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CYP24A1 and *CYP27B1* polymorphisms, concentrations of vitamin D metabolites, and odds of colorectal adenoma recurrence

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Abstract

Development of colorectal adenoma and cancer are associated with low circulating 25-hydroxyvitamin D (25(OH)D) levels. However, less is known regarding colorectal neoplasia risk and variation in *CYP27B1* or *CYP24A1*, genes encoding the enzymes responsible for the synthesis and catabolism of 1α,25-hydroxyvitamin D (1,25(OH)$_2$D). This study examined associations between *CYP27B1* and *CYP24A1* polymorphisms, circulating 25(OH)D and 1,25(OH)$_2$D concentrations, and colorectal adenoma recurrence in a pooled sample from two clinical trials (n=1,188). Nominal associations were observed between increasing copies of the *T* allele in *CYP24A1 rs927650* and 25(OH)D concentrations (p=0.02); as well as colorectal adenoma recurrence, with ORs (95% CIs) of 1.30 (0.99–1.70) and 1.38 (1.01–1.89) for heterozygotes and minor allele homozygotes, respectively (p=0.04). In addition, a statistically significant relationship between *CYP24A1 rs35051736*, a functional polymorphism, and odds for advanced colorectal adenoma recurrence was observed (p<0.001). Further, *nominally statistically significant* interactions were observed between *rs2296241* and 25(OH)D as well as *rs2762939* and 1,25(OH)$_2$D ($p_{interaction}$ = 0.10, respectively). Overall, *CYP24A1* polymorphisms may influence the development of advanced lesions, and modify the effect of vitamin D metabolites on adenoma recurrence. Further study is necessary to characterize the differences between *circulating vitamin D metabolite measurements* compared to cellular level activity in relation to *cancer risk*.

Keywords

Colorectal adenoma; Vitamin D; Adenoma recurrence; 1,25(OH)$_2$D; 25(OH)D

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Introduction

The relationship between vitamin D metabolites and colorectal neoplasia has been studied extensively. In epidemiological research, the biomarker most commonly employed is 25-hydroxycholecalciferol [25(OH)D], which reflects both dietary intake and endogenous synthesis of vitamin D. Although some studies have shown no association between 25(OH)D and colorectal adenoma (1–6), meta-analyses have revealed significant inverse relationships between 25(OH)D and both colorectal adenoma (7–9) and cancer (10–12). While 25(OH)D is the most abundant circulating metabolite of vitamin D, hydroxylation by the enzyme CYP27B1 is required for the formation of 1,25-dihydroxycholecalciferol [1,25$(OH)_2$D], which is the active, hormonal form of vitamin D (13). Although 1,25$(OH)_2$D is not as frequently studied as 25(OH)D in epidemiological work, it has been demonstrated to have anti-carcinogenic effects in colon cells (14, 15), and is the primary metabolite under investigation in experimental models of cancer (16–19). Further, our group and others have previously reported associations between concentrations of 1,25$(OH)_2$D and colorectal adenoma (1, 20, 21). Therefore, it is of interest to investigate the genetic factors that may alter the concentration of this hormone, and how they relate to odds of colorectal neoplasia.

As mentioned above, CYP27B1 is responsible for production of 1,25$(OH)_2$D from the precursor molecule, 25(OH)D. In turn, the primary enzyme involved in 1,25$(OH)_2$D catabolism is CYP24A1, which begins the process of metabolizing 1,25$(OH)_2$D by hydroxylating the molecule at the C24 position to form 1,24,25$(OH)_3$D. This molecule can no longer bind to or activate the vitamin D receptor, and in turn is further catabolized until it can be excreted (22). Our group has shown that single nucleotide polymorphisms (SNPs) in *CYP27B1* and *CYP24A1* have functional effects that ultimately alter the concentration of 1,25$(OH)_2$D available in colon cancer cell lines (23) such that a more active isotype of CYP27B1 and a less active form of CYP24A1 may have the net effect of increasing 1,25$(OH)_2$D concentrations at the tissue level, and vice versa. We therefore sought to assess the effect of this variation in a human population.

The importance of CYP27B1 and CYP24A1 in local intracrine modulation of 1,25$(OH)_2$D is clear, and several epidemiological studies of genetic polymorphisms in *CYP27B1* or *CYP24A1* and either circulating concentrations of vitamin D metabolites (24–26) or risk for colorectal cancer (27–30) have been conducted. However, to date, there are no published studies of the association between variation in these genes and risk of the precursor lesions to colorectal cancer. Therefore, the goal of the current work was to conduct the first investigation of genetic variation in *CYP27B1* or *CYP24A1*, circulating concentrations of 25(OH)D and 1,25$(OH)_2$D, and odds of colorectal adenoma recurrence.

Methods

Study population

Participants in the present study were drawn from two randomized, double-blind, placebo-controlled clinical trials, the Wheat Bran Fiber (WBF) Trial (31) and the Ursodeoxycholic Acid (UDCA) Trial (32), which have been described in detail elsewhere. Briefly, the objective of the WBF trial was to compare the effect of a high-fiber vs. a low-fiber cereal supplement on adenoma recurrence among 1310 individuals from Phoenix, Arizona who had undergone colonoscopy and had one or more adenoma(s) removed. No differences in adenoma recurrence rates were observed between treatment groups (32). The UDCA trial employed a design similar to that of the WBF trial, and compared the effect of UDCA vs. placebo on adenoma recurrence among 1192 patients that had a prior polyp removed at colonoscopy. No main effect of UDCA on adenoma recurrence was observed (31). All participants who completed these clinical trials, had available serum for analysis of 25(OH)D, and had genotype data for *CYP24A1* and *CYP27B1* were eligible for the current study (n=1,188). A subset of these participants also had measured circulating concentrations of 1,25$(OH)_2$D (n=828). The WBF and UDCA studies were approved by the University of Arizona Human Subjects Committee and local hospital committees, and written informed consent was obtained from each participant prior to study enrollment.

Data collection

Self-administered baseline questionnaires were employed to capture dietary, sociodemographic, and medical history data for all participants in the WBF and UDCA trials. Dietary data were collected using the Arizona Food Frequency Questionnaire (AFFQ), which was developed based on the food frequency section of the National Cancer Institute's Health Habits and History Questionnaire (33), and modified to reflect the diet in southwestern Arizona.

Information for adenoma characteristics such as number, size, location, and histology were collected for both baseline and recurrent adenomas. As described previously (31), data were obtained via medical records and pathology reports for each study participant. The study design for both the WBF and UDCA trials required all participants to have had at least one adenoma at baseline. After removal of these lesions, recurrent adenomas were defined as any colorectal adenoma detected at colonoscopy at least six months after randomization. Adenomas were classified as advanced if they had a diameter of 1 cm or more, and/or tubulovillous or villous histology (at least 25% villous). Among subjects who had more than one adenoma, the size and characterization of the histologic type were based on the largest and/or most advanced adenoma.

Selection of CYP27B1 and CYP24A1 SNPs

The SNPs for the present study were selected *a priori* based on either prior reports of associations with colorectal cancer or circulating concentrations of vitamin D metabolites. In addition, SNPs that were investigated in our previously-published work, which revealed the functional effects of variants in the two genes (23), were considered for inclusion. Several SNPs in the latter group, particularly in *CYP27B1*, were excluded from the present analysis due to the high prevalence of monomorphs, most likely resulting from suspected pathogenic effects of the variation (*rs28934604*; *rs58915677*; *rs2229103*).

Genotyping was conducted though a service contract with BioServe Biotechnologies, Ltd [Beltsville, MD] using the MassARRAY iPLEX™ platform [Sequenom Laboratories, San Diego, CA], which employs a PCR process and mass spectrometry-based system, followed by a single-base extension reaction. A total of 24 DNA samples from the Coriell

Polymorphism Discovery Panel were run in duplicate validate each plex group assay. PCR and extension primers were synthesized for the SNPs of interest at BioServe and checked using a MALDI-TOF mass spectrometer and Sequenom's OligoCHECK software. Additional quality control checks, including duplicate control and negative control results were established, and a further quality control measure was conducted in 10% of the study samples, which were run in duplicate and compared. SNPs were considered to have failed the platform and dropped from the analysis if the assay yielded inaccurate or no control data based on published allele frequencies, or if call rates were < 80%. One SNP (rs3787557) was dropped from the present analysis for the latter reason.

Analysis of vitamin D metabolite levels

Measurement of 25(OH)D and 1,25(OH)$_2$D concentrations was performed at Heartland Assays (Ames, IA). A competitive chemiluminescence immunoassay was employed for assessment of 25(OH)D concentrations (34), and included quality assurance and quality control measures such as 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. For 1,25(OH)$_2$D concentrations, a ^{125}I-based radioimmunoassay was employed, as has been described in detail previously (35). The coefficient of variation was 11.5% for 1,25(OH)$_2$D analysis. All analyses were conducted in a blinded fashion. For assessment of statistical interactions between SNPs and concentrations and 25(OH)D, study participants were classified using Institute of Medicine criteria (36). Vitamin D deficiency was defined as circulating 25(OH)D concentrations <12 ng/ml; while inadequacy included those with 25(OH)D levels ≥ 12 and < 20 ng/ml. Participants with 25(OH)D levels at or above 20 ng/ml were classified as adequate. For 1,25(OH)$_2$D, tertiles for circulating concentrations were created based on the population distribution of the metabolite.

Statistical Analysis

In order to evaluate trends in associations between *CYP27B1* and *CYP24A1* SNPs and vitamin D metabolites, linear regression was employed, with age and sex included as covariates. A logarithmic transformation was applied to the 25(OH)D variable to conform to the assumptions of normality. To test the association of SNPs with adenoma recurrence and advanced recurrence, multinomial logistic regression models which included age and sex as covariates were used. If a minor allele heterozygote was absent from an outcome, p-values were obtained via calculation of a z-score and conducting a two-tailed z test. Potentially confounding variables examined included race, body mass index, study, and dietary intake of vitamin D; however, none changed the point estimate by 10% or greater and they were excluded from the final model. To evaluate the relationships between SNPs, colorectal adenoma recurrence, and features of advanced colorectal adenomas, logistic regression modeling was employed, including the same covariates as the previous model. Finally, to test for interactions between SNPs and vitamin D metabolites in relation to adenoma recurrence, we constructed interaction terms for each SNP and 25(OH)D, and 1,25(OH)$_2$D, and evaluated the terms within logistic regression models. When considering the statistical significance of results, a conservative Bonferroni adjustment for 15 tests set significance threshold of α=0.0033. All analyses were conducted in R Statistical software (37) as well as Stata [version 9.0, Stata Corporation, College Station, TX].

Results

Descriptive characteristics of the study population are shown in Table 1. The mean (± sd) age of participants was 65.6 ± 8.5 years, with 68.3% of the participants being male and 93.3% white. The mean (± sd) concentrations of 25(OH)D and 1,25$(OH)_2$D were 27.7 ± 11.4 ng/ml and 33.9 ± 11.3 pg/ml, respectively. A total of 519 (43.7%) individuals had a recurrent adenoma during study follow-up, and 28.6% of those had an advanced adenoma recurrence. *Of adenoma recurrences, 28.1% were in the distal colorectum only; 45.9% were in the proximal colon, and 26.0% of those who had a recurrence had adenomas in both locations*. Supplemental Table 1 presents the genomic location of SNPs, along with respective allele frequencies and genotype missing rates.

Table 2 shows the mean concentrations of 25(OH)D and 1,25$(OH)_2$D by each *CYP27B1* and *CYP24A1* SNP genotype. A suggestive relationship between the *CYP24A1* SNP *rs927650* and concentrations of 25(OH)D was observed, with levels of 26.9 ± 8.5, 27.5 ± 12.8, and 29.1 ± 11.3 for the *CC*, *TC*, and *TT* genotypes, respectively. The results for the association between SNPs, overall colorectal adenoma recurrence, and advanced adenoma recurrence are presented in Table 3. There was a nominally statistically significant association between the *CYP24A1* SNP *rs927650* and odds for any adenoma recurrence, with ORs (95% CIs) of 1.30 (0.99–1.70) and 1.38 (1.01–1.89) for each additional copy of the *T* allele, as compared to wild-type homozygotes (p-trend=0.04). In addition, there was a statistically significant relationship between the *CYP24A1* SNP *rs35051736* and odds for advanced recurrence (p<0.0001); this association arose because the minor allele (*A*) was completely absent in individuals with advanced lesions (n=162).

Table 4 shows the results for the associations between *CYP24A1* and *CYP27B1* SNPs and features of advanced colorectal adenoma recurrence. There was a nominally statistically significant association between increasing number of copies of the *G* allele in *CYP24A1 rs2296241* and higher odds for recurrent adenomas with villous histology, with ORs (95% CIs) of 1.19 (0.68–2.06) and 1.98 (1.12–3.50) for *AG* and *GG* genotypes, respectively, compared to *AA* (p-trend=0.02). Further, the minor allele for the *CYP24A1* SNP *rs35051736* was again absent among those presenting with a large or villous adenoma upon follow-up; however, these findings did not reach statistical significance.

We next sought to determine whether there were interactions between SNPs in *CYP27B1* and *CYP24A1* and vitamin D metabolite concentrations in relation to adenoma recurrence. Logistic regression analyses revealed *nominally* statistically significant interactions between *CYP24A1* SNP *rs2296241* and 25(OH)D (p-interaction=0.10), and *rs2762939* in the same gene and 1,25$(OH)_2$D (p-interaction=0.10). Further exploration of these interactions is presented in Table 5. The results suggest that increasing concentrations of 25(OH)D were significantly associated with reduced odds for adenoma recurrence only among those with at least one copy of the minor allele in *rs2296241*. Among individuals with the *AA* genotype, the ORs (95% CIs) for adenoma recurrence were 0.84 (0.27–2.61) for those with inadequate

25(OH)D concentrations (≥12 and <20 ng/ml) and 0.78 (0.27–2.30) for those with adequate levels (>20 ng/ml), respectively, compared to participants with vitamin D deficiency (<12 ng/ml). For heterozygotes, the ORs (95% CIs) were 0.23 (0.07–0.72) and 0.21 (0.07–0.62) for those who were inadequate and adequate, respectively, compared to deficient individuals. Finally, compared to those who were vitamin D deficient, participants who were homozygous for the minor allele and who had inadequate 25(OH)D concentrations had an OR (95% CI) of 0.13 (0.02–0.68) for adenoma recurrence; while those who were adequate had an OR (95% CI) of 0.18 (0.03–0.91). With regard to *CYP24A1 rs2762939*, the results suggest only a modestly reduced odds for adenoma recurrence among those with higher concentrations of 1,25(OH)$_2$D and at least one copy of the minor allele.

Discussion

The findings of the present study demonstrated a suggestive association between the *CYP24A1* SNP r*s927650* and concentrations of 25(OH)D, but no relationship between SNPs in *CYP27B1* or *CYP24A1* and blood levels of 1,25(OH)$_2$D. A nominally statistically significant association was observed for *CYP24A1 rs927650* and odds of overall adenoma recurrence; while *CYP24A1 rs35051736* was significantly associated with odds of advanced recurrence. Associations between *CYP27B1* and *CYP24A1* SNPs and features of advanced recurrent adenoma recurrence were observed. Statistically significant interactions were observed between the *CYP24A1* SNPs *rs2296241* and *rs2762939* and concentrations of 25(OH)D and 1,25(OH)$_2$D, respectively, in relation to overall adenoma recurrence. These findings indicate that the complexity of the vitamin D pathway may require further gene/ environment investigations in order to fully clarify any role of the vitamin D pathway in colorectal neoplasia.

The relationship between low circulating 25(OH)D concentrations and increased risk of colorectal neoplasia has been reported consistently (7, 9–12); though less common, studies of circulating 1,25(OH)$_2$D concentrations have also revealed associations (1, 20, 21). The results for the main effects associations between 25(OH)D(38) and 1,25(OH)$_2$D (21) and colorectal adenomas in the present study population have been reported previously; however, the relationship between genetic variation in the vitamin D pathway genes *CYP27B1* and *CYP24A1*, concentrations of these metabolites, and adenoma recurrence had not yet been investigated.

Several studies of *CYP27B1* and *CYP24A1* SNPs and colorectal cancer have been conducted, with inconsistent results (27–30). Three studies showed no associations between SNPs in these genes and overall risk for colorectal cancer (27, 29, 30), while another report demonstrated a nominal relationship between the *CYP24A1* polymorphism *rs4809958* and risk for colon cancer (28). In the present study, no association between *rs4809958* and overall adenoma recurrence, nor any adenoma characteristic, was observed, which is consistent with the majority of the published literature for colorectal cancer.

We observed a suggestive relationship between *CYP24A1 rs927650* and overall adenoma recurrence. This SNP was previously identified as part of a haplotype that was significantly related to follicular thyroid cancer compared to controls (39); however, the directionality was the opposite of what was observed in the present study. Another *CYP24A1* SNP of interest in the present work was *rs35051736*, for which there were no participants who were homozygous for the minor allele. This is perhaps not surprising given that this SNP is located two amino acids from another that was identified in a case-series to be associated with idiopathic infantile hypercalcemia (40). There was a complete absence of heterozygotes among participants with advanced adenomas, as well as among those with the component features of advanced lesions; namely, large or villous adenomas. In the polymorphic isotype of *rs35051736*, an uncharged glutamine is substituted for the original positively-charged arginine, resulting in possible changes in enzymatic activity. In prior work by our group, we employed site-directed mutagenesis to observe the functional effects of this SNP, and found a 31% reduction in activity of *CYP24A1* in colon cancer cell lines (23). Because this enzyme is responsible for commencing the catabolism of 1,25$(OH)_2$D, these results suggest that the presence of the *A* allele may result in greater concentrations of 1,25$(OH)_2$D at the cellular level (14, 15), which may in turn inhibit the development of colorectal neoplasia (16–19). Nonetheless, our results show that this finding did not extend to circulating blood concentrations of 1,25$(OH)_2$D, where no differences in this metabolite were observed in association by *rs35051736* genotype, nor did we find an association between blood levels of 1,25$(OH)_2$D and villous histology in a previously-conducted study within the same study population (21). These findings underscore the importance of further characterizing the differences in blood measurements of vitamin D metabolites compared to activity at the cellular level in relation to the development of cancer.

We also observed significant associations between two *CYP24A1* SNPs, *rs2296241* and *rs35051736*, and odds for recurrent adenomas with advanced features. In the present study, increasing copies of the *G* allele in *rs2296241* were related to increased odds for lesions with villous histology, a finding with similar directionality to another report showing that increasing copies of the *G* allele were associated with reduced overall survival among patients with head and neck cancers (41). In contrast, results of other studies have indicated that the presence of the *G* allele is protective, with reports showing that *rs2296241* heterozygotes were at reduced risk for oral cancer (42), and for prostate-cancer specific deaths (43).

We observed no associations between *CYP27B1* SNPs and concentrations of either 25(OH)D or 1,25$(OH)_2$D, in contrast to other published studies (26, 44–46). The *CYP27B1* SNP most commonly reported to have a significant relationship with 25(OH)D in prior epidemiological studies is *rs10877012* (26, 45, 46). However, the direction of the association between genotypes and 25(OH)D varied between the studies, the minor allele frequency is relatively rare, and limited or no functional data are available; therefore, this variant was not selected for the present study. In addition, a suggestive trend between the *CYP24A1* SNP *rs927650* and circulating concentrations of 25(OH)D was found which has not been reported previously, and this SNP was also related to colorectal adenoma recurrence. However, the alleles associated with higher 25(OH)D concentrations were also related to increased risk for colorectal adenoma recurrence, which was unexpected given well-documented inverse association between 25(OH)D and colorectal neoplasia. Nonetheless, our prior work has shown that there was no association between 25(OH)D and overall adenoma recurrence in our study population, which partially explain this apparently contradictory finding (47). A prior genome-wide association study conducted with approximately 30,000 participants reported an significant relationship between *rs6013897* and concentrations of 25(OH)D (24); however, we observed no associations for this SNP in our study population. Another investigation reported associations for *rs2244719, rs17219315*, and *rs2296241* (48), the latter of which was included in the current work; however, no relationship between this SNP and concentrations of either metabolite was observed. These findings highlight the potential differences in SNP/outcome associations that may be detected depending on the population under study; specifically, these prior reports have included participants from differing racial or ethnic groups, and/or with conditions such as diabetes or asthma.

In addition to the investigation of the main effects for *CYP24A1* and *CYP27B1* in adenoma recurrence and circulating vitamin D metabolite concentrations, interactions between these SNPs, 25(OH)D, and 1,25(OH)$_2$D in relation to adenoma recurrence were studied. Significant interactions between *CYP24A1 rs2296241* and 25(OH)D levels, and between *CYP24A1 rs2762939* and 1,25(OH)$_2$D, were observed. For *rs2296241*, the data indicated that the inverse association between 25(OH)D and overall colorectal adenoma recurrence was stronger among those carrying at least one *G* allele. However, these results are not consistent with the findings for this SNP in relation to villous adenoma recurrence, which showed that increasing copies of the *G* allele was related to increased odds for these advanced lesions. As noted above, statistically significant results for another SNP, *rs2762939* have been reported in several studies, but the directionality of any association remains inconsistent. We found that a modestly stronger inverse association between 1,25(OH)D and adenoma recurrence in the presence of at least on minor allele in *rs2762939*. This SNP has previously been reported to be associated with risk of non-Hodgkin lymphoma (49) and prostate cancer progression (43), though again there is inconsistency in the directionality of the associations.

There were both strengths and limitations to this study. Strengths include that this was the first investigation of *CYP27B1* and *CYP24A1* SNPs in relation to colorectal adenoma recurrence and was conducted in a large sample size that allowed for consideration of gene/environment interactions between specifically-selected polymorphisms and vitamin D metabolite concentrations. Limitations include the relative homogeneity of the study population with regard to race and ethnicity, particularly given the known variation in vitamin D metabolite concentrations and genotype frequencies by racial or ethnic group (50).

In summary, the findings of the present study suggest that the CYP24A1 SNP rs927650 may be related to both circulating concentrations of 25(OH)D as well as to odds of recurrent colorectal neoplasia. In addition, there were modest indications that at least one genetic variant with known functional effects (23) may be related to odds for the development of advanced colorectal adenomas. The rarity of this variant would require a larger study population in order to confirm any associations with confidence. Finally, the results of this work highlight the need to continue to clarify the differences between the results of epidemiological studies of circulating vitamin D metabolites and genetic variants, and experimental work at the cellular level.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Characteristics of the study population (n=1,188).

Characteristic	
Mean age, y ± SD	65.6 ± 8.5
Sex, Male, n (%)	811 (68.3%)
White, n (%)	1108 (93.3%)
Mean 25(OH)D (ng/ml ± SD)	27.7 ± 11.4
Mean 1,25$(OH)_2$D (pg/ml ± SD)	33.9 ± 11.3
Adenoma recurrence, n (%)	519 (43.7%)
Advanced adenoma recurrence, n (% of adenoma recurrence)	148 (28.6%)
Distal only recurrence, n (% of adenoma recurrence)	146 (28.1%)
Proximal only recurrence[1], n (% of adenoma recurrence)	238 (45.9%)
Both distal and proximal recurrence, n (% of adenoma recurrence)	135 (26.0%)

Table 2

Concentrations of vitamin D metabolites 25(OH)D (n=1,188) and 1,25(OH)$_2$D (n=828), by *CYP27B1* and *CYP24A1* genotypes.

SNP	25(OH)D (ng/ml; ± sd)	p-trend[1]	1,25(OH)$_2$D (pg/ml; ± sd)	p-trend[1]
CYP24A1				
rs6013905				
TT	27.5 ± 10.3		34.3 ± 11.8	
TC	28.7 ± 14.0		33.4 ± 10.6	
CC	25.1 ± 8.9	0.49	34.1 ± 12.3	0.37
rs 2585428				
GG	28.5 ± 14.1		34.1 ± 11.2	
AG	27.8 ± 10.1		33.9 ± 11.5	
AA	26.9 ± 10.2	0.09	33.8 ± 11.8	0.76
rs2296241				
AA	27.4 ± 9.8		33.7 ± 11.5	
AG	28.0 ± 12.9		34.1 ± 11.6	
GG	27.7 ± 10.5	0.69	34.3 ± 11.4	0.57
rs2762939				
GG	27.8 ± 12.2		33.9 ± 11.8	
CG	28.0 ± 10.5		34.4 ± 11.4	
CC	26.4 ± 9.5	0.64	32.6 ± 9.2	0.89
rs35051736				
GG	27.8 ± 11.5		34.0 ± 11.5	
GA	25.7 ± 8.9	0.49	33.7 ± 9.8	0.93
rs6022999				
AA	27.5 ± 9.8		33.6 ± 11.2	
AG	28.8 ± 14.4		34.4 ± 11.9	
GG	25.7 ± 8.5	0.73	35.3 ± 12.1	0.23
rs4809958				
TT	27.5 ± 10.2		34.1 ± 11.8	
GT	28.5 ± 13.9		33.5 ± 10.6	
GG	26.7 ±+ 9.3	0.32	34.3 ± 12.0	0.63
rs276942				
AA	27.8 ± 11.6		34.2 ± 11.4	
AG	27.1 ± 9.8		33.3 ± 12.0	
GG	22.7 ± 7.1	0.23	30.5 ± 12.0	0.31
rs927650				
CC	26.9 ± 8.5		33.8 ± 11.3	
TC	27.5 ± 12.8		33.9 ± 11.5	
TT	29.1 ± 11.3	0.02	34.3 ± 11.8	0.63
rs6013897				

SNP	25(OH)D (ng/ml; ± sd)	p-trend[1]	1,25(OH)$_2$D (pg/ml; ± sd)	p-trend[1]
TT	27.9 ± 12.1		33.8 ± 10.9	
AT	26.8 ± 8.8		33.9 ± 13.1	
AA	27.0 ± 9.1	0.21	36.4 ± 12.8	0.25
rs4809960				
TT	27.7 ± 10.4		34.0 ± 11.2	
TC	27.6 ± 13.2		33.8 ± 12.2	
CC	27.8 ± 9.6	0.97	34.1 ± 9.9	0.96
CYP27B1				
rs4646536				
TT	28.1 ± 12.8		33.6 ± 11.5	
CT	27.4 ± 10.0		34.2 ± 11.4	
CC	27.0 ± 9.9	0.22	34.4 ± 11.5	0.40

[1]P-trend calculated using regression modeling with categorical variables for each SNP included as a continuous variable.

Table 3

Adjusted[1] ORs (95% CIs) for the association between SNPs in *CYP27B1* and *CYP24A1*, overall colorectal adenoma recurrence, and advanced recurrence.

SNP	Any recurrence (n, %)	OR (95% CI)	Advanced recurrence (n, %)	OR (95% CI)
CYP24A1				
rs6013905				
TT	370 (43.8)	1.00	106 (12.6)	1.00
TC	159 (43.4)	0.97 (0.76–1.25)	47 (12.8)	1.01 (0.69–1.47)
CC	14 (43.8)	1.01 (0.49–2.07)	2 (6.3)	0.51 (0.12–2.22)
p-trend[2]		0.87		0.68
rs 2585428				
GG	193 (46.7)	1.00	56 (13.6)	1.00
AG	241 (42.6)	0.85 (0.66–1.10)	66 (11.7)	0.80 (0.54–1.19)
AA	112 (42.8)	0.86 (0.63–1.18)	35 (14.1)	0.93 (0.58–1.48)
p-trend		0.28		0.64
rs2296241				
AA	157 (42.4)	1.00	49 (13.3)	1.00
AG	237 (42.7)	1.01 (0.77–1.32)	58 (10.5)	0.79 (0.52–1.21)
GG	147 (47.3)	1.22 (0.90–1.66)	49 (15.8)	1.31 (0.84–2.04)
p-trend		0.21		0.27
rs2762939				
GG	319 (43.8)	1.00	94 (12.9)	1.00
CG	216 (46.6)	1.11 (0.88–1.40)	64 (13.8)	1.11 (0.78–1.59)
CC	25 (30.9)	0.59 (0.36–0.97)	5 (6.2)	0.40 (0.16–1.03)
p-trend		0.43		0.36
rs35051736				
GG	553 (43.9)	1.00	162 (12.8)	1.00
GA	9 (60.0)	1.98 (0.70–5.62)	0 (0.0)	--
p-value[3]		0.20		<0.001
rs6022999				
AA	348 (44.6)	1.00	101 (13.0)	1.00
AG	181 (44.3)	0.98 (0.77–1.25)	54 (13.2)	1.01 (0.70–1.46)
GG	28 (35.9)	0.70 (0.43–1.13)	6 (7.7)	0.52 (0.22–1.24)
p-trend		0.29		0.34
rs4809958				
TT	388 (44.6)	1.00	114 (13.1)	1.00
GT	159 (43.4)	0.95 (0.74–1.21)	45 (12.3)	0.91 (0.62–1.34)
GG	15 (44.1)	0.98 (0.49–1.96)	3 (8.8)	0.66 (0.19–2.29)
p-trend		0.70		0.48
rs276942				
AA	470 (43.4)	1.00	130 (12.0)	1.00

SNP	Any recurrence (n, %)	OR (95% CI)	Advanced recurrence (n, %)	OR (95% CI)
AG	64 (48.1)	1.19 (0.83–1.71)	20 (15.0)	1.36 (0.79–2.32)
GG	1 (12.5)	0.20 (0.02–1.66)	0 (0.00)	--
p-value[3]		0.92		0.37
rs927650				
CC	136 (39.2)	1.00	40 (11.5)	1.00
TC	274 (45.6)	1.30 (0.99–1.70)	74 (12.3)	1.19 (0.78–1.82)
TT	148 (47.1)	1.38 (1.01–1.89)	46 (14.7)	1.46 (0.91–2.34)
p-trend		0.04		0.11
rs6013897				
TT	430 (44.6)	1.00	122 (12.7)	1.00
AT	88 (41.9)	0.90 (0.66–1.22)	62 (12.4)	0.94 (0.59–1.50)
AA	29 (40.9)	0.85 (0.51–1.39)	19 (14.1)	1.02 (0.50–2.10)
p-trend		0.37		0.92
rs4809960				
TT	314 (43.5)	1.00	86 (11.9)	1.00
TC	203 (44.7)	1.04 (0.82–1.32)	61 (13.4)	1.14 (0.79–1.64)
CC	38 (44.7)	1.05 (0.67–1.66)	14 (16.5)	1.42 (0.75–2.70)
p-trend		0.72		0.25
CYP27B1				
rs4646536				
TT	268 (45.3)	1.00	80 (13.5)	1.00
CT	226 (42.4)	0.89 (0.70–1.13)	65 (12.2)	0.86 (0.60–1.24)
CC	60 (45.8)	1.04 (0.71–1.53)	15 (11.5)	0.88 (.48–1.61)
p-trend		0.75		0.47

[1] Models adjusted for age and sex.

[2] P-trend calculated using logistic regression modeling with categorical variables for each SNP included as a continuous variable.

[3] P-value calculated with multinomial logistic regression and computation of a z-score and a two-tailed z-test.

Table 4

Adjusted[1] ORs (95% CIs) for the association between SNPs in *CYP27B1* and *CYP24A1* and advanced features of recurrent colorectal adenomas.

SNP	Multiplicity ≥3 adenoma (n, %)	OR (95% CI)	Large size ≥1 cm (n, %)	OR (95% CI)	Villous histology[2] (n, %)	OR (95% CI)
CYP24A1						
rs6013905						
TT	173 (20.5)	1.00	71 (8.7)	1.00	59 (7.0)	1.00
TC	67 (18.3)	0.84 (0.61–1.06)	29 (7.9)	0.90 (0.58–1.42)	30 (8.2)	1.18 (0.75–1.86)
CC	7 (21.9)	1.10 (0.46–2.62)	1 (3.1)	0.35 (0.05–2.58)	2 (6.3)	0.89 (0.21–3.83)
p-trend[3]		0.46		0.36		0.62
rs 2585428						
GG	82 (19.9)	1.00	34 (8.2)	1.00	38 (9.2)	1.00
AG	111 (19.6)	0.99 (0.72–1.37)	45 (8.0)	0.97 (0.61–1.54)	40 (7.1)	0.75 (0.47–1.20)
AA	56 (21.4)	1.12 (0.76–1.65)	26 (9.9)	1.24 (0.73–2.13)	14 (5.4)	0.56 (0.30–1.06)
p-trend		0.60		0.48		0.06
rs2296241						
AA	70 (18.9)	1.00	37 (10.0)	1.00	21 (5.7)	1.00
AG	114 (20.5)	1.12 (0.80–1.56)	36 (6.5)	0.62 (0.39–1.01)	37 (6.7)	1.19 (0.68–2.06)
GG	63 (20.3)	1.10 (0.74–1.61)	31 (10.0)	1.00 (0.61–1.66)	22 (10.6)	1.98 (1.12–3.50)
p-trend		0.62		0.90		0.02
rs2762939						
GG	134 (18.4)	1.00	60 (8.2)	1.00	62 (8.5)	1.00
CG	107 (23.0)	1.32 (0.99–1.77)	48 (10.3)	1.28 (0.86–1.90)	29 (6.3)	0.71 (0.45–1.13)
CC	11 (13.6)	0.74 (0.38–1.44)	3 (3.7)	0.44 (0.13–1.43)	4 (4.9)	0.57 (0.20–1.62)
p-trend		0.49		0.94		0.10
rs35051736						
GG	250 (19.8)	1.00	109 (8.6)	1.00	94 (7.5)	1.00
GA	5 (33.3)	2.17 (0.72–6.52)	0 (0.0)	--	0 (0.0)	--
p-value[4]		0.17		0.27		0.23
rs6022999						
AA	158 (20.2)	1.00	68 (8.7)	1.00	63 (8.1)	1.00

SNP	Multiplicity ≥3 adenoma (n, %)	OR (95% CI)	Large size ≥1 cm (n, %)	OR (95% CI)	Villous histology[2] (n, %)	OR (95% CI)
AG	79 (19.3)	0.94 (0.69–1.27)	38 (9.3)	1.07 (0.71–1.63)	27 (6.6)	0.80 (0.50–1.28)
GG	16 (20.5)	1.01 (0.56–1.82)	4 (5.1)	0.57 (0.20–1.62)	2 (2.6)	0.30 (0.07–1.26)
p-trend		0.81		0.63		0.08
rs4809958						
TT	181 (20.8)	1.00	79 (9.1)	1.00	63 (7.2)	1.00
GT	68 (18.6)	0.85 (0.62–1.17)	29 (7.9)	0.86 (0.55–1.33)	28 (7.7)	1.05 (0.66–1.68)
GG	7 (20.6)	0.98 (0.42–2.13)	2 (5.9)	0.62 (0.15–2.64)	2 (5.9)	0.80 (0.19–3.40)
p-trend		0.41		0.37		0.99
rs276942						
AA	216 (19.9)	1.00	92 (8.5)	1.00	70 (6.5)	1.00
AG	27 (20.3)	0.97 (0.62–1.53)	7 (5.3)	0.60 (0.27–1.32)	18 (13.5)	2.27 (1.30–3.95)
GG	0.00	--	0 (0.0)	--	0 (0.0)	--
p-value[4]		0.59		0.13		0.02
rs927650						
CC	64 (18.4)	1.00	29 (8.4)	1.00	20 (5.8)	1.00
TC	128 (21.3)	1.19 (0.85–1.68)	46 (7.7)	0.91 (0.56–1.48)	44 (7.3)	1.29 (0.75–2.23)
TT	60 (19.1)	1.04 (0.70–1.55)	34 (10.8)	1.34 (0.79–2.25)	29 (9.2)	1.66 (0.92–3.01)
p-trend		0.82		0.27		0.09
rs6013897						
TT	196 (20.3)	1.00	82 (8.5)	1.00	72 (7.5)	1.00
AT	38 (18.1)	0.87 (0.59–1.28)	16 (7.6)	0.89 (0.51–1.56	14 (6.7)	0.89 (0.49–1.61)
AA	15 (21.3)	1.04 (0.57–1.89)	7 (9.9)	1.16 (0.51–2.61)	6 (8.5)	1.12 (0.47–2.69)
p-trend		0.76		0.97		0.98
rs4809960						
TT	141 (19.5)	1.00	56 (7.8)	1.00	53 (7.4)	1.00
TC	95 (20.9)	1.08 (0.81–1.45)	41 (9.0)	1.17 (0.77–1.78)	33 (7.3)	0.98 (0.62–1.54)
CC	16 (18.8)	0.96 (0.53–1.71)	11 (12.9)	1.78 (0.89–3.55)	7 (8.2)	1.14 (0.50–2.59)
p-trend		0.82		0.13		0.88
CYP27B1						
rs4646536						

SNP	**Multiplicity ≥3 adenoma (n, %)**	**OR (95% CI)**	**Large size ≥1 cm (n, %)**	**OR (95% CI)**	**Villous histology[2] (n, %)**	**OR (95% CI)**
TT	118 (19.9)	1.00	55 (9.3)	1.00	46 (7.8)	1.00
CT	111 (20.8)	1.06 (0.79–1.42)	44 (8.3)	0.88 (0.58–1.33)	36 (6.8)	0.86 (0.55–1.35)
CC	22 (16.8)	0.82 (0.49–1.36)	11 (8.4)	0.91 (0.46–1.80)	8 (6.1)	0.78 (0.36–1.70)
p-trend		0.72		0.62		0.43

[1]Models adjusted for age and sex.

[2]Villous histology was present if adenoma exhibited tubulovillous or villous histology (at least 25% villous).

[3]P-trend calculated using logistic regression modeling with categorical variables for each SNP included as a continuous variable.

[4]P-value calculated with multinomial logistic regression and computation of a z-score and a two-tailed z-test

Table 5

Adjusted[1] odds ratios (95% CIs) for *CYP24A1 rs2296241* and *rs2762939* genotype and colorectal adenoma recurrence, by vitamin D metabolite concentration.

	CYP24A1 rs2296241 **(OR, 95% CI)**		
25(OH)D (ng/ml)	*AA*	*AG*	*GG*
<12	1.00	1.00	1.00
≥12 and <20	0.84 (0.27–2.61)	0.23 (0.07–0.72)	0.13 (0.02–0.68)
≥ 20	0.78 (0.27–2.30)	0.21 (0.07–0.62)	0.18 (0.03–0.91)
p-trend	0.65	0.03	0.56
p-interaction			0.10
	CYP24A1 rs2762939 OR (95% CI)		
	GG	*GC*	*CC*
1,25(OH)$_2$D(pg/ml)			
< 28.3	1.00	1.00	1.00
≥ 28.3 and <38.2	0.71 (0.45–1.12)	0.64 (0.36–1.14)	0.62 (0.16–2.38)
≥ 38.2	0.98 (0.63–1.53)	0.54 (0.30–0.95)	0.49 (0.10–2.46)
p-trend	0.94	0.03	0.37
p-interaction			0.10

[1] Models adjusted for age and sex.