisms and circulating measures of 25(OH)D (adjusted $P < 0.01$) and *CASR* SNP rs1042636 and proximal colorectal neoplasia (adjusted $P = 0.01$).

Conclusions: These results show a possible association between variation in *CASR* and odds of colorectal neoplasia as well as the potential role of variation in *GC* with circulating 25(OH)D concentrations.

Impact: Additional research is warranted to determine the mechanism of *GC* genotype in influencing 25(OH)D concentrations and to further elucidate the role of *CASR* in colorectal neoplasia. *Cancer Epidemiol Biomarkers Prev; 21(2); 368–75. ©2011 AACR.*

Introduction

Colorectal cancer is the second leading cause of cancer-related deaths in the United States and more than 147,000 newly diagnosed cases are expected annually (1, 2). There is a long-standing model showing the development of colorectal carcinoma from adenomas following the accumulation of mutations in colorectal cells (2–4). Lifestyle factors including diet, obesity, and physical activity are associated with risk of sporadic colorectal cancer (5–7), and there is strong epidemiologic evidence to support a relationship between low vitamin D status as well as low dietary calcium intake and increased risk of colorectal neoplasia (8–10). The Gc-globulin (*GC*) and calcium-sensing receptor (*CASR*) genes play significant roles in regulating both the vitamin D endocrine system and calcium homeostasis; however, it is not yet clear how variation in these genes affect circulating concentration of vitamin D metabolites or risk of colorectal cancer.

Gc-globulin (GC) primarily functions to transport vitamin D metabolites in the plasma (11–14). GC is a serum α_2-globulin (11) and binds to many vitamin D metabolites, although the greatest affinity is for 25(OH)D (12, 13). The *GC* is highly polymorphic with more than 120 known variants (11), and there are 2 commonly studied polymorphisms, rs7041 and rs4588 (14, 15). These polymorphisms result in amino acid changes that produce different isoforms of the GC protein, and the population distribution of each varies by race and ethnicity (11, 14, 16). These isoforms alter affinity of GC for vitamin D metabolites,

Authors' Affiliations: [1]Department of Epidemiology and Biostatistics, Mel and Enid Zuckerman College of Public Health, [2]Arizona Cancer Center, University of Arizona, Tucson; [3]Division of Mathematical and Natural Sciences, Arizona State University, Glendale; and [4]Department of Basic Medical Sciences, University of Arizona College of Medicine-Phoenix, Arizona

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

Corresponding Author: Elizabeth A. Hibler, Arizona Cancer Center, 1515 N. Campbell Avenue, Tucson, AZ 85724. Phone: 520-444-0905; Fax: 520-626-9275; E-mail: ehibler@azcc.arizona.edu

doi: 10.1158/1055-9965.EPI-11-0916

and it is hypothesized that this could affect the delivery of vitamin D at the tissue or cellular levels (14). Several studies have shown associations between these polymorphisms or their phenotypic alleles and circulating 25(OH)D concentrations in populations with varied age, gender, and race/ethnicity (17–19). Furthermore, 25(OH)D concentrations have been associated with both colorectal adenoma incidence and recurrence (10, 20). However, it is not yet clear whether there are additional polymorphisms associated with these outcomes or how the genetic variation translates to disease risk at the population level. In addition to *GC*, the *CASR* is another gene in this pathway that may also be important to the etiology of colorectal neoplasia.

The CASR has a primary role in calcium homeostasis by sensing extracellular blood calcium levels, which are maintained within a narrow range (21–25). The CASR is part of the superfamily of G-protein–coupled receptors (23, 26, 27), and expression of CASR has been identified in cells of the gastrointestinal tract and bone (21–23, 27). Observational studies have reported associations between calcium intake and adenoma recurrence (28, 29). Furthermore, a clinical trial by Baron and colleagues showed a significantly decreased risk of colorectal adenoma recurrence in individuals randomized to a daily calcium supplement, and additional studies have provided evidence for a reduced risk of colorectal cancer with higher dietary calcium intake (8, 28, 30). There are 2 functional vitamin D response elements (VDRE) located in the promoter regions of *CASR* (25, 26, 31), and researchers have identified *CASR* polymorphisms associated with different colorectal neoplasia outcomes (32, 33). However, there is not yet unequivocal evidence of a role for *CASR* variation in vitamin D metabolite level regulation or risk of neoplasia in the colon. Therefore, the goal of this study was to assess associations between genetic variation in *GC* and *CASR* and vitamin D metabolite concentrations as well as colorectal neoplasia outcomes.

Materials and Methods

The analyses were conducted using data from participants from the Ursodeoxycholic acid (UDCA) and Wheat Bran Fiber (WBF) trials conducted at the Arizona Cancer Center, which have been previously described (34–36). The UDCA trial was a phase III randomized, double-blind, placebo-controlled trial conducted to test the effect of UDCA on recurrence of colorectal neoplasia (34). The study population included Arizona residents ($N = 1{,}192$) between 40 and 80 years of age with a history of removal of one or more colorectal adenomas (>3 mm in diameter) during a colonoscopy within 6 months of study enrollment (34). The WBF trial was also a double-blind phase III clinical trial conducted at the University of Arizona, Tucson, AZ, to measure the effects of high (13.5 g/d) versus low (2.0 g/d) WBF intake for 3 years on colorectal adenoma recurrence (36). A total of 1,310 participants competed this trial and included individuals between 40 and 80 years of age, of both genders, who had removal of one or more colorectal adenoma ($\geq$3 mm) at colonoscopy within 3 months prior to study enrollment (36). There were 1,530 participants in a pooled sample with complete recurrence and genotype data [UDCA, $N = 896$ (58.5%); WBF, $N = 634$ (41.4%)]; however, the sample was further restricted to 1,439 individuals who reported white race. This sample restriction was necessary because there were not enough individuals of varied race/ethnicity to allow us to address the issue of population stratification. Vitamin D metabolite levels were measured in a random sample of 619 White participants from the UDCA trial only (34, 37). The University of Arizona Human Subjects Protection Program approved both the UDCA and WBF trials. Informed consent was obtained for all subjects prior to enrollment.

Genotyping and outcome ascertainment

Participants were genotyped using the Illumina Golden Gate platform (Illumina), and tagSNPs were selected from HapMap data release #16c.1, June 2005, on NCBI B34 assembly, dbSNP b124. SNP selection methodology and genotyping for this project have been previously described (38, 39). The final statistical analysis included 25 *GC* and 35 *CASR* single-nucleotide polymorphisms (SNP). Data on the size, location, and histology of identified adenomas were collected for participants of both trials (35). For the UDCA trial, all outcome data were collected and coded from medical records of colonoscopy, sigmoidoscopy, or surgical resections by individuals trained in abstraction (34). For the WBF trial, the results from each colonoscopy reported were collected using standard abstraction guidelines (36). Any identified adenomas were referred to as metachronous colorectal neoplasia to account for the possibility of adenomas missed at the baseline colonoscopy in addition to adenoma recurrence (38). All lesions were classified as either proximal, defined as being located proximal to the splenic flexure; or distal, defined as occurring distal to the splenic flexure and including lesions in the rectum (38). The WBF and UDCA trials also used the Arizona Food Frequency Questionnaire (AFFQ) to measure dietary intake for participants. The AFFQ was used in the WBF trial as a screening tool for eligibility and used to measure dietary intake at baseline in the UDCA trial (36, 37). Individuals were instructed to report intake from the previous 12 months.

Vitamin D metabolite measurement

Vitamin D metabolite concentrations were measured from baseline samples of UDCA participants and analyzed at the University of South Carolina, Columbia, SC, in the laboratory of Dr. Bruce Hollis using the radioimmunoassay (RIA) method, as previously described in detail (40, 41). The laboratory used several QA/QC measures including a pooled serum sample analyzed with batches of study samples to monitor analytic precision and identify possible laboratory shifts over time as well as to test duplicates in different batches. The coefficient of

variation was less than 7.0% for 25(OH)D analyses and 11.5% for 1,25$(OH)_2$D analysis (42, 43). All analyses were conducted in a blinded fashion.

Statistical analysis

The outcome measures of circulating 25(OH)D and 1,25$(OH)_2$D concentrations were analyzed as continuous variables, whereas measures of colorectal neoplasia were dichotomous variables. There is currently no single standard method for analysis of high-dimensional genetic data, and therefore 4 techniques were used: principal components analysis (PCA), analysis of individual SNPs through regression models, classification and regression trees (CART), and random forest analyses (38). Principal components analysis is a method that assesses the overall gene-level associations, and the principal components represent a linear transformation of the original SNP data that explain variation within a specific genetic locus (44). Principal component analyses were generated using the SNP data that explain 80% of the variance at each locus and then modeled with colorectal neoplasia outcomes in logistic regression models (44). The gene-level associations were also compared with the results of individual SNP-level analysis. Finally, CART and random forest analysis were used to identify predictive polymorphisms for each outcome.

Individual polymorphisms were also examined through regression models testing the additive, dominant, and recessive modes of inheritance, with a multiple comparisons adjustment applied (45). The "*P* values adjusted for multiple correlated tests" (P_{ACT}) multiple comparisons adjustment was developed by Conneely and Boehnke (45). This approach has been published previously and, briefly, is described as a comparison of the observed test statistics directly with their asymptotic distribution, using numerical integration (45). Any significant SNPs identified in the pooled sample were also evaluated separately in the UDCA and WBF populations to ensure that no heterogeneity of effect was present. Interaction was assessed using a likelihood ratio test comparing a model with an interaction term (study) to a model without the interaction term ($\alpha = 0.10$). Potential confounding factors assessed were gender, study (WBF vs. UDCA trial), and age. Confounding was evaluated by comparing the difference between the crude versus adjusted estimates, and none of the above variables were included in the model because the adjusted estimate did not change by more than 10%.

In addition to testing associations, methods were used to identify polymorphisms that were predictive of colorectal neoplasia outcomes. CART and random forests are tree-based analysis methods used to identify patterns in high-dimensional genetic data (46). These are nonparametric methods for prediction using recursive partitioning that offer the advantages of not requiring model specification prior to analysis and also allow for testing interactions between SNPs (46, 47). The results identified a set of SNPs that were most strongly predictive of outcomes in this population. Data analysis for all aims was completed using STATA SE version 10.1 and R version 2.9.1, and all statistical tests were two-sided with $\alpha = 0.05$. No method is yet considered accepted practice in the field, thus the results must be carefully interpreted in the context of identifying associations through principal components and regression analyses versus prediction through CART and random forest modeling.

Results

Baseline characteristics of the participants are shown in Table 1 and have been previously described in detail (36). The current analysis was completed using a pooled sample of participants from the UDCA and WBF trials ($N = 2{,}502$) restricted to individuals with complete data for adenoma recurrence and genotype for the selected polymorphisms ($N = 1{,}530$). The sample was further restricted to those who reported White race ($N = 1{,}439$). For vitamin D metabolite analysis, data were available for a total of 619 participants of the UDCA trial. There were 475 participants with complete recurrence and genotype data, which was then again restricted to individuals who reported White race because of insufficient data to account for potential population stratification ($N = 404$). The final sample population for genetic analysis had mean age of 66.0 ± 8.3 years, mean body mass index of 27.9 ± 4.6 kg/m^2, and 66.1% were male (Table 1). The vitamin D metabolite subset also included older, male adults (mean age $= 65.5 \pm 8.6$, 66.1% male) with mean body mass index of 28.4 kg/m^2. Dietary intake was similar between samples with respect to total energy ($1{,}963.0 \pm 780.0$ and $2{,}037.5 \pm 858.6$, respectively), fat intake (64.0 ± 30.9 and 63.5 ± 32.8, respectively), and calcium intake (971.0 ± 460.4 vs. $1{,}026.8 \pm 513.4$). Vitamin D supplement use was moderately higher (75.2%) among the subset with vitamin D metabolite data versus the pooled gene analysis sample (66.3%). The subset of the UDCA trial was comparable with the pooled UDCA plus WBF participant sample used for the genetic analysis with respect to the selected characteristics.

Polymorphic variation and circulating concentration of vitamin D metabolites

Several *GC* polymorphisms were statistically significantly associated with circulating 25(OH)D concentration; in contrast, there were no significant associations between *CASR* polymorphisms and circulating vitamin D metabolite levels with any of the analysis methods applied (Table 2, Supplementary Tables S1–S4). In the principal components analysis, statistically significant gene-level associations were observed between *GC* and 25(OH)D concentration ($P < 0.001$; Table 2), whereas no significant associations were observed between variation in *GC* and circulating 1,25$(OH)_2$D concentrations ($P = 0.35$). The analysis at the individual SNP level (Supplementary Tables S1 and S2) identified 7 *GC* polymorphisms that were significantly related to circulating

Table 1. Baseline characteristics of study population

Characteristics	Pooled UDCA and WBF ($N = 1,530$)	Vitamin D subset ($N = 475$)
Trial participant, *n* (%)		
UDCA	896 (58.6)	475 (100.0)
WBF	634 (41.4)	N.A.
Age, mean ± SD, y	66.0 ± 8.3	65.5 ± 8.6
Sex, male, *n* (%)	1,027 (66.1)	314 (66.1)
Race, White, *n* (%)	1,439 (94.1)[a]	404 (85.2)
Body mass index, mean ± SD, kg/m^2	27.9 ± 4.6	28.5 ± 4.9
Aspirin use, *n* (%)	443 (29.0)	134 (28.2)
Ever smoker, *n* (%)	998 (65.2)[a]	316 (66.5)[b]
Current smoker, *n* (%)	179 (11.7)	61 (12.8)
Total fat, g/d ± SD	64.0 ± 30.9	63.5 ± 32.8
Energy, kcal/d ± SD	1,963.0 ± 780.0	2,037.5 ± 858.6
Calcium intake, g/d ± SD	971.0 ± 460.4	1,026.8 ± 513.4
Vitamin D supplement use, *n* (%)	1,015 (66.3)	357 (75.2) ± 9.2
Previous polyps, *n* (%)	612 (40.0)	202 (42.5)
Family history of colorectal cancer, *n* (%)	360 (23.5)	129 (27.2)
Any metachronous neoplasia, *n* (%)	693 (45.3)	188 (39.6)
Proximal metachronous neoplasia, *n* (%)	493 (32.2)	132 (27.8)[b]
Distal metachronous neoplasia, *n* (%)	363 (23.7)	103 (21.7)[b]

[a]Missing for race, $N = 15$; ever-smoker, $N = 20$; proximal and distal neoplasia, $N = 11$.
[b]Missing for race, $N = 9$; ever-smoker, $N = 8$; proximal and distal neoplasia, $N = 2$.

25(OH)D levels, using the additive model of inheritance, but not with 1,25$(OH)_2$D. Following multiple comparisons adjustments, the SNPs that were significantly associated with circulating 25(OH)D were: rs7041 ($P = 0.02$), rs222035 ($P = 0.02$), rs842999 ($P = 0.05$), rs1155563 ($P \leq 0.001$), rs12512631 ($P = 0.02$), rs16846876 ($P = 0.001$), and rs17467825 ($P < 0.001$; Supplementary Table S1). The *GC* polymorphisms rs12512631 and rs842999 were not in Hardy–Weinberg equilibrium in this population ($P < 0.001$, data not shown). Analysis of correlation coefficients for these SNPs showed that rs7041, rs222035, and rs842999 are highly correlated ($r^2 > 0.92$) and rs1155563 is in strong correlation with rs17467825 ($r^2 = 0.86$).

The results from the prediction models using CART and random forest methods identified *GC* polymorphisms that may be predictive of 25(OH)D levels. The CART analysis identified rs17467825 as the only SNP predictive of 25(OH)D concentration; however, no polymorphisms were identified as predictive of circulating 1,25$(OH)_2$D. The random forest analysis identified similar SNPs to the single SNP level as analysis for prediction of 25(OH)D concentration, although none of the importance measures were statistically significant. The two most commonly studied polymorphisms (rs7041 and rs4588) as well as correlated SNPs (rs842999, rs11155563, and rs17467825) appear to be consistently important for both associations with and prediction of 25(OH)D levels.

Polymorphic variation and metachronous colorectal neoplasia

The results of this analysis provide no evidence of an association between *GC* and colorectal neoplasia, as none of the statistical methods used revealed a statistically significant relationship. In principal components analyses for *CASR*, there were no statistically significant gene-level associations between *CASR* and distal ($P = 0.57$), proximal ($P = 0.17$), or villous ($P = 0.39$) colorectal neoplasia, nor for overall recurrence ($P = 0.85$) as shown in Table 3. However, a single polymorphism, rs1042636, was identified through the SNP-level analysis as associated with decreased odds of proximal colorectal neoplasia, following the multiple comparisons adjustment (Supplementary Tables S5–S12). The rs1042636 polymorphism was significantly associated with odds of proximal metachronous colorectal neoplasia in both additive ($P = 0.02$) and dominant ($P = 0.01$) modes of inheritance. Analysis by rs1042636 genotype showed that one copy of the *G* allele led to decreased odds for metachronous adenomas in both the UDCA and WBF trial participants [OR (95% confidence interval, CI) = 0.67 (0.42–1.06) and 0.34 (0.19–0.61), respectively; data not shown]. In contrast, when using the CART approach, there were no *CASR* polymorphisms that were predictive of colorectal neoplasia at any site. The most predictive *CASR* polymorphism in random forest analysis was rs1042636, although no SNPs were

Table 2. Association between genetic variation in *GC* and *CASR* and circulating 25(OH)D and $1,25(OH)_2D$ concentrations

	25(OH)D Mean change (95% CI)	$1,25(OH)_2D$ Mean change (95% CI)
GC		
PC1	−1.23 (−1.78 to 0.68)	−0.57 (−1.18 to 0.04)
PC2	−0.12 (−0.67 to 0.71)	0.19 (−0.86 to 0.62)
PC3	0.47 (0.30–2.02)	−0.23 (−0.49 to 1.43)
PC4	−0.04 (−0.54 to 1.92)	−0.13 (−1.39 to 1.31)
LRT *P*[a]	<0.001	0.35
CASR		
PC1[b]	0.12 (−0.37 to 0.61)	−0.11 (−0.64 to 0.42)
PC2	0.09 (−0.48 to 0.66)	0.01 (−0.60 to 0.62)
PC3	−0.43 (−1.08 to 0.22)	−0.28 (−0.97 to 0.41)
PC4	0.48 (−0.34 to 1.3)	0.88 (0.01–1.75)
PC5	−0.16 (−1.12 to 0.8)	−0.02 (−1.04 to 1.00)
LRT *P*[a]	0.62	0.45

[a]*P* value for each model is from a likelihood ratio test (LRT) with degrees of freedom equal to the number of principal components and is a test of gene-level associations.
[b]An 80% explained-variance threshold is used for including principal components (PC) in the model.

significant using a standardized importance score. Overall, this analysis provided modest evidence that *CASR* polymorphism *rs1042636* may be related to proximal colorectal neoplasia in the single SNP analysis approach only.

Discussion

This study provides evidence for associations between polymorphisms in *GC* and circulating 25(OH)D concentrations, whereas there was no relationship for *CASR* and

Table 3. Association between genetic variation in *CASR* and *GC* and measures of metachronous colorectal neoplasia

	Colorectal neoplasia outcomes (*N* = 1,439)			
	Distal[a], *N* = 343 (24.0%) OR (95% CI)	Proximal[a], *N* = 473 (33.1%) OR (95% CI)	Recurrence, *N* = 660 (45.9%) OR (95% CI)	Villous, *N* = 107 (7.5%) OR (95% CI)
GC				
PC1[b]	0.98 (0.90–1.06)	1.00 (0.93–1.07)	0.99 (0.92–1.05)	0.90 (0.80–1.03)
PC2	1.03 (0.94–1.13)	0.97 (0.89–1.05)	0.99 (0.92–1.07)	1.01 (0.88–1.16)
PC3	0.97 (0.85–1.09)	0.96 (0.86–1.07)	0.95 (0.86–1.06)	0.88 (0.72–1.09)
PC4	0.95 (0.79–1.14)	0.95 (0.81–1.12)	0.96 (0.82–1.12)	1.00 (0.75–1.35)
LRT *P*[c]	0.87	0.83	0.86	0.41
CASR				
PC1[a]	1.03 (0.97–1.12)	1.05 (0.99–1.13)	1.03 (0.97–1.10)	1.01 (0.90–1.14)
PC2	0.98 (0.91–1.07)	1.00 (0.93–1.07)	1.01 (0.94–1.08)	0.99 (0.87–1.12)
PC3	1.00 (0.92–1.10)	0.92 (0.85–1.00)	0.95 (0.88–1.03)	0.96 (0.83–1.12)
PC4	0.95 (0.86–1.06)	1.03 (0.93–1.13)	1.02 (0.93–1.12)	1.10 (0.92–1.31)
PC5	1.10 (0.97–1.25)	1.01 (0.90–1.14)	1.07 (0.96–1.20)	1.03 (0.83–1.27)
LRT *P*[c]	0.57	0.17	0.39	0.85

[a]The colorectal neoplasia outcome categories are not mutually exclusive.
[b]An 80% explained-variance threshold is used for including principal components (PC) in the model.
[c]*P* value for each model is from a likelihood ratio test (LRT) with degrees of freedom equal to the number of principal components and is a test of gene-level associations.

this vitamin D metabolite. A modest relationship was observed for polymorphisms in *CASR* and odds of metachronous colorectal neoplasia, but no association between *GC* and colorectal lesions was observed. In addition, neither gene was related to circulating concentrations of $1,25(OH)_2D$. Overall, the evidence justifies further study of these associations in larger, more diverse populations.

Polymorphic variation in GC and CASR and vitamin D metabolites

The present analysis identified an overall gene-level association between *GC* and circulating 25(OH)D concentrations, and the individual SNP analysis identified rs7041, rs222035, rs842999, rs1155563, rs12512631, rs16846876, and rs17467825 as *GC* polymorphisms associated with variation in circulating 25(OH)D concentration. However, a simple correlation matrix revealed that rs7041, rs222035, and rs842999 are highly correlated, and rs1155563 is in strong correlation with rs17467825 (data not shown). Analysis of HapMap data also determined that although rs4588 was not included in our data set, there were two SNPs included (rs1155563 and rs17467825) in high linkage disequilibrium (LD) with rs4588 (LD = 0.83 and 1.0, respectively). The present study therefore supports previous reports that two commonly studied *GC* polymorphisms rs7041 and rs4588, are significantly associated with circulating 25(OH)D concentrations (17, 48). We observed a similar trend to that described by Sinotte and colleagues where increasing copies of the rare G allele for rs4588 led to a decline in circulating 25(OH)D (15). Furthermore, our work identified similar trends and significant associations with 3 of the 4 polymorphisms identified by Ahn and colleagues (rs7041, rs12512631, and rs1155563; ref. 18). Ahn and colleagues also identified rs2282679 and, although it was not included in our data set, it is another SNP in high linkage disequilibrium with rs4588 and that association was evaluated through analysis of rs1155563 and rs17467825 in our sample. These results provide support for previously published results and also identify novel polymorphisms that should be further evaluated for functional effects. There are scarce data on the functional effects of polymorphisms in *GC*, although recent evidence suggest that these polymorphisms affect affinity of GC for binding vitamin D metabolites (14). This evidence justifies further analysis of the effects of *GC* polymorphisms on the vitamin D endocrine system in both molecular and population-based studies.

The current work did not identify an association between *GC* and $1,25(OH)_2D$ concentration or any gene-level association or individual polymorphisms in *CASR* that are associated with circulating 25(OH)D or $1,25(OH)_2D$ concentration. We speculate this could be due, in part, to the close homeostatic control of $1,25(OH)_2D$ concentration related to bone health (49), and it is also possible that the study did not have sufficient power to observe associations with changes on the picogram per milliliter scale on which $1,25(OH)_2D$ concentration is measured. Furthermore, with respect to *GC* specifically, it is known that the affinity of GC for $1,25(OH)_2D$ is lower than for 25(OH)D and thus polymorphic variation in the *GC* gene could be less likely associated with circulating concentration of the "free" hormone. While the majority of previous studies related to *CASR* examined the association with calcium levels and few have analyzed its relationship with vitamin D metabolites, prior work has found no associations between intracellular domain polymorphisms A986S (rs1801725), R990G (rs1042636), Q1011E (rs1801726), and 25(OH)D or $1,25(OH)_2D$ concentration (27, 50), suggesting that *CASR* may not play a large role in regulation of vitamin D metabolite concentrations.

Polymorphic variation in GC and CASR and colorectal neoplasia

There were no significant gene-level or individual SNP-level associations identified between *GC* and colorectal neoplasia recurrence. The results of the present study support those of the one other published report examining this relationship in the colon. Poynter and colleagues reported no significant associations between colorectal neoplasia and variation in either *GC* or *VDR* (51). It is possible that *GC* genotype is an effect modifier of the relationship between vitamin D and colorectal neoplasia; however, this sample did provide sufficient statistical power for testing that association. Theoretically, genetic changes in *GC* affecting the ability of the expressed protein to bind to or release vitamin D metabolites and thus influencing their delivery to colon cells (14) could also alter risk for colorectal neoplasia, but currently data on this subject are sparse and require further investigation.

With regard to *CASR*, the present study identified a single polymorphism associated with increased odds of proximal colorectal neoplasia. Although there was no statistically significant gene-level association between *CASR* and any type of colorectal neoplasia in principal components analysis, the individual SNP analysis identified rs1042636 as statistically significantly associated with odds of proximal colorectal neoplasia in both additive and dominant models of inheritance. This polymorphism has previously been studied for associations with colorectal neoplasia and is located in coding region of exon 7 (25). Peters and colleagues identified rs1042636 as part of a *CASR* diplotype associated with advanced colorectal neoplasia; however, the authors pointed out that it was unlikely that this polymorphism was driving the association (33). In contrast, Jacobs and colleagues recently found no association between any *CASR* polymorphisms and risk of colorectal neoplasia in analysis of participants of the Colon Cancer Family Registry (52). The European Prospective Investigation into Cancer and Nutrition (EPIC) study found no association between colorectal neoplasia and *CASR* polymorphisms, whereas Bacsi and colleagues reported increased risk of colorectal cancer for individuals with the rs1801725 TT genotype (32, 50). Finally, proximal colorectal neoplasia risk was significantly associated with variation in rs10934578, rs2270916, rs12485716, and rs4678174 in a study by Dong

and colleagues (26). No statistically significant associations between these polymorphisms and colorectal neoplasia at any site were observed in our pooled sample. However, it is possible that rs1042636 is in linkage disequilibrium with another polymorphism that alters the functional effects of *CASR* in a way that directly effects differentiation of cells. It is also possible that differences in the populations studied, such as race or ethnicity, may have affected the results. These results support a possible difference of colorectal neoplasia risk by anatomic location in the colon but are in contrast to results of previous observational studies and must be interpreted with caution.

There are limitations of these data that should be addressed in future research. The sample for these analyses only included individuals whose self-reported race/ethnicity was white, and it would be beneficial to also test these hypotheses in a population more representative of the overall U.S. population in terms of race/ethnicity and gender distributions (43). Furthermore, the sample was not large enough to appropriately assess effect modification by study and these associations should be evaluated in a larger population. There were also limitations to the statistical methods used for analysis. The statistical adjustment used to account for multiple comparisons may have been too stringent to allow for identification of the modest effects often observed for SNPs. In addition, interpretability across methods is challenging when the results of prediction models do not correspond to what is identified in association studies and thus we feel additional work is necessary to identify the best approaches for analysis of genetic data.

Conclusions

In summary, GC and CASR are proteins that have previously been linked to variation in circulating vitamin D metabolite concentrations and risk of colorectal neoplasia. The results of the present study confirm that *GC* polymorphisms are associated with circulating 25(OH)D concentrations. The role of GC as the transport protein for vitamin D metabolites is well established; however, there is still debate for whether these polymorphisms affect cell-level functions of the vitamin D endocrine system or whether these observed associations translate to disease risk in populations. These results also suggested a modest potential association between polymorphic variation in *CASR* and odds for proximal colorectal neoplasia, with no relationship observed for *GC*. It has been shown that altered biologic function of these proteins could allow neoplastic cells to progress; however, in population-level studies, associations are equivocal. Furthermore, considering the known variation in *GC* and *CASR* by race/ethnicity, these associations should be evaluated in larger, more diverse populations to better determine whether any subgroups of the population are at increased risk because of genetic variability.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work was supported in part by National Cancer Institute grants (CA-41108, CA-23074, and CA-140285), the Specialized Program of Research Excellence (SPORE) in Gastrointestinal Cancer (CA95060), and a Career Development award from the National Cancer Institute (1K07CA10629-01A1 to E.T. Jacobs).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 27, 2011; revised November 22, 2011; accepted November 25, 2011; published OnlineFirst December 5, 2011.

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin 2009;59:225–49.
2. Li FY, Lai MD. Colorectal cancer, one entity or three. J Zhejiang Univ Sci B 2009;10:219–29.
3. Cho KR, Vogelstein B. Suppressor gene alterations in the colorectal adenoma-carcinoma sequence. J Cell Biochem Suppl 1992;16G: 137–41.
4. Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development. N Engl J Med 1988;319:525–32.
5. Harriss DJ, Atkinson G, Batterham A, George K, Cable NT, Reilly T, et al. Lifestyle factors and colorectal cancer risk (2): a systematic review and meta-analysis of associations with leisure-time physical activity. Colorectal Dis 2009;11:689–701.
6. Lieberman DA. Clinical practice. Screening for colorectal cancer. N Engl J Med 2009;361:1179–87.
7. Martinez ME, Marshall JR, Giovannucci E. Diet and cancer prevention: the roles of observation and experimentation. Nat Rev Cancer 2008;8: 694–703.
8. Baron JA, Beach M, Mandel JS, van Stolk RU, Haile RW, Sandler RS, et al. Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. N Engl J Med 1999;340:101–7.
9. Lee JE, Li H, Chan AT, Hollis BW, Lee IM, Stampfer MJ, et al. Circulating levels of vitamin D and colon and rectal cancer: the Physicians' Health Study and a meta-analysis of prospective studies. Cancer Prev Res (Phila) 2011;4:735–43.
10. Wei MY, Garland CF, Gorham ED, Mohr SB, Giovannucci E. Vitamin D and prevention of colorectal adenoma: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2008;17:2958–69.
11. Gomme PT, Bertolini J. Therapeutic potential of vitamin D-binding protein. Trends Biotechnol 2004;22:340–5.
12. Haddad J. The vitamin D binding protein and its clinical significance. In: Holick MF, editor. Vitamin D: physiology, molecular biology, and clinical applications. Totowa, NJ: Humana Press, Inc.; 1999. p. 101–7.
13. Norman AW, Ishizuka S, Okamura WH. Ligands for the vitamin D endocrine system: different shapes function as agonists and antagonists for genomic and rapid response receptors or as a ligand for the plasma vitamin D binding protein. J Steroid Biochem Mol Biol 2001;76:49–59.
14. Speeckaert M, Huang G, Delanghe JR, Taes YE. Biological and clinical aspects of the vitamin D binding protein (Gc-globulin) and its polymorphism. Clin Chim Acta 2006;372:33–42.
15. Sinotte M, Diorio C, Berube S, Pollak M, Brisson J. Genetic polymorphisms of the vitamin D binding protein and plasma concentrations

of 25-hydroxyvitamin D in premenopausal women. Am J Clin Nutr 2009;89:634–40.
16. Fu L, Yun F, Oczak M, Wong BY, Vieth R, Cole DE. Common genetic variants of the vitamin D binding protein (DBP) predict differences in response of serum 25-hydroxyvitamin D [25(OH)D] to vitamin D supplementation. Clin Biochem 2009;42:1174–7.
17. Abbas S, Linseisen J, Slanger T, Kropp S, Mutschelknauss EJ, Flesch-Janys D, et al. The Gc2 allele of the vitamin D binding protein is associated with a decreased postmenopausal breast cancer risk, independent of the vitamin D status. Cancer Epidemiol Biomarkers Prev 2008;17:1339–43.
18. Ahn J, Albanes D, Berndt SI, Peters U, Chatterjee N, Freedman ND, et al. Vitamin D-related genes, serum vitamin D concentrations and prostate cancer risk. Carcinogenesis 2009;30:769–76.
19. McGrath JJ, Saha S, Burne TH, Eyles DW. A systematic review of the association between common single nucleotide polymorphisms and 25-hydroxyvitamin D concentrations. J Steroid Biochem Mol Biol 2010;121:471–7.
20. Hartman TJ, Albert PS, Snyder K, Slattery ML, Caan B, Paskett E, et al. The association of calcium and vitamin D with risk of colorectal adenomas. J Nutr 2005;135:252–9.
21. Egbuna OI, Brown EM. Hypercalcaemic and hypocalcaemic conditions due to calcium-sensing receptor mutations. Best Pract Res Clin Rheumatol 2008;22:129–48.
22. Magno AL, Ward BK, Ratajczak T. The calcium-sensing receptor: a molecular perspective. Endocr Rev 2011;32:3–30.
23. Saidak Z, Mentaverri R, Brown EM. The role of the calcium-sensing receptor in the development and progression of cancer. Endocr Rev 2009;30:178–95.
24. Tfelt-Hansen J, Brown EM. The calcium-sensing receptor in normal physiology and pathophysiology: a review. Crit Rev Clin Lab Sci 2005;42:35–70.
25. Yun FH, Wong BY, Chase M, Shuen AY, Canaff L, Thongthai K, et al. Genetic variation at the calcium-sensing receptor (CASR) locus: implications for clinical molecular diagnostics. Clin Biochem 2007;40:551–61.
26. Dong LM, Ulrich CM, Hsu L, Duggan DJ, Benitez DS, White E, et al. Genetic variation in calcium-sensing receptor and risk for colon cancer. Cancer Epidemiol Biomarkers Prev 2008;17:2755–65.
27. Harding B, Curley AJ, Hannan FM, Christie PT, Bowl MR, Turner JJ, et al. Functional characterization of calcium sensing receptor polymorphisms and absence of association with indices of calcium homeostasis and bone mineral density. Clin Endocrinol (Oxf) 2006;65:598–605.
28. Carroll C, Cooper K, Papaioannou D, Hind D, Pilgrim H, Tappenden P. Supplemental calcium in the chemoprevention of colorectal cancer: a systematic review and meta-analysis. Clin Ther 2010;32:789–803.
29. Gonzalez CA, Riboli E. Diet and cancer prevention: contributions from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Eur J Cancer 2010;46:2555–62.
30. Chan AT, Giovannucci EL. Primary prevention of colorectal cancer. Gastroenterology 2010;138:2029–43.e10.
31. Liu G, Hu X, Chakrabarty S. Vitamin D mediates its action in human colon carcinoma cells in a calcium-sensing receptor-dependent manner: downregulates malignant cell behavior and the expression of thymidylate synthase and survivin and promotes cellular sensitivity to 5-FU. Int J Cancer 2010;126:631–9.
32. Bacsi K, Hitre E, Kosa JP, Horvath H, Lazary A, Lakatos PL, et al. Effects of the lactase 13910 C/T and calcium-sensor receptor A986S G/T gene polymorphisms on the incidence and recurrence of colorectal cancer in Hungarian population. BMC Cancer 2008;8:317.
33. Peters U, Chatterjee N, Yeager M, Chanock SJ, Schoen RE, McGlynn KA, et al. Association of genetic variants in the calcium-sensing receptor with risk of colorectal adenoma. Cancer Epidemiol Biomarkers Prev 2004;13:2181–6.
34. Alberts DS, Martinez ME, Hess LM, Einspahr JG, Green SB, Bhattacharyya AK, et al. Phase III trial of ursodeoxycholic acid to prevent colorectal adenoma recurrence. J Natl Cancer Inst 2005;97:846–53.
35. Alberts DS, Martinez ME, Roe DJ, Guillen-Rodriguez JM, Marshall JR, van Leeuwen JB, et al. Lack of effect of a high-fiber cereal supplement on the recurrence of colorectal adenomas. Phoenix Colon Cancer Prevention Physicians' Network. N Engl J Med 2000;342:1156–62.
36. Martinez ME, Reid ME, Guillen-Rodriguez J, Marshall JR, Sampliner R, Aickin M, et al. Design and baseline characteristics of study participants in the Wheat Bran Fiber trial. Cancer Epidemiol Biomarkers Prev 1998;7:813–6.
37. Jacobs ET, Alberts DS, Benuzillo J, Hollis BW, Thompson PA, Martinez ME. Serum 25(OH)D levels, dietary intake of vitamin D, and colorectal adenoma recurrence. J Steroid Biochem Mol Biol 2007;103:752–6.
38. Egan JB, Thompson PA, Ashbeck EL, Conti D, Duggan D, Hibler EA, et al. Genetic polymorphisms in vitamin D receptor VDR/RXRA influence the likelihood of colon adenoma recurrence. Cancer Res 2010;70:1496–504.
39. Levine AJ, Figueiredo JC, Won L, Poynter JN, Conti D, Duggan DJ, et al. Genetic Variability in the MTHFR gene and colorectal cancer risk in the Colorectal Cancer Family Registry. Cancer Epidemiol Biomarkers Prev 2009;19:89–100.
40. Hollis BW, Kamerud JQ, Selvaag SR, Lorenz JD, Napoli JL. Determination of vitamin D status by radioimmunoassay with an 125I-labeled tracer. Clin Chem 1993;39:529–33.
41. Hollis BW. Assay of circulating 1,25-dihydroxyvitamin D involving a novel single-cartridge extraction and purification procedure. Clin Chem 1986;32:2060–3.
42. Jacobs E, Martinez ME, Buckmeier J, Lance P, May M, Jurutka P. Circulating fibroblast growth factor-23 is associated with increased risk for metachronous colorectal adenoma. J Carcinog 2011;10:3.
43. Jacobs ET, Alberts DS, Foote JA, Green SB, Hollis BW, Yu Z, et al. Vitamin D insufficiency in southern Arizona. Am J Clin Nutr 2008;87:608–13.
44. Gauderman WJ, Murcray C, Gilliland F, Conti DV. Testing association between disease and multiple SNPs in a candidate gene. Genet Epidemiol 2007;31:383–95.
45. Conneely KN, Boehnke M. So many correlated tests, so little time! Rapid adjustment of P values for multiple correlated tests. Am J Hum Genet 2007;81:1158–68.
46. Garcia-Magarinos M, Lopez-de-Ullibarri I, Cao R, Salas A. Evaluating the ability of tree-based methods and logistic regression for the detection of SNP-SNP interaction. Ann Hum Genet 2009;73:360–9.
47. Zaykin DV, Young SS. Large recursive partitioning analysis of complex disease pharmacogenetic studies. II. Statistical considerations. Pharmacogenomics 2005;6:77–89.
48. Dusso AS, Brown AJ, Slatopolsky E. Vitamin D. Am J Physiol Renal Physiol 2005;289:F8–28.
49. Norman AW. From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health. Am J Clin Nutr 2008;88:491S–9S.
50. Jenab M, McKay J, Bueno-de-Mesquita HB, van Duijnhoven FJ, Ferrari P, Slimani N, et al. Vitamin D receptor and calcium sensing receptor polymorphisms and the risk of colorectal cancer in European populations. Cancer Epidemiol Biomarkers Prev 2009;18:2485–91.
51. Poynter JN, Jacobs ET, Figueiredo JC, Lee WH, Conti DV, Campbell PT, et al. Genetic variation in the vitamin D receptor (VDR) and the vitamin D-binding protein (GC) and risk for colorectal cancer: results from the Colon Cancer Family Registry. Cancer Epidemiol Biomarkers Prev 2010;19:525–36.
52. Jacobs ET, Martinez ME, Campbell PT, Conti DV, Duggan D, Figueiredo JC, et al. Genetic variation in the retinoid X receptor and calcium-sensing receptor and risk of colorectal cancer in the Colon Cancer Family Registry. Carcinogenesis 2010;31:1412–6.