Carcinogenesis vol.31 no.8 pp.1412–1416, 2010
doi:10.1093/carcin/bgq127
Advance Access publication June 17, 2010

Genetic variation in the retinoid X receptor and calcium-sensing receptor and risk of colorectal cancer in the Colon Cancer Family Registry

Elizabeth T.Jacobs[1,2,*], Maria E.Martínez[1,2], Peter T.Campbell[3], David V.Conti[4], David Duggan[5], Jane C.Figueiredo[4], Robert W.Haile[4], Elizabeth C.LeRoy[1,2], Jenny N.Poynter[4,6], Patricia A.Thompson[1,2] and John A.Baron[7]

[1]Arizona Cancer Center, University of Arizona, Tucson, AZ, USA, [2]Mel and Enid Zuckerman Arizona College of Public Health, Arizona Cancer Center, University of Arizona, Tucson, AZ 85724-5024, USA, [3]Epidemiology Research Program, American Cancer Society, Atlanta, GA, USA, [4]Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA, [5]Translational Genomics Research Institute, Phoenix, AZ, USA, [6]Division of Pediatric Epidemiology and Clinical Research, University of Minnesota, Minneapolis, MN, USA and [7]Departments of Medicine and Community and Family Medicine, Dartmouth Medical School, Hanover, NH, USA

[*]To whom correspondence should be addressed. Tel: +520 626 0341; Fax: +520 626 9275;
Email: jacobse@u.arizona.edu

Genetic variants in the calcium/vitamin D metabolic pathway may be related to risk for colorectal cancer. While several investigations of vitamin D receptor (*VDR*) polymorphisms and colorectal cancer have been conducted, no studies to date have evaluated the association of genetic variation in the heterodimer partner for VDR, the retinoid X receptor (RXR). Another important gene in this pathway is the calcium-sensing receptor (*CASR*). Employing a discordant-sibship case–control design, we examined the association between single nucleotide polymorphisms (SNPs) in *RXRA* and *CASR* and risk for colorectal cancer overall and by colorectal subsite and microsatellite instability (MSI) status using data from the Colon Cancer Family Registry. No gene-level relationships between *RXRA* or *CASR* and colorectal cancer overall were observed. However, for *RXRA* SNP rs7861779, a high-interest SNP selected for study *a priori*, there was a statistically significantly increased risk for proximal colorectal cancer among those with at least one *A* allele [odds ratio (OR) = 1.42; 95% confidence interval (CI) = 1.03–1.97]. Another selected *RXRA* SNP, rs12004589, was significantly associated with risk of MSI-high cancers (OR = 2.27; 95% CI = 1.13–4.56). Additionally, *CASR* SNP rs1801726 was significantly associated with a reduced risk for rectal cancer (OR = 0.53; 95% CI = 0.29–0.96). These results provide support that *RXRA* SNPs rs7861779 and rs12004589 and *CASR* SNP rs1801726 may be important markers for colorectal neoplasia. Further work is needed to elucidate their role in the carcinogenic pathway.

Introduction

Recently, the relationship between calcium, vitamin D, and colorectal cancer has garnered a great deal of attention (1–3), and genetic variation in metabolic pathways for these nutrients may play a role in colorectal carcinogenesis (3). Within the context of the Colon Cancer Family Registry, an investigation of two key genes in this pathway, the vitamin D receptor (*VDR*) and the vitamin D binding protein (*GC*), has recently been completed, and no main effects for these genes were reported (4). However, there are several other key genes in the calcium/vitamin D pathway that may be of importance, including the retinoid X receptor (*RXR*) and the calcium-sensing receptor (*CASR*) (3).

The RXR is part of the steroid nuclear receptor super-family, the members of which have been identified as potential targets for the prevention and treatment of several cancers (5–7); its ligands include 9-*cis* retinoic acid and an array of RXR-specific ligands called rexinoids (8). RXR can form homodimers or heterodimerize with other nuclear receptors, including the VDR and the peroxisome proliferator-activator receptor (PPAR) (9,10). Previous work from a study of colorectal adenoma demonstrated that genetic variation in *RXRA*, an isoform of *RXR*, was associated with the risk of colorectal adenoma recurrence (11); however, to our knowledge, there are currently no studies of polymorphisms in *RXRA* and risk for colorectal cancer.

Another gene of interest in this pathway is the *CASR*. In the promoter region of *CASR*, vitamin D response elements are present (12), providing support for mechanistic interactions between vitamin D and calcium. *CASR* may also independently have a role in colorectal carcinogenesis (3,13). Higher intake of calcium has been associated with reduced risk for colorectal neoplasms (2,14), and the CASR has a critical role in the maintenance of calcium homeostasis (15). Expression of this receptor has been shown to be reduced in colon cancer cells, and this decrease may result in the inability of these cells to utilize calcium in a protective manner (15). Genetic variation in *CASR* has been shown to be related to risk of colon cancer (16), particularly of the proximal colon (13), and advanced stage rectal cancer (17). However, to date, no studies have evaluated the association between genetic variation in *RXR* and *CASR* and colorectal cancer by microsatellite instability (MSI) status. The purpose of this study was therefore to comprehensively evaluate the relationship between genetic variation in *RXRA* and *CASR* and colorectal cancer within participants from the Colon Cancer Family Registry and to assess whether these relationships varied by colorectal subsite or by MSI status.

Abbreviations: *CASR*, calcium-sensing receptor; CI, confidence interval; LD, linkage disequilibrium; MSI, microsatellite instability; OR, odds ratio; PC, principal component; PPAR, peroxisome proliferator-activator receptor; *RXR*, retinoid X receptor; SNP, single nucleotide polymorphism; *VDR*, vitamin D receptor.

Materials and methods

Study population

Participants were men and women drawn from the Colon Cancer Family Registry, as described in detail elsewhere (18). The Colon Cancer Family Registry includes six recruitment centers: Cancer Care Ontario (Toronto, Canada), Fred Hutchinson Cancer Research Center (Seattle, WA, USA), Mayo Clinic (Rochester, MN, USA), University of Hawaii (Honolulu, HI, USA), University of Melbourne (Melbourne, Australia) and the University of Southern California Consortium (Los Angeles, CA, USA). The Colon Cancer Family Registry included both population-based and clinic-based recruitment, with the former conducted via cancer registries and the latter identified at high-risk cancer clinics (18). In the current work, we present data from the population-based participants only. Proband cases were enrolled in the study and subsequently asked to assist with recruitment of relatives into the registry as either additional cases or controls (18). Self-administered questionnaires or interviews were employed for ascertainment of such information as demographics, race and ethnicity, personal and familial history of cancer, medical history, reproductive history, physical activity and use of alcohol and tobacco (18). Informed consent from all study participants was obtained at each study center, and the protocol was approved at the individual Colon Cancer Family Registry sites.

Assessment of MSI

A total of 10 markers were employed for evaluation of MSI status for colorectal cancer cases (ACTC, BAT25, BAT26, BAT40, BAT34C4, D5S346, D17S250, D18S55, D10S197 and MYCL). Cases were classified as MS stable, MSI low and MSI high if 0% of loci were unstable, >0 to <30% of loci were unstable or ≥30% of loci were unstable, respectively (18,19). MSI status was assigned only in those cases with a valid result for at least four markers (18,19).

Genotyping

Genotyping was performed on the Illumina Golden Gate platform (Illumina®, San Diego, CA, USA). Briefly, DNA was activated with streptavidin/biotin and

added to a hybridization mixture. After hybridization, the samples were washed, followed by extension, ligation and cleanup. Universal primers labeled with cyanine3 or cyanine5 were utilized for polymerase chain reaction labeling of the DNA. The labeled DNA was then allowed to hybridize with the Sentrix Array Matrix and placed in the BeadArray Reader for quantification of the fluorescence signal. Analysis of the output from the fluorescence reading was managed using Bead Studio software (Illumina®).

Single nucleotide polymorphism (SNP) selection was performed using Haploview Tagger to identify bin tags from a Caucasian European (CEU) population. Initial tag SNPs and linkage disequilibrium (LD) blocks were identified from HapMap data release #16c.1, June 2005, on NCBI B34 assembly, dbSNP b124. Tag SNPs from these data were identified utilizing the following criteria: minor allele frequency $> 5\%$; pairwise $r^2 > 0.95$ and at least 60 bp distance between neighboring SNPs (20,21). SNPs located at the 5′ and 3′ ends of an LD block were also included. SNPs with little or no LD were selected from HapMap or dbSNP at a density of 1 per kb. SNPs were considered to have failed genotyping if they met at least one of the following: Illumina GenTrain Score < 0.4, 10% GC score < 0.25, AB T Dev > 0.1239, call frequency < 0.95, intra-plate replicate errors > 2, parent–parent–child errors > 2 or discordance with HapMap > 3. Participants were genotyped for a total of 41 SNPs in *RXRA* and 40 SNPs in *CASR*. Prior to the statistical analysis, all SNPs that failed the above criteria or were monomorphic were excluded from the data set (*RXRA* = 12; *CASR* = 4), leaving a total of 29 SNPs in *RXRA* and 36 SNPs in *CASR* for inclusion in the final analyses.

Statistical analyses

A discordant-sibship case–control study design was employed for the current work, which was restricted to the population-based participants. Any probands or their relatives who were diagnosed with invasive colorectal cancer were counted as cases, whereas unaffected siblings with no personal history of colorectal cancer were controls. All participants from the population-based Colon Cancer Family Registry who had available genetic data for SNPs in *RXRA* and *CASR* were included in the current analyses, yielding a total of 1802 cases and 2874 controls.

Principal components (PCs) analysis was employed to evaluate the overall association between variants in each gene and risk of colorectal cancer. First, PCs were computed as uncorrelated linear combinations of the original SNP data. These PCs are computed such that the first PC has maximal variance, the second maximizes the remaining variance and so forth. The PCs were modeled using conditional logistic regression with the unique sibship identifier as the matching factor, utilizing an 80% explained-variance threshold in determining how many PCs to include in the models (22). A *P*-value for the overall gene-outcome association was obtained from a likelihood ratio test comparing the model with PCs versus a model containing only age and sex, with degrees of freedom equal to the number of PCs. The data were further explored using PC analysis through stratification by cancer location (proximal, distal and rectal) and MSI status (low/stable and high). Additionally, *a priori*, we selected two high-interest SNPs in *RXRA* [rs7861779 (11) and rs12004589 (11)] and four high-interest SNPs in *CASR* [rs1042636 (13), rs12485716 (13), rs1801725 (16,17) and rs4678174 (13)] for analysis using a log-additive model. These SNPs were specifically chosen because they or an SNP with high LD were reported in the literature to have a statistically significant association with colorectal neoplasia (11,13,16,17). Single-SNP analyses were then performed on the remaining SNPs considering additive, recessive and dominant modes of inheritance. To ensure a correct type I error rate, we adjusted for the multiple correlated tests using the approach presented by Conneely and Boehnke (23). Variables examined for potential confounding included age, sex, body mass index, physical activity and baseline tumor site; however, none changed the model by 10% or greater (24). All models were therefore adjusted for age and sex only.

Results

Table I presents selected characteristics of the participants with genotype data in the Colon Cancer Family Registry. A total of 1747 population-based sibships were included in the current work. Data for MSI status were available for a total of 1182 of the cases, with 9.8% classified as MSI high, 8.4% as MS low, 47.5% as MS stable (Table I). There were no material differences in age, race, body mass index or physical activity between the cases and controls; however, a larger proportion of cases were men.

Results for PCs analyses conducted to evaluate whether there was an overall gene-level relationship between *RXRA* or *CASR* and colorectal cancer are presented in Table II, along with results for pre-selected *RXRA* and *CASR* SNPs. There were no significant gene-level associations between *RXRA* and *CASR* and colorectal cancer overall nor were there any single-SNP associations for either gene among SNPs selected *a priori* (Table II) or in an analysis of all single SNPs in each gene (data not shown).

Table I. Selected characteristics of cases and sibling controls from population-based participants in the Colon Cancer Family Registry

	Cases (n = 1802)	Sibling controls (n = 2874)
Person characteristic		
Mean age ± SD	53.5 ± 10.9	54.0 ± 11.8
Sex, no. (%)		
Male	924 (51.3)	1278 (44.5)
Female	878 (48.7)	1596 (55.5)
Race, no (%)		
Non-hispanic white	1576 (87.5)	2507 (87.2)
Black	32 (1.8)	42 (1.5)
Asian	69 (3.8)	113 (3.9)
Other[a]	104 (5.8)	189 (6.6)
Unknown/missing	21 (1.2)	23 (0.8)
BMI (kg/m^2)[b]		
15–18 (underweight)	22 (1.2)	25 (0.9)
18–25 (normal)	628 (34.9)	1153 (40.1)
25–30 (overweight)	667 (37.0)	1035 (36.0)
30+ (obese)	422 (23.4)	592 (20.6)
Unknown/missing	63 (3.5)	69 (2.4)
Physical activity (MET hours)[c]		
0–6 (inactive)	438 (24.3)	666 (23.2)
6.1–20 (less active)	491 (27.3)	776 (27.0)
20.1–44 (active)	412 (22.9)	641 (22.3)
44+ (very active)	377 (20.9)	631 (22.0)
Unknown/missing	84 (4.7)	160 (5.6)
Tumor characteristics		
Site, no. (%)		
Right colon	595 (33.0)	—
Left colon	525 (29.1)	
Rectum	593 (32.9)	
Unknown/missing	89 (4.9)	
MSI, no. (%)[d]		
MSS	855 (47.5)	—
MSI-L	151 (8.4)	
MSI-H	176 (9.8)	
Unknown/missing	620 (34.4)	

MET, metabolic equivalent; MSS, microsatellite stable.
[a]Includes individuals who self-identified themselves as hispanic, native, Hawaiian/Pacific Islander and mixed race.
[b]Self-reported weight and height 2 years prior to questionnaire completion date used to calculate body mass index (BMI).
[c]Average weekly total lifetime MET hours.
[d]Not all individuals were tested for MSI.

Table III presents findings for PCs analyses by colorectal subsite. No significant gene-level relationships for *RXRA* or *CASR* and proximal, distal or rectal cancers were observed. However, in analyses of single SNPs selected *a priori*, for *RXRA* SNP rs7861779, there was a statistically significant relationship with proximal colon cancer [odds ratio (OR) = 1.42; 95% confidence interval (CI) = 1.03–1.97; $P = 0.03$]. Additionally, *CASR* SNP rs1801726 was statistically significantly related to reduced risk for rectal cancer (OR = 0.53; 95% CI = 0.29–0.96). When conducting the analyses of all SNPs in *CASR* and after adjusting for multiple comparisons, one SNP (rs17203502) was statistically significantly related to proximal colorectal cancer in the recessive inheritance model (OR = 0.55; 95% CI = 0.40–0.78; supplementary Table I is available at *Carcinogenesis* Online), whereas no SNPs in *RXRA* were significantly associated at any colorectal subsite (supplementary Table II is available at *Carcinogenesis* Online). As shown in Table IV, there were no significant gene-level relationships between *RXRA* and *CASR* and risk for colorectal cancer by MSI status, though one of the *RXRA* SNPs selected *a priori* (rs12004589) was associated with MSI-high cancers (OR = 2.27;

95% CI = 1.13–4.56). No significant relationships for any of the pre-selected *CASR* SNPs were observed by tumor MSI status; further, after analyses of all available SNPs in *RXRA* and *CASR* and adjustment for multiple comparisons, no statistically significant associations were observed by MSI status (supplementary Tables III and IV are available at *Carcinogenesis* Online).

Table II. Association between *RXRA* and *CASR* and colorectal cancer in population-based participants in the Colon Cancer Family Registry

	OR[a]	95% CI	
RXRA			
PC1	1.00	0.97–1.04	
PC2	1.00	0.92–1.08	
PC3	1.00	0.92–1.10	
PC4[b]	1.00	0.88–1.13	
LRT *P*-value[c]			1.00
CASR			
PC1	1.00	0.95–1.05	
PC2	0.99	0.93–1.05	
PC3	0.96	0.89–1.02	
PC4	1.08	1.00–1.17	
PC5[b]	1.04	0.95–1.14	
LRT *P*-value[c]			0.21
RXRA SNPs[d]			
rs7861779	0.98	0.82–1.18	
rs12004589	0.99	0.82–1.21	
CASR SNPs[d]			
rs1042636	0.90	0.73–1.10	
rs12485716	0.95	0.82–1.08	
rs1801726	0.86	0.62–1.18	
rs4678174	0.92	0.81–1.05	

[a]Adjusted for age and sex and matched for center and race.
[b]An 80% explained-variance threshold is used for including PCs 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 PCs.
[d]ORs were estimated assuming a log-additive model.

Discussion

The results of the current work do not provide support for a gene-level relationship between *RXRA* and *CASR* and risk for colorectal cancer. However, the associations observed for one *CASR* SNP and two *RXRA* SNPs selected *a priori* are of interest. Carriage of at least one *A* allele at *RXRA* rs7861779 was shown to be associated with higher risk of proximal colorectal cancer, whereas the presence of the *A* allele at *RXRA* rs12004589 was linked with higher risk of MSI-high cancers. Additionally, the high-interest *CASR* SNP rs1801726 was significantly related to reduced risk of rectal cancer.

While the study of genetic variation in nuclear receptors has most often focused on the heterodimer partners of RXR such as VDR and PPAR, evidence supports a role for RXR itself in carcinogenesis. Ligands for RXRα, an isoform expressed in the colonic epithelium, enhance the interaction between RXR and β-catenin and induce β-catenin degradation (25,26). In combination with PPAR ligands, RXR agonists act synergistically to inhibit growth in colon cancer cell lines (27). RXR ligands also alter the cellular response to the VDR ligand $1,25(OH)_2D_3$. In Caco-2 cells, the addition of RXR agonists increased the proliferative response, whereas in HT-29 cells, the response was blocked (28). Hence, experimental work indicates that RXR has an active role in carcinogenesis, and this role may vary by a complex network of potential heterodimer partner selections.

In previous work, carriage of at least one *A* allele for *RXRA* rs7861779 was shown to be associated with reduced odds of proximal colorectal adenoma (11), whereas the results of the current work show increased risk of proximal colorectal cancer with presence of at least one of these alleles. Any mechanism for explaining why *RXRA* would have different roles in adenoma as compared with cancer is purely speculative, particularly as rs7861779 is located within an intron. Although intronic SNPs can potentially affect alternative splicing of RNA (29–31), it is possible that this SNP is in LD with a site of genetic variation, which exerts a conformational change in the resultant protein, which in turn has biological effects. If this were the case, the functional change might be related to the capacity of RXRα to heterodimerize with specific nuclear receptor partners such as VDR and PPAR, both of which are of interest to colorectal carcinogenesis. The RXR/PPAR heterodimer is permissive and therefore can be

Table III. Association between *RXRA* and *CASR* and colorectal cancer in population-based families in the Colon Cancer Family Registry

	Proximal colon cancer (cases = 595)			Distal colorectal cancer (cases = 525)			Rectal cancer (cases = 593)		
	OR[a]	95% CI		OR[a]	95% CI		OR[a]	95% CI	
RXRA									
PC1	1.00	0.94–1.07		1.01	0.94–1.08		0.99	0.93–1.06	
PC2	0.83	0.74–0.98		1.17	1.01–1.35		1.04	0.91–1.20	
PC3	0.98	0.84–1.15		0.90	0.76–1.06		1.07	0.91–1.26	
PC4[b]	1.07	0.87–1.32		0.94	0.84–1.35		0.86	0.68–1.10	
LRT *P*-value[c]			0.23			0.20			0.65
CASR									
PC1	0.99	0.91–1.07		0.99	0.91–1.09		1.03	0.94–1.12	
PC2	1.05	0.94–1.16		1.01	0.90–1.12		0.92	0.82–1.02	
PC3	0.89	0.79–1.00		1.03	0.92–1.16		0.98	0.88–1.10	
PC4	1.13	0.99–1.29		1.07	0.93–1.23		1.04	0.90–1.21	
PC5[b]	1.12	0.96–1.31		1.06	0.90–1.25		0.96	0.82–1.11	
LRT *P*-value[c]			0.07			0.92			0.61
RXRA SNPs[d]									
rs7861779	1.42	1.03–1.97		0.79	0.57–1.10		0.87	0.64–1.19	
rs12004589	1.16	0.83–1.63		0.76	0.53–1.09		1.03	0.74–1.44	
CASR SNPs[d]									
rs1042636	0.83	0.58–1.19		0.90	0.62–1.30		0.97	0.68–1.39	
rs12485716	1.01	0.80–1.29		0.93	0.72–1.20		0.93	0.73–1.18	
rs1801726	0.86	0.49–1.51		1.21	0.69–2.14		0.53	0.29–0.96	
rs4678174	0.99	0.78–1.25		0.97	0.76–1.23		0.84	0.67–1.06	

[a]Adjusted for age and sex and matched for center and race.
[b]An 80% explained-variance threshold is used for including PCs 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 PCs.
[d]ORs were estimated assuming a log-additive model.

Table IV. Association between *RXRA* and *CASR* and colorectal cancer in the Colon Cancer Family Registry

	MSI low/stable (cases = 1006)			MSI high (cases = 176)		
	OR[a]	95% CI		OR[a]	95% CI	
RXRA						
PC1	1.02	0.97–1.07		0.93	0.82–1.05	
PC2	1.01	0.92–1.12		0.93	0.72–1.21	
PC3	0.97	0.86–1.09		0.96	0.71–1.30	
PC4[b]	1.04	0.88–1.24		1.22	0.84–1.78	
LRT *P*-value[c]			0.86			0.61
CASR						
PC1	1.03	0.97–1.10		0.97	0.83–1.14	
PC2	0.98	0.91–1.07		1.01	0.84–1.22	
PC3	0.97	0.89–1.06		1.02	0.83–1.24	
PC4	0.99	0.90–1.10		1.16	0.89–1.51	
PC5[b]	1.03	0.91–1.15		0.90	0.67–1.21	
LRT *P*-value[c]			0.91			0.85
RXRA SNPs[d]						
rs7861779	0.95	0.75–1.20		1.30	0.73–2.30	
rs12004589	0.90	0.70–1.16		2.27	1.13–4.56	
CASR SNPs[d]						
rs1042636	0.82	0.63–1.07		1.27	0.66–2.43	
rs12485716	1.03	0.86–1.23		0.86	0.58–1.30	
rs1801726	0.91	0.60–1.39		1.20	0.32–4.52	
rs4678174	1.01	0.85–1.21		0.88	0.59–1.32	

[a]Adjusted for age and sex and matched for center and race.
[b]An 80% explained-variance threshold is used for including PCs 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 PCs.
[d]ORs were estimated assuming a log-additive model.

activated by ligands for either receptor; provision of ligands simultaneously exerts a synergistic effect of cell growth inhibition and apoptosis induction above the activity of the ligands alone (32). If the structure of RXRα was changed such that it exhibited decreased affinity for PPAR heterodimerization and/or preference for an alternate receptor, this might lead to cancer progression. In turn, the same conformational change might be protective from the formation of new adenomas in the colorectum by increasing the affinity of RXR for VDR and thus activation of the latter, which may have a critical role in the maintenance of normal colon tissue after removal of adenomas (33,34). Overall, while the results of the present study were contrary to what was expected based on prior work involving adenomas, the repeated finding of an association for this particular SNP and proximal colorectal neoplasia indicates that it may be marking a region of *RXRA* that has the potential for activity in proximal colon carcinogenesis.

While it is unclear as to why *RXRA* rs7861779 has been found to be associated with proximal and not distal lesions, neoplasia of these colorectal subsites may represent biologically disparate pathologies with potentially distinct genetic etiologies (35). PPARγ, one isoform of an *RXRA* heterodimer partner, exhibits different patterns of expression in the proximal and distal colon and regulates different genes within each colorectal subsite (36). In addition, chromosomal instability is more often described in the distal region, and genetic instability and MSI are more often found in proximal neoplasia (37). Interestingly, an *RXRA* SNP in strong LD with rs7861779 and rs12004589 was significantly associated with MSI-high lesions in the current work. MSI is associated with defective mismatch repair (38), most often because of methylation of mutL humolog 1, a mismatch repair protein (19). Previous work has shown that RXR interacts with the T:G mismatch-specific thymine-DNA glycolylase, which has a critical role in initiating the repair of methylated DNA (39). Though the potential mechanism of action is currently unknown, this finding provides more evidence that these SNPs may be marking a functional change that affects colorectal neoplasia of a particular biological pathway, possibly involving MSI.

With regard to *CASR*, many epidemiological studies have reported that calcium intake is related to a reduced risk for colorectal neoplasia (2). In a randomized, placebo-controlled intervention trial, Baron *et al.* (14) demonstrated that daily supplementation with calcium carbonate significantly reduced the risk of adenoma recurrence compared with the placebo group. The CASR is critical for maintenance of circulating calcium concentrations. This receptor is present in colon epithelial tissue, but in colon cancer, this expression is decreased (15). The promoter region of *CASR* contains vitamin D response elements (12), supporting a mechanistic relationship between vitamin D and calcium, and genetic variation in *CASR* may increase or reduce calcium absorption and therefore have implications in colorectal carcinogenesis (3). There have been several prior studies of variation in this gene and risk for colorectal cancers. In work by Dong *et al.* (13), the authors reported that whereas there were no associations with colorectal cancer overall, several *CASR* SNPs were significantly associated with proximal lesions (rs10934578, rs12485716, rs4678174 and rs2270916); while in the current work, these SNPs were not related to colorectal cancer. Another high-interest SNP in *CASR* is rs1801725; two studies reported a significant link with colorectal cancer (16,17), whereas others found no relationship (13,40,41). Specifically, Speer *et al.* (17) reported an association between this SNP and more advanced rectal tumors, and the results of the current work also reveal a significant association for this SNP that was observed only with rectal cancers. When conducting analyses of all *CASR* SNPs and adjusting for multiple comparisons, rs17203502 was significantly associated with risk for proximal colorectal cancer for the recessive mode of inheritance (supplementary Table I is available at *Carcinogenesis* Online). In another study of colorectal cancer, this intronic SNP was not associated with any cancer or proximal lesions; however, in that work, only one mode of inheritance was investigated (13).

Whereas there are several published reports for *CASR* and colorectal neoplasia, to our knowledge only one study, discussed above (11), has evaluated association of *RXRA* and colorectal lesions. However, there are two published papers investigating *RXRA* and other cancer sites. In one study, genetic variation in *RXRA* and risk for prostate cancer was investigated and no relationship was observed (42), though only 11 SNPs in *RXRA* were included in the work. In another study, no significant associations between two *RXRA* SNPs (rs1536475 and rs1805343) and biliary tract cancers were detected (43); these SNPs were not significantly related to colorectal cancer risk in the current work, nor with metachronous colorectal adenoma in another study (11).

A limitation of this work includes the lack of statistical power for investigating racial or ethnic differences in the reported associations. Because of relatively low numbers of non-white study participants, we could not conduct robust stratified analyses. Given the strong relationship between vitamin D and race/ethnicity, replication of these findings in a more diverse population is necessary.

In summary, the current work provides support for a role of *CASR* and *RXRA* in the development of colorectal cancer. The results for *CASR* support prior findings of a possible role in rectal cancers specifically, whereas those for *RXRA* provide further evidence of an important functional role in the proximal colon, which may be related in part to tumor MSI status.

Supplementary material

Supplementary Tables I–IV can be found at http://carcin.oxfordjournals.org/

Funding

National Cancer Institute, National Institutes of Health under RFA # CA-95-011 and through cooperative agreements with the Australasian Colorectal Cancer Family Registry (U01 CA097735); University of Southern California Familial Colorectal Neoplasia

Collaborative Group (U01 CA074799); Mayo Clinic Cooperative Family Registry for Colon Cancer Studies (U01 CA074800); Ontario Registry for Studies of Familial Colorectal Cancer (U01 CA074783); Seattle Colorectal Cancer Family Registry (U01 CA074794); University of Hawaii Colorectal Cancer Family Registry (U01 CA074806); National Cancer Institute grants (CA-41108, CA-23074, CA-77145); Specialized Program of Research Excellence in Gastrointestinal Cancer (CA95060); Career Development Awards from the National Cancer Institute (1K07CA10629-01A1 to E.T.J., T32 CA009142 to J.N.P.).

Acknowledgements

Conflict of Interest Statement: None declared.

References

1. Davis,C.D. (2008) Vitamin D and cancer: current dilemmas and future research needs. *Am. J. Clin. Nutr.*, **88**, 565S–569S.
2. Holt,P.R. (2008) New insights into calcium, dairy and colon cancer. *World J. Gastroenterol.*, **14**, 4429–4433.
3. Jacobs,E.T. *et al*. (2005) Vitamin D activity and colorectal neoplasia: a pathway approach to epidemiologic studies. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 2061–2063.
4. Poynter,J.N.J. *et al*. Genetic variation in the vitamin D receptor (VDR) and the vitamin D binding protein (GC) and risk of colorectal cancer: results from the Colon Cancer Family Registry. *Cancer Epidemiol. Biomarkers Prev.*, **19**, 525–536.
5. Fan,Y.Y. *et al*. (2003) Chemopreventive n-3 fatty acids activate RXRalpha in colonocytes. *Carcinogenesis*, **24**, 1541–1548.
6. Albrechtsson,E. *et al*. (2002) The expression of retinoic acid receptors and the effects *in vitro* by retinoids in human pancreatic cancer cell lines. *Pancreas*, **25**, 49–56.
7. Jiang,S.Y. *et al*. (1999) Expression of nuclear retinoid receptors in normal, premalignant and malignant gastric tissues determined by *in situ* hybridization. *Br. J. Cancer*, **80**, 206–214.
8. Howe,L.R. (2007) Rexinoids and breast cancer prevention. *Clin. Cancer Res.*, **13**, 5983–5987.
9. Chawla,A. *et al*. (2001) Nuclear receptors and lipid physiology: opening the X-files. *Science*, **294**, 1866–1870.
10. Haussler,M.R. *et al*. (2008) Vitamin D receptor: molecular signaling and actions of nutritional ligands in disease prevention. *Nutr. Rev.*, **66**, S98–S112.
11. Egan,J.B.T. *et al*. Genetic variation in VDR and RXR and risk for colorectal adenoma recurrence. *Cancer Res.*, **70**, 1496–1504.
12. Canaff,L. *et al*. (2002) Human calcium-sensing receptor gene. Vitamin D response elements in promoters P1 and P2 confer transcriptional responsiveness to 1,25-dihydroxyvitamin D. *J. Biol. Chem.*, **277**, 30337–30350.
13. Dong,L.M. *et al*. (2008) Genetic variation in calcium-sensing receptor and risk for colon cancer. *Cancer Epidemiol. Biomarkers Prev.*, **17**, 2755–2765.
14. Baron,J.A. *et al*. (1999) Calcium supplements for the prevention of colorectal adenomas. *N. Engl. J. Med.*, **340**, 101–107.
15. Saidak,Z. *et al*. (2009) The role of the calcium-sensing receptor in the development and progression of cancer. *Endocr. Rev.*, **30**, 178–195.
16. Bacsi,K. *et al*. (2008) 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*, **8**, 317.
17. Speer,G. *et al*. (2002) Calcium-sensing receptor A986S polymorphism in human rectal cancer. *Int. J. Colorectal Dis.*, **17**, 20–24.
18. Newcomb,P.A. *et al*. (2007) Colon Cancer Family Registry: an international resource for studies of the genetic epidemiology of colon cancer. *Cancer Epidemiol. Biomarkers Prev.*, **16**, 2331–2343.
19. Poynter,J.N. *et al*. (2008) Molecular characterization of MSI-H colorectal cancer by MLHI promoter methylation, immunohistochemistry, and mismatch repair germline mutation screening. *Cancer Epidemiol. Biomarkers Prev.*, **17**, 3208–3215.
20. (2003) The International HapMap Project. *Nature*, **426**, 789–796.
21. de Bakker,P.I. *et al*. (2005) Efficiency and power in genetic association studies. *Nat. Genet.*, **37**, 1217–1223.
22. Gauderman,W.J. *et al*. (2007) Testing association between disease and multiple SNPs in a candidate gene. *Genet. Epidemiol.*, **31**, 383–395.
23. Conneely,K.N. *et al*. (2007) So many correlated tests, so little time! rapid adjustment of P values for multiple correlated tests. *Am. J. Hum. Genet.*, **81**, 1158–1168.
24. Mickey,R.M. *et al*. (1989) The impact of confounder selection criteria on effect estimation. *Am. J. Epidemiol.*, **129**, 125–137.
25. Xiao,J.H. *et al*. (2003) Adenomatous polyposis coli (APC)-independent regulation of beta-catenin degradation via a retinoid X receptor-mediated pathway. *J. Biol. Chem.*, **278**, 29954–29962.
26. Dillard,A.C. *et al*. (2008) Retinol increases beta-catenin-RXRalpha binding leading to the increased proteasomal degradation of beta-catenin and RXRalpha. *Nutr. Cancer*, **60**, 97–108.
27. Cesario,R.M. *et al*. (2006) Differentiation and growth inhibition mediated via the RXR:PPARgamma heterodimer in colon cancer. *Cancer Lett.*, **240**, 225–233.
28. Kane,K.F. *et al*. (1996) Antiproliferative responses to two human colon cancer cell lines to vitamin D3 are differently modified by 9-cis-retinoic acid. *Cancer Res.*, **56**, 623–632.
29. ElSharawy,A. *et al*. (2006) SNPSplicer: systematic analysis of SNP-dependent splicing in genotyped cDNAs. *Hum. Mutat.*, **27**, 1129–1134.
30. Fujimaru,M. *et al*. (1998) Two mutations remote from an exon/intron junction in the beta-hexosaminidase beta-subunit gene affect 3'-splice site selection and cause Sandhoff disease. *Hum. Genet.*, **103**, 462–469.
31. Webb,K.E. *et al*. (2003) The 4830C>A polymorphism within intron 5 affects the pattern of alternative splicing occurring within exon 6 of the thrombopoietin gene. *Exp. Hematol.*, **31**, 488–494.
32. Yamazaki,K. *et al*. (2007) Synergistic effects of RXR alpha and PPAR gamma ligands to inhibit growth in human colon cancer cells—phosphorylated RXR alpha is a critical target for colon cancer management. *Gut*, **56**, 1557–1563.
33. Fedirko,V. *et al*. (2009) Effects of vitamin d and calcium on proliferation and differentiation in normal colon mucosa: a randomized clinical trial. *Cancer Epidemiol. Biomarkers Prev.*, **18**, 2933–2941.
34. Fedirko,V. *et al*. (2009) Effects of vitamin D and calcium supplementation on markers of apoptosis in normal colon mucosa: a randomized, double-blind, placebo-controlled clinical trial. *Cancer Prev. Res*, **2**, 213–223.
35. Jacobs,E.T. *et al*. (2007) Diet, gender, and colorectal neoplasia. *J. Clin. Gastroenterol.*, **41**, 731–746.
36. Su,W. *et al*. (2007) Differential expression, distribution, and function of PPAR-gamma in the proximal and distal colon. *Physiol. Genomics*, **30**, 342–353.
37. Gervaz,P. *et al*. (2004) Two colons-two cancers: paradigm shift and clinical implications. *J. Surg. Oncol.*, **88**, 261–266.
38. Grady,W.M. *et al*. (2008) Genomic and epigenetic instability in colorectal cancer pathogenesis. *Gastroenterology*, **135**, 1079–1099.
39. Um,S. *et al*. (1998) Retinoic acid receptors interact physically and functionally with the T:G mismatch-specific thymine-DNA glycosylase. *J. Biol. Chem.*, **273**, 20728–20736.
40. Fuszek,P. *et al*. (2004) Relationship between serum calcium and CA 19-9 levels in colorectal cancer. *World J. Gastroenterol.*, **10**, 1890–1892.
41. Jenab,M. *et al*. (2009) Vitamin D receptor and calcium sensing receptor polymorphisms and the risk of colorectal cancer in European populations. *Cancer Epidemiol. Biomarkers Prev.*, **18**, 2485–2491.
42. Ahn,J. *et al*. (2009) Vitamin D-related genes, serum vitamin D concentrations, and prostate cancer risk. *Carcinogenesis*, **30**, 769–776.
43. Chang,S.C. *et al*. (2008) Polymorphism of genes related to insulin sensitivity and the risk of biliary tract cancer and biliary stone: a population-based case-control study in Shanghai, China. *Carcinogenesis*, **29**, 944–948.

Received April 14, 2010; revised May 25, 2010; accepted June 11, 2010