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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]

[1]Department of Epidemiology and Biostatistics, Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, AZ, USA 85724

[2]Arizona Cancer Center, University of Arizona, Tucson, AZ, USA 85724

[3]Division of Mathematical and Natural Sciences, Arizona State University, Glendale, Arizona 85069

[4]Department of Basic Medical Sciences, University of Arizona College of Medicine-Phoenix, Arizona 85004.

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=1439) 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, 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 demonstrate 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.

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

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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.

Keywords

GC; CASR; polymorphism; colorectal neoplasia; vitamin D

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 demonstrating 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 greater than 120 known variants (11), and there are two 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 and it is hypothesized that this could affect the delivery of vitamin D at the tissue or cellular levels (14). Several studies have demonstrated 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 (20, 21). However, it is not yet clear if 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 calcium-sensing receptor (CASR) has a primary role in calcium homeostasis by sensing extracellular blood calcium levels, which are maintained within a narrow range (22-26). The CASR is part of the superfamily of G-protein coupled receptors (24, 27, 28) and expression of CASR has been identified in cells of the gastrointestinal tract and bone (22-24, 28). Observational studies have reported associations between calcium intake and adenoma recurrence (29, 30). Furthermore, a clinical trial by Baron et al. demonstrated 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 (29, 31, 32). There are two functional vitamin D response elements (VDRE) located in the promoter regions of *CASR* (26, 27, 33) and researchers have identified *CASR* polymorphisms associated with different colorectal neoplasia outcomes (34, 35). 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 performed 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 (36-38). 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 (36). The study population included Arizona residents (N = 1192) between 40 to 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 (36). The WBF trial was also a double-blind phase III clinical trial conducted at the University of Arizona to measure the effects of high (13.5 g/day) versus low (2.0 g/day) WBF intake for 3 years on colorectal adenoma recurrence (38). A total of 1310 participants competed this trial and included individuals between 40 to 80 years of age, of both genders, who had removal of one or more colorectal adenoma (> 3 mm) at colonoscopy within 3 months prior to study enrollment (38). There were 1530 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 1439 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 (36, 39). 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®, San Diego, CA) 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 (40, 41). The final statistical analysis included 25 *GC* and 35 *CASR* SNPs. Data on the size, location, and histology of identified adenomas were collected for participants of both trials (37). 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 (36). For the WBF trial, the results from each colonoscopy reported were collected using standard abstraction guidelines (38). 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 (40). 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 (40). The WBF and UDCA trials also utilized 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 (38, 39). 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 in the lab of Dr. Bruce Hollis using the radioimmunoassay (RIA) method, as previously described in detail (42, 43). The laboratory utilized several QA/QC measures including a pooled serum sample analyzed with batches of study samples to monitor analytical precision and identify possible laboratory

shifts over time as well as testing duplicates in different batches. The coefficient of variation was less than 7.0% for 25(OH)D analyses and 11.5% for $1,25(OH)_2D$ analysis (44, 45). All analyses were conducted in a blinded fashion.

Statistical Analysis

The outcome measures of circulating 25(OH)D and $1,25(OH)_2D$ concentrations were analyzed as continuous variables; while measures of colorectal neoplasia were dichotomous variables. There is currently no single standard method for analysis of high-dimensional genetic data, and therefore four techniques were employed; principal components (PC) analysis, analysis of individual SNPs through regression models, classification and regression trees (CART), and random forest analyses (40). PC analysis is a method that assesses the overall gene-level associations and the PCs represent a linear transformation of the original SNP data that explain variation within a specific genetic locus (46). PCs 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 (46). The gene-level associations were also compared to 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 (47). The “p values adjusted for multiple correlated tests” (P_{ACT}) multiple comparisons adjustment was developed by Conneely and Boehnke (47). This approach has been published previously and, briefly, is described as a comparison of the observed test statistics directly with their asymptotic distribution, utilizing numerical integration (47). 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 versus 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 percent.

In addition to testing associations, methods were used to identify polymorphisms that were predictive of colorectal neoplasia outcomes. Classification and regression trees (CART) and random forests are tree-based analysis methods used to identify patterns in high-dimensional genetic data (48). These are non-parametric 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 (48, 49). 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 RF modeling.

Results

Baseline characteristics of the participants are shown in Table 1 and have been previously described in detail (38). The current analysis was completed using a pooled sample of participants from the UDCA and WBF trials (N = 2502) restricted to individuals with complete data for adenoma recurrence and genotype for the selected polymorphisms (N =

1530). The sample was further restricted to those who reported white race (N = 1439). 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 due to 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 BMI 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 ± 8.6, 66.1% male) with mean BMI of 28.4 kg/m^2. Dietary intake was similar between samples with respect to total energy (1963.0 ± 780.0 and 2037.5 ± 858.6, respectively), fat intake (64.0 ± 30.9 and 63.5 ± 32.8, respectively), and calcium intake (971.0 ± 460.4 versus 1026.8 ± 513.4). Vitamin D supplement use was moderately higher (75.2%) amongst the subset with vitamin D metabolite data versus the pooled gene analysis sample (66.3%). The subset of the UDCA trial was comparable to 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, Supplemental Tables 1-4). In the principal components analysis, statistically significant gene-level associations were observed between *GC* and 25(OH)D concentration ($p < 0.001$) (Table 2), while 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 (Supplemental Tables 1-2) identified seven *GC* polymorphisms that were significantly related to circulating 25(OH)D levels, using the additive model of inheritance, but not with 1,25(OH)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 ($p = < 0.001$), rs12512631 ($p = 0.02$), rs16846876 ($p = 0.001$), and rs17467825 ($p < 0.001$) (Supplemental Table 1). 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 demonstrated that rs7041, rs222035, and rs842999 are highly correlated ($r^2 > 0.92$) and rs1155563 in strong correlation with rs17467825 ($r^2 = 0.86$).

The results from the prediction models using CART and RF 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 RF analysis identified similar SNPs to the single-SNP level as analysis for prediction of 25(OH)D concentration, though 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 employed revealed a statistically significant relationship. In principal components analyses for *CASR*, there were no statistically significant gene-level associations between *CASR* and either 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 (Supplemental Tables 5-12). 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 demonstrated that one copy of the *G* allele led to decreased odds for metachronous adenomas in both the UDCA and WBF trial participants (OR (95% C.I.) = 0.67 (0.42-1.06) and 0.34 (0.19-0.61), respectively; data not shown). In contrast, when employing the CART approach, there were no *CASR* polymorphisms that were predictive of colorectal neoplasia at any site. The most predictive *CASR* polymorphism in RF analysis was rs1042636, though no SNPs were 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, while there was no relationship for *CASR* and 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 dataset, there were two SNPs included (rs1155563 and rs17467825) in high linkage disequilibrium 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, 50). We observed a similar trend to that described by Sinotte et al. 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 three of the four polymorphisms identified by Ahn et al. (rs7041, rs12512631, and rs1155563) (18). Ahn et al. also identified rs2282679 and, though it was not included in our dataset, 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*, though 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 (51) and it 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 to be 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 (28, 52), 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 et al. reported no significant associations between colorectal neoplasia and variation in either *GC* or *VDR* (53). 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 (26). Peters et al. 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 (35). In contrast, Jacobs et al. recently found no association between any *CASR* polymorphisms and risk of colorectal neoplasia in analysis of participants of the Colon Cancer Family Registry (54). The EPIC study found no association between colorectal neoplasia and *CASR* polymorphisms; while Basci et al. reported increased risk of colorectal cancer for individuals with the rs1801725 TT genotype (34, 52). Finally, proximal colorectal neoplasia risk was significantly associated with variation in rs10934578, rs2270916, rs12485716, and rs4678174 in a study by Dong et al. (27). 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 anatomical 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 (45). 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 if 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 demonstrated 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 in order to better determine if any subgroups of the population are at increased risk due to genetic variability.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Baseline characteristics of study population

Characteristics	Pooled UDCA and WBF N =1530	Vitamin D Subset N =475
Trial Participant		
UDCA, n, (%)	896 (58.6)	475 (100.0)
WBF, n, (%)	634 (41.4)	N.A.
Mean age, y ± SD	66.0 ± 8.3	65.5 ± 8.6
Sex, Male, n (%)	1027 (66.1)	314 (66.1)
Race, White, n (%)	1439 (94.1)[1]	404 (85.16.5)
Mean BMI, kg/m^2 ± SD	27.9 ± 4.6	28.5 ± 4.9
Aspirin use, n (%)	443 (29.0)	134 (28.21)
Ever smoker, n (%)	998 (65.2)[1]	316 (66.5)[2]
Current smoker, n (%)	179 (11.7)	61 (12.8)
Total Fat, g/day ± SD	64.0 ± 30.9	63.5 ± 32.8
Energy, kcal/day ± SD	1963.0 ± 780.0	2037.5 ± 858.6
Calcium intake, g/day ± SD	971.0 ± 460.4	1026.8 ± 513.4
Vitamin D supplement use, n (%)	1015 (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)[2]
Distal metachronous neoplasia, n (%)	363 (23.7)	103 (21.7)[2]

[1]Missing for race, N = 15; ever smoker , N = 20; proximal and distal neoplasia, N =11.

[2]Missing for race, N = 9; ever smoker, N = 8; proximal and distal neoplasia, N = 2.

Table 2

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

	25(OH)D			$1,25(OH)_2D$		
	Mean change	**95% CI**		**Mean change**	**95% CI**	
GC						
PC1	−1.23	−1.78-0.68		−0.57	−1.18-0.04	
PC2	−0.12	−0.67-0.71		0.19	−0.86-0.62	
PC3	0.47	0.30-2.02		−0.23	−0.49-1.43	
PC4	−0.04	−0.54-1.92		−0.13	−1.39-1.31	
LRT p-value[2]			<0.001			0.35
CASR						
PC1[1]	0.12	−0.37-0.61		−0.11	−0.64-0.42	
PC2	0.09	−0.48-0.66		0.01	−0.60-0.62	
PC3	−0.43	−1.08-0.22		−0.28	−0.97-0.41	
PC4	0.48	−0.34-1.3		0.88	0.01-1.75	
PC5	−0.16	−1.12-0.8		−0.02	−1.04-1.00	
LRT p-value[2]			0.62			0.45

[1] An 80% explained-variance threshold is used for including principal components (PC) in the model.

[2] 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.

Table 3

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

	Colorectal Neoplasia Outcomes (N = 1439)											
	Distal[3] N = 343 (24.0%)			Proximal[3] N = 473 (33.1 %)			Recurrence N = 660 (45.9 %)			Villous N = 107 (7.5%)		
	OR	95% CI		OR	95% CI		OR	95% CI		OR	95% CI	
GC												
PC1[1]	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-value[2]			0.87			0.83			0.86			0.41
CASR												
PC1[1]	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-value[2]			0.57			0.17			0.39			0.85

[1] An 80% explained-variance threshold is used for including principal components (PC) in the model.

[2] 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.

[3] The colorectal neoplasia outcome categories are not mutually exclusive.