

Association of Genetic Variants in the Calcium-Sensing Receptor with Risk of Colorectal Adenoma

Ulrike Peters,^{1,2,3} Nilanjan Chatterjee,¹ Meredith Yeager,⁴ Stephen J. Chanock,⁴ Robert E. Schoen,⁵ Katherine A. McGlynn,¹ Timothy R. Church,⁶ Joel L. Weissfeld,⁵ Arthur Schatzkin,¹ and Richard B. Hayes¹

¹Department of Health and Human Services, Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Rockville, Maryland; ²Cancer Prevention Research Program, Fred Hutchinson Cancer Research Center, Seattle, Washington; ³Department of Epidemiology, University of Washington, Seattle, Washington; ⁴Department of Health and Human Services, Core Genotype Facility, National Cancer Institute, NIH, Gaithersburg, Maryland; ⁵University of Pittsburgh, Pittsburgh, Pennsylvania; and ⁶Division of Environmental and Occupational Health, University of Minnesota, Minneapolis, Minnesota

Abstract

Objective: Evidence suggests that calcium prevents colorectal cancer, possibly mediated through the calcium-sensing receptor (CASR). We assessed the associations between CASR gene variants and risk for colorectal adenoma, a cancer precursor. We further investigated gene-diet interactions between the CASR variants and calcium intake on adenoma risk.

Methods: Individuals with advanced distal adenomas ($n = 716$) and controls with a negative sigmoidoscopy exam ($n = 729$) were randomly selected from participants in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Three nonsynonymous variants in the intracellular signaling region of CASR (A986S, R990G, Q1011E) were analyzed by Taqman.

Results: Compared with the most common diplotype (haplotype pair), the odds ratios for advanced adenoma were 0.80 [95% confidence interval (CI), 0.60-1.06], 0.79 (95% CI, 0.55-1.13), and 0.56 (95% CI, 0.36-0.88) for the other three common diplotypes (>5% frequency). Although calcium intake was inversely associated with adenoma risk, CASR diplotypes did not modify this association. However, the power to investigate interactions was limited.

Conclusion: Variants in the CASR intracellular signaling region were significantly associated with the risk of advanced adenoma. (Cancer Epidemiol Biomarkers Prev 2004;13(12):2181-6)

Introduction

Several recent cohort studies (1-6) and three randomized intervention trials (7, 8) report inverse associations between calcium intake and colorectal tumors; the calcium-sensing receptor (CASR) may mediate this chemopreventive effect of calcium (9-12). CASR detects extracellular calcium and has a key role in calcium homeostasis and other diverse and cell type-specific functions, including regulation of cell differentiation, proliferation, and apoptosis (9-16). The expression patterns of CASR support its involvement in colorectal tumor development; CASR expression is high in normal colonic epithelial cells, is lower in well-differentiated colon cancer tissue, and is greatly reduced in undifferentiated carcinomas (9, 17, 18). Consistent with this finding, calcium inhibits the proliferation of well-differentiated but not poorly differentiated cells (19).

CASR has three parts, a large extracellular domain (about 600 amino acids) containing the calcium-binding

site, a transmembrane domain (about 250 amino acids) spanning seven subunits, and a carboxyl-terminal intracellular tail (about 220 amino acids) containing the signal transduction region. The intracellular tail of CASR affects several important functions of this receptor, including its level of cell surface expression, the rate at which the CASR desensitizes after calcium exposure, and the capacity of signal transduction into the cell (20, 21). CASR is directly regulated by protein kinase C phosphorylation (22-24), with three of five putative protein kinase C phosphorylation sites located in the intracellular tail (25-27).

Rare mutations, located almost exclusively in the extracellular and transmembrane domain, lead to familial hypocalcemic hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia (28-31). In the search for these rare mutations, common genetic variants (polymorphisms) in CASR have been identified (32), which may alter function or expression of CASR. We investigated the three common nonsynonymous single nucleotide polymorphisms (SNP) in the coding region of the intracellular CASR tail (32) in relation to risk of colorectal adenoma, because of the importance of this region for cellular signal transduction.

We also investigated whether the CASR variants modify the effect of calcium on colorectal adenoma. Because the promoter region of CASR contains a vitamin D

Received 3/29/04; revised 5/27/04; accepted 6/7/04.

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

Requests for reprints: Ulrike Peters, Cancer Prevention Research Program, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue N, M4-B402 Seattle, WA 98109-1024. Phone: 206-667-2450; Fax: 206-667-7850. E-mail: upeters@fhcrc.org

Copyright © 2004 American Association for Cancer Research.

receptor (VDR) response element, enabling vitamin D through VDR to regulate *CASR* transcription (33), we also considered interaction between *CASR* polymorphisms, serum vitamin D, and a variant in the *VDR* gene.

Materials and Methods

This case-control study was nested within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO Trial), a multisite investigation (Birmingham, AL, Denver, CO, Detroit, MI, Honolulu, HI, Marshfield, WI, Minneapolis, MN, Pittsburgh, PA, Salt Lake City, UT, St. Louis, MO, and Washington, DC) of the effectiveness of cancer screening, early markers of cancer, and the etiology of cancer (34, 35). Participants, ages 55 to 74 years, who were randomly assigned to the screening group, were offered a flexible sigmoidoscopy examination of the distal colon (60 cm) at study entry. If polyps or other suspect lesions were identified, participants were referred for further colonoscopy and surgery, if indicated. All available medical and pathologic reports on follow-up were obtained and coded by trained medical record abstractors without a centralized pathology review. The institutional review boards of the U.S. National Cancer Institute and the 10 screening centers approved the study and all participants provided informed consent.

Study Population. Cases and controls for this study were drawn from the participants randomly assigned to the screening arm of the PLCO Trial between September 1993 and September 1999, who filled out the risk factor questionnaire, had a successful sigmoidoscopy (insertion to at least 50 cm with >90% of mucosa visible or a suspect lesion identified), and provided a blood sample for use in etiologic studies ($n = 42,037$). Of these participants, we excluded 4,834 with a self-reported history of ulcerative colitis, Crohn's disease, familial polyposis, colorectal polyps, Gardner's syndrome, or cancer, except basal-cell skin cancer. We randomly selected 772 of 1,234 cases with at least one distal advanced colorectal adenoma (≥ 1 cm, high-grade dysplasia, or villous elements including tubulovillous adenoma) and 777 of 26,651 control participants, with a negative sigmoidoscopy screening (i.e., no polyp or other suspect lesion), matched to the cases by gender and ethnicity.

Genotype Analysis. DNA was extracted from buffy coat or whole blood samples. We used Taqman (Applied Biosystems, Foster City, CA) to analyze three SNPs in exon 7 of the *CASR* gene, A986S (rs1801725), R990G (rs1042636), and Q1011E (rs1801726). For validation purposes, we resequenced approximately 600 bp upstream and downstream of the three SNPs in a multiethnic group ($n = 102$; ref. 36). This sequence information was used to design primers for the Taqman assay (36). We only used the Taqman assay if genotype results were 100% concordant with sequencing results for the same 102 individuals (36). Because of insufficient DNA yields, DNA that did not amplify, or failure of unambiguous genotyping for 56 cases and 48 controls, genotype results were available for 716 cases and 729 controls (success rate of 95.1%, 98.3%, and 98.4% for A986S, R990G, and Q1011E, respectively). Genotype results of blinded quality control samples (40 partici-

pants assayed two to four times, total 136 results) were 100% concordant. Genotype analysis for the *VDR* TaqI polymorphism was reported in detail elsewhere (37).

Other Factors. At baseline, participants filled out a risk factor questionnaire, including questions on demographic factors, personal, and family medical history, physical activity, weight, height, and smoking. Dietary intake for the year before enrollment was assessed, with a 137-item food frequency questionnaire, including an additional 14 questions on supplement use. To calculate daily nutrient intake from the diet, we multiplied the daily frequency of each food item by the nutrient value of the gender-specific portion size (38) using the U.S. Department of Agriculture nutrient database (39). To calculate total nutrient intake, we combined dietary and supplemental nutrient intake. Details about analysis of serum vitamin D levels (25-hydroxyvitamin D and 1,25 dihydroxyvitamin D) have been reported elsewhere (37).

Statistical Analysis. We tested whether each SNP was in Hardy-Weinberg equilibrium among the controls. As estimates of linkage disequilibrium between SNPs, we calculated D' (40) and r^2 (41). We used PHASE 2.0.2 to reconstruct the most likely pair of haplotypes (diplotypes) and their probability for each participant (42, 43). As a global test for association in the genomic region, we used the likelihood ratio statistic to test if the overall frequencies of the most likely diplotypes differed between cases and controls.

Genotype-specific and diplotype-specific prevalence odds ratio (OR) for colorectal adenoma and 95% confidence intervals (CI) were calculated by logistic regression using the genotype or the most likely diplotype as covariate and adjusting for age, screening center, gender, and ethnicity. Other potential risk factors for colorectal tumors (educational attainment, smoking, alcohol intake, aspirin use, ibuprofen use, physical activity, body mass index, energy intake, fiber intake, red meat intake, and folate intake), either separately or together, did not change the risk estimates for *CASR* by >10% and were not included in the analysis.

We investigated whether the associations of colorectal adenoma with calcium intake, vitamin D serum levels, and a *VDR* polymorphism were modified by *CASR* variants, by stratified analyses and tests for multiplicative interaction, with inclusion of cross-product terms in the regression models. The statistical significance of interaction terms was investigated by comparing the log-likelihood statistics of the main effect model with the joint effects model. On the basis of our earlier analysis (2), we adjusted the analysis of association between calcium intake and adenoma risk for age, screening center, gender, ethnicity, education, smoking, alcohol intake, aspirin use, ibuprofen use, physical activity, body mass index, and intake of energy, fiber, red meat, and folate. We adjusted the analysis of association between serum vitamin D levels and adenoma risk for age, screening center, gender, ethnicity, month of blood draw (37).

Results

Advanced adenoma cases were slightly older and less well-educated than controls (Table 1), the distributions

Table 1. Characteristics of the study population

Characteristics	Cases	Controls
N	716	729
Age, n (%)		
55-59 years	237 (33.1%)	336 (46.1%)
60-64 years	230 (32.1%)	186 (25.5%)
65-69 years	155 (21.7%)	136 (18.7%)
70-74 years	94 (13.1%)	71 (9.7%)
Female, n (%)	215 (30.3%)	228 (31.3%)
Ethnic origin, n		
Non-Hispanic Black	21 (2.9%)	23 (3.2%)
Non-Hispanic White	673 (94.0%)	684 (93.8%)
Others	22 (3.1%)	22 (3.0%)
Education, n (%)		
<12 years of school	68 (9.5%)	47 (6.5%)
12 years of school/high school equivalent	170 (23.7%)	170 (23.3%)
Some college	258 (36.0%)	235 (32.2%)
College and above	220 (30.7%)	277 (38.0%)

by gender and ethnic origin were similar because we matched on these variables. About 94% of the study participants were non-Hispanic White and 69% were male.

The minor allelic frequency in controls was 14.5%, 8.5%, and 5.6% for A986, R990, and Q1011, respectively. All three genotypes were in Hardy-Weinberg equilibrium ($P = 0.92$ for A986S, $P = 0.69$ for R990G, and $P = 0.62$ for Q1011E, calculated for non-Hispanic White controls). No single genotype was significantly associated with risk for advanced colorectal adenoma (Table 2).

Four haplotypes explained 100% of the genetic variation: haplotype 000 consisted of the three common variants (A986, R990, Q1011); haplotypes 100, 010, and 001 were characterized, respectively, by one polymorphic variant at codon 986, 990, and 1011 (Table 3). Each phase call was assigned with more than 95% probability. The probability of the most likely haplotype pair (diplotype) was >0.99 for 93.8% of the participants, >0.95 for 95.1% of the participants, and >0.80 for 99.3% of the participants. Four common diplotypes (000/000, 000/100, 000/010, and 000/001) covered 91.4% of the study population. Because the frequency of any of the other six possible diplotypes was low (<2.5%), we did not assess adenoma risk associated with these diplotypes separately. Overall, the

Table 2. Association between genotypes of CASR and advanced colorectal adenomas

CASR genotype	Cases, n (%)	Controls, n (%)	OR (95% CI)
A986S			
GG	545 (76.8)	531 (73.1)	1.00
GT	148 (20.9)	179 (24.7)	0.82 (0.64-1.06)
TT	17 (2.4)	16 (2.2)	1.07 (0.52-2.19)
R990G			
AA	590 (85.1)	580 (83.9)	1.00
AG	94 (13.6)	104 (15.1)	0.92 (0.68-1.26)
GG	9 (1.3)	7 (1.0)	1.17 (0.36-3.79)
Q1011E			
CC	646 (91.1)	647 (89.1)	1.00
CG	58 (8.2)	77 (10.6)	0.76 (0.53-1.11)
GG	5 (0.7)	2 (0.3)	2.35 (0.44-12.6)

NOTE: ORs adjusted for age, gender, ethnic origin, and study center.

Table 3. Haplotype frequency of CASR reconstructed from genotype data

Haplotype	Cases, frequency (SE)	Controls, frequency (SE)
000 = A986, R990, Q1011	0.743 (0.0025)	0.741 (0.0026)
100 = 986S, R990, Q1011	0.128 (0.0010)	0.145 (0.0011)
010 = A986, 990G, Q1011	0.081 (0.0021)	0.085 (0.0024)
001 = A986, R990, 1011E	0.048 (0.0012)	0.056 (0.0007)

NOTE: A986S and R990G, $r^2 = 0.018$, $D' = -0.99$, $P = 0.002$; A986S and Q1011E, $r^2 = 0.010$, $D' = -1.00$, $P = 0.0003$; R990G and Q1011E, $r^2 = 0.004$, $D' = -0.86$, $P = 0.13$.

frequency of the four common diplotypes differed between cases of advanced colorectal adenoma and controls (likelihood ratio test 9.012, 4 *df*, $P = 0.06$). Compared with participants carrying the most common diplotype (000/000), adenoma risk was significantly reduced for participants carrying the 000/001 diplotype (OR 0.56) and tended (nonsignificantly) to be reduced for carriers of the 000/100 and 000/010 diplotypes (Table 4). This analysis is equivalent to comparing heterozygotes for a given polymorphism with a reference group that is a homozygote for the common allele of all three variants. Excluding participants with a probability for the most likely haplotype of <0.99 ($n = 90$) resulted in similar risk estimates as shown in Table 4: (000/100) versus (000/000) OR 0.77, 95% CI, 0.58-1.02; (000/010) versus (000/000) OR 0.78, 95% CI, 0.54-1.12; and (000/001) versus (000/000) OR 0.51, 95% CI, 0.33-0.78.

We found a protective association between total calcium intake and advanced colorectal adenoma risk, with a 21% risk reduction per 1,000 mg increase in calcium intake (OR 0.79, Table 5). This association was similar to results for the entire study population, which also showed that a linear coefficient fits well with the calcium-adenoma association (2). Stratification by CASR diplotype resulted in similar protective calcium-adenoma associations for participants carrying 000/000 or 000/100. The association between calcium intake and advanced adenoma risk varied for participants carrying 000/010 or 000/001 diplotypes, but the small number of cases and controls resulted in less precise risk estimates. No multiplicative interaction coefficients between total calcium intake and the CASR diplotypes were below $P = 0.2$. Furthermore, we found that the association between serum vitamin D levels or VDR TaqI genotype and adenoma risk did not substantially vary by CASR diplotype (data not

Table 4. Association between diplotypes of CASR and advanced colorectal adenomas

CASR diplotype	Cases (n)	Controls (n)	OR (95% CI)
000/000	410	369	1.00
000/100	132	151	0.80 (0.60-1.06)
000/010	71	84	0.79 (0.55-1.13)
000/001	39	64	0.56 (0.36-0.88)
All other diplotypes*	63	61	0.99 (0.67-1.46)

NOTE: ORs adjusted for age, gender, ethnic origin, and study center. 000 = A986, R990, Q1011; 100 = 986S, R990, Q1011; 010 = A986, 990G, Q1011; 001 = A986, R990, 1011E.

*Frequency of any of the other six possible diplotypes was <2.5%

shown, main effects of vitamin D and *VDR* were reported elsewhere; ref. 37).

Risk estimates presented here included all participants and were adjusted for ethnic origin. Restricting the analysis to non-Hispanic Whites resulted in risk estimates very similar to those presented (data not shown).

Discussion

We found a significant association between *CASR* diplotypes and advanced colorectal adenoma. Risks were not specific to one of the three genotypes studied, suggesting that the association between genetic variants in *CASR* and adenoma risk may depend on the diplotypes patterns studied, or linked genetic variants upstream or downstream of these three SNPs.

CASR is suggested to be important for mediating the anticarcinogenic effect of calcium (9-12). Lamprecht and Lipkin (12) recently reviewed the molecular mechanisms of *CASR* in colorectal cancer, indicating that specific signaling pathways involved in cell growth and differentiation are activated by calcium through *CASR*, including promotion of E-cadherin expression, suppression of β -catenin/T cell factor (9) activation, and activation of the p38 mitogen-activated protein kinase cascade (44). The intracellular signaling region of *CASR* which we studied interacts with filamin-A, linking *CASR*-mediated activation to the mitogen-activated protein kinase cascades (45, 46). *CASR* is expressed on both the basolateral and luminal surfaces, indicating that *CASR* senses changes in calcium concentrations in the colon lumen as well as in blood (12, 47, 48); and Kalley et al. (10, 11) showed that the cell proliferative effect of low intestinal calcium concentrations is likely mediated by *CASR* via protein kinase C-signaling activation leading to up-regulation of c-myc expression. Low calcium levels can occur particularly in the large intestine due to increased fractional absorption of calcium in the small intestine—from 20% to 70%—if calcium intake is low.

From an evolutionary standpoint, the R990 (common) variant seems functionally relevant, as evidenced by cross-species evolutionary conservation (49). Based on physical principles, the change from arginine (R) to glycine (G) at codon 990 results in a radical amino acid change, from a positively charged to a hydrophilic base, whereas the amino acid changes in the two other SNPs

are more conservative (Ala⁹⁸⁶Ser, hydrophobic to hydrophilic; Gln¹⁰¹¹Glu, hydrophilic to negatively charged; ref. 50). Thus, 990G could be related to altered function in *CASR* in a manner that influences risk for colorectal adenoma, however, our study does not point specifically to the R990G polymorphism, rather it suggests that one or more of the minor variants (986S, 990G, 1011E), or other unmeasured polymorphisms in linkage disequilibrium with them, are associated with reduced risk for colorectal adenoma.

Ours is the first study to evaluate *CASR* variants in relation to colorectal adenoma risk. A small study investigated the association between *CASR* A986S and rectal cancer in 56 cases and 112 controls, however, the data in two reports (51, 52) are conflicting, and it is not clear whether the A986S variant influences the risk for rectal cancer.

There is growing experimental (53) and epidemiological evidence (1-8), including recent evidence from this study population, that high calcium intake protects from colorectal tumors (2). This study shows no substantial interrelation between *CASR* gene variants, calcium intake and colorectal adenoma risk. Our study is limited by sample size for the evaluation of *CASR*-calcium interactions; however, the negative results might indicate that the gene variants in the intracellular signaling region operate functionally independently from the extracellular part of this receptor, which binds calcium. Some studies showed lower serum calcium levels in homozygous A986 carriers (55-57), whereas others did not find an effect on serum calcium concentrations (58-60).

It is widely recognized that vitamin D induces differentiation and apoptosis in normal and tumor colonic cells (12), and a small number of observational studies including ours (37) suggest a reduced risk of colorectal tumors with increasing levels of serum vitamin D. We found no substantial interaction for vitamin D and *VDR* genotype with *CASR* genotypes or diplotypes; however, such interactions may be more likely with genetic variants in the promoter region of *CASR*, where the *VDR* response element is located.

We focused on our analysis of left-sided adenoma. Because differences in the associations with right-sided and left-sided colorectal neoplasia have been reported for some risk factors, including calcium (5, 8, 61), the extrapolation of our findings with all adenomas (right-sided and left-sided) or right-sided adenoma should be done with caution.

Ninety of the cases in our analysis had a hyperplastic polyp as well as an advanced adenoma. Because the association between *CASR* diplotypes and advanced adenoma with and without hyperplastic polyps was similar (data not shown), the observed *CASR* diplotypes-adenoma association cannot be explained by the inclusion of adenoma cases with concomitant hyperplastic polyps.

We used the most likely pair of haplotypes in the standard logistic regression analysis to estimate relative risk. Because of the inaccuracy in haplotype imputation (62), our approach could potentially underestimate the true effect. Such an error could bias the risk estimate if the true effect of the gene was due to a haplotype and not to specific variants. However, given the rarity of the alleles

Table 5. Association between calcium (1,000 mg/day) and advanced colorectal adenoma stratified by *CASR* diplotypes

<i>CASR</i> diplotypes	Calcium intake, OR (95% CI)
Overall	0.79 (0.61-1.02)
000/000	0.68 (0.47-0.99)
000/100	0.69 (0.39-1.23)
000/010	1.49 (0.57-3.90)
000/001	0.36 (0.07-1.88)
Other diplotypes	0.97 (0.28-3.36)

NOTE: ORs adjusted for age, gender, ethnic origin, study center, energy intake, educational attainment, tobacco use, alcohol intake, aspirin and ibuprofen use, physical activity, body mass index, and intake of red meat, folate, and dietary fiber. Range of the 10th to 90th percentile of calcium intake in controls (567-2,021 mg/day).

and the strong linkage disequilibrium, there was little ambiguity in imputing haplotype pairs for each participant and the possibility of such error in our analysis was minimal. Furthermore, excluding participants with <0.99 probability of the most likely haplotype pair did not change the risk estimates for *CASR* diplotypes.

The study design enabled us to randomly select cases and controls derived from the same source population and screened following a standardized procedure, in particular, cases were not screened based on symptoms. The large number of cases identified in the study population allowed us to focus our analysis on advanced adenoma. Because these lesions are more likely to progress to colorectal cancer, they are a particularly meaningful intermediate outcome for studying risk factors for colorectal cancer. Cases were identified from the initial baseline screening, so we cannot distinguish the effect of genetic variants in *CASR* on the temporal component of adenoma formation.

In conclusion, we found a significant association between advanced colorectal adenoma and *CASR* diplotypes comprised of three nonsynonymous polymorphic variants in the intracellular signaling region of *CASR*, supporting laboratory evidence suggesting a major role for *CASR* in the chemopreventive effects of calcium. It will be important to further investigate genetic variations in *CASR* in separate study populations, expanding genetic characterization to the entire functional region of *CASR*, including the calcium binding and the vitamin D-related regulatory regions, and to more fully characterize the gene variant-function correlates of this gene.

References

- McCullough ML, Robertson AS, Rodriguez C, et al. Calcium, vitamin D, dairy products, and risk of colorectal cancer in the Cancer Prevention Study II Nutrition Cohort (United States). *Cancer Causes Control* 2003;14:1–12.
- Peters U, Chatterjee N, McGlynn KA, et al. Calcium intake and colorectal adenoma in a colorectal cancer early detection program. *Am J Clin Nutr*. 2004;80:1358–65.
- Pietinen P, Malila N, Virtanen M, et al. Diet and risk of colorectal cancer in a cohort of Finnish men. *Cancer Causes Control* 1999;10:387–96.
- Sellers TA, Bazyk AE, Bostick RM, et al. Diet and risk of colon cancer in a large prospective study of older women: an analysis stratified on family history (Iowa, United States). *Cancer Causes Control* 1998;9:357–67.
- Wu K, Willett WC, Fuchs CS, Colditz GA, Giovannucci EL. Calcium intake and risk of colon cancer in women and men. *J Natl Cancer Inst* 2002;94:437–46.
- Zheng W, Anderson KE, Kushi LH, et al. A prospective cohort study of intake of calcium, vitamin D, and other micronutrients in relation to incidence of rectal cancer among postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 1998;7:221–5.
- Baron JA, Beach M, Mandel JS, et al. Calcium supplements for the prevention of colorectal adenomas. Calcium Polyp Prevention Study Group. *N Engl J Med* 1999;340:101–7.
- Bonithon-Kopp C, Kronborg O, Giacosa A, Rath U, Faivre J. Calcium and fibre supplementation in prevention of colorectal adenoma recurrence: a randomised intervention trial. European Cancer Prevention Organisation Study Group. *Lancet* 2000;356:1300–6.
- Chakrabarty S, Radjendirane V, Appelman H, Varani J. Extracellular calcium and calcium sensing receptor function in human colon carcinomas: promotion of E-cadherin expression and suppression of β -catenin/TCF activation. *Cancer Res* 2003;63:67–71.
- Kallay E, Kifor O, Chattopadhyay N, et al. Calcium-dependent c-myc proto-oncogene expression and proliferation of Caco-2 cells: a role for a luminal extracellular calcium-sensing receptor. *Biochem Biophys Res Commun* 1997;232:80–3.
- Kallay E, Bajna E, Wrba F, Kriwanek S, Peterlik M, Cross HS. Dietary calcium and growth modulation of human colon cancer cells: role of the extracellular calcium-sensing receptor. *Cancer Detect Prev* 2000;24:127–36.
- Lamprecht SA, Lipkin M. Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. *Nat Rev Cancer* 2003;3:601–14.
- Berridge MJ, Bootman MD, Roderick HL. Calcium signalling: dynamics, homeostasis and remodelling. *Nat Rev Mol Cell Biol* 2003;4:517–29.
- Chattopadhyay N, Brown EM. Cellular “sensing” of extracellular calcium (Ca²⁺(o)): emerging roles in regulating diverse physiological functions. *Cell Signal* 2000;12:361–6.
- Cullen PJ, Lockyer PJ. Integration of calcium and Ras signalling. *Nat Rev Mol Cell Biol* 2002;3:339–48.
- Hofer AM, Brown EM. Extracellular calcium sensing and signalling. *Nat Rev Mol Cell Biol* 2003;4:530–8.
- Sheinin Y, Kallay E, Wrba F, Kriwanek S, Peterlik M, Cross HS. Immunocytochemical localization of the extracellular calcium-sensing receptor in normal and malignant human large intestinal mucosa. *J Histochem Cytochem* 2000;48:595–602.
- Gama L, Baxendale-Cox LM, Breitwieser GE. Ca²⁺-sensing receptors in intestinal epithelium. *Am J Physiol* 1997;273:C1168–75.
- Buras RR, Shabahang M, Davoodi F, et al. The effect of extracellular calcium on colonocytes: evidence for differential responsiveness based upon degree of cell differentiation. *Cell Prolif* 1995;28:245–62.
- Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. *Physiol Rev* 2001;81:229–97.
- Gama L, Breitwieser GE. A carboxyl-terminal domain controls the cooperativity for extracellular Ca²⁺ activation of the human calcium sensing receptor. A study with receptor-green fluorescent protein fusions. *J Biol Chem* 1998;273:29712–8.
- Bai M, Trivedi S, Lane CR, Yang Y, Quinn SJ, Brown EM. Protein kinase C phosphorylation of threonine at position 888 in Ca²⁺-sensing receptor (CaR) inhibits coupling to Ca²⁺ store release. *J Biol Chem* 1998;273:21267–75.
- Hjalm G, MacLeod RJ, Kifor O, Chattopadhyay N, Brown EM. Filamin-A binds to the carboxyl-terminal tail of the calcium-sensing receptor, an interaction that participates in CaR-mediated activation of mitogen-activated protein kinase. *J Biol Chem* 2001;276:34880–7.
- Jiang YF, Zhang Z, Kifor O, Lane CR, Quinn SJ, Bai M. Protein kinase C (PKC) phosphorylation of the Ca²⁺-sensing receptor (CaR) modulates functional interaction of G proteins with the CaR cytoplasmic tail. *J Biol Chem* 2002;277:50543–9.
- Bai M, Trivedi S, Lane CR, Yang Y, Quinn SJ, Brown EM. Protein kinase C phosphorylation of threonine at position 888 in Ca²⁺-sensing receptor (CaR) inhibits coupling to Ca²⁺ store release. *J Biol Chem* 1998;273:21267–75.
- Hjalm G, MacLeod RJ, Kifor O, Chattopadhyay N, Brown EM. Filamin-A binds to the carboxyl-terminal tail of the calcium-sensing receptor, an interaction that participates in CaR-mediated activation of mitogen-activated protein kinase. *J Biol Chem* 2001;276:34880–7.
- Jiang YF, Zhang Z, Kifor O, Lane CR, Quinn SJ, Bai M. Protein kinase C (PKC) phosphorylation of the Ca²⁺ o-sensing receptor (CaR) modulates functional interaction of G proteins with the CaR cytoplasmic tail. *J Biol Chem* 2002;277:50543–9.
- Conley YP, Finegold DN, Peters DG, Cook JS, Oppenheim DS, Ferrell RE. Three novel activating mutations in the calcium-sensing receptor responsible for autosomal dominant hypocalcemia. *Mol Genet Metab* 2000;71:591–8.
- Hendy GN, D'Souza-Li L, Yang B, Canaff L, Cole DE. Mutations of the calcium-sensing receptor (CASR) in familial hypocalcemic hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia. *Hum Mutat* 2000;16:281–96.
- Hendy GN. Molecular mechanisms of primary hyperparathyroidism. *Rev Endocr Metab Disord* 2000;1:297–305.
- Brown EM, Hebert SC. Calcium-receptor-regulated parathyroid and renal function. *Bone* 1997;20:303–9.
- Health H III, Odelberg S, Jackson CE, et al. Clustered inactivating mutations and benign polymorphisms of the calcium receptor gene in familial benign hypocalcemic hypercalcemia suggest receptor functional domains. *J Clin Endocrinol Metab* 1996;81:1312–7.
- Canaff L, Hendy GN. 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* 2002;277:30337–50.
- Gohagan JK, Prorok PC, Hayes RB, Kramer BS. The Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial of the

- National Cancer Institute: history, organization, and status. *Control Clin Trials* 2000;21:251–72S.
35. Hayes RB, Reding D, Kopp W, et al. Etiologic and early marker studies in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. *Control Clin Trials* 2000;21:349–55S.
 36. National Cancer Institute. Cancer Genome Anatomy Project, SNP500Cancer Database. 2004 [cited 2004 Mar]. Available from: <http://snp500cancer.nci.nih.gov>.
 37. Peters U, Hayes RB, Chatterjee N, et al. Circulating vitamin D metabolites, polymorphism in vitamin D receptor and colorectal adenoma risk. *Cancer Epidemiol Biomarkers Prev* 2004;13:546–52.
 38. Subar AF, Ziegler RG, Thompson FE, et al. Is shorter always better? Relative importance of questionnaire length and cognitive ease on response rates and data quality for two dietary questionnaires. *Am J Epidemiol* 2001;153:404–9.
 39. Tippet KS, Cypel YS. Design and operation: the continuing survey of food intakes by individuals and the diet and health knowledge survey, 1994–96. In: Continuing survey of food intakes by individuals 1994–96, Nationwide Food Surveys Rep. No. 96-1. U.S. Department of Agriculture, Agricultural Research Service; 1997.
 40. Lewontin RC. The interaction of selection and linkage. I. General considerations: heterotic models. *Genetics* 1964;49:49–67.
 41. Hill WG, Robertson A. The effect of linkage on limits to artificial selection. *Genet Res* 1966;8:269–94.
 42. Stephens M, Smith NJ, Donnelly P. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* 2001;68:978–89.
 43. Stephens M, Donnelly P. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. *Am J Hum Genet* 2003;73:1162–9.
 44. Hobson SA, Wright J, Lee F, McNeil SE, Bilderback T, Rodland KD. Activation of the MAP kinase cascade by exogenous calcium-sensing receptor. *Mol Cell Endocrinol* 2003;200:189–98.
 45. Awata H, Huang C, Handlogten ME, Miller RT. Interaction of the calcium-sensing receptor and filamin, a potential scaffolding protein. *J Biol Chem* 2001;276:34871–9.
 46. Hjaln G, MacLeod RJ, Kifor O, Chattopadhyay N, Brown EM. Filamin-A binds to the carboxyl-terminal tail of the calcium-sensing receptor, an interaction that participates in CaR-mediated activation of mitogen-activated protein kinase. *J Biol Chem* 2001;276:34880–7.
 47. Chattopadhyay N, Cheng I, Rogers K, et al. Identification and localization of extracellular Ca(2+)-sensing receptor in rat intestine. *Am J Physiol* 1998;274:G122–30.
 48. Cheng SX, Okuda M, Hall AE, Geibel JP, Hebert SC. Expression of calcium-sensing receptor in rat colonic epithelium: evidence for modulation of fluid secretion. *Am J Physiol Gastrointest Liver Physiol* 2002;283:G240–50.
 49. Garrett JE, Capuano IV, Hammerland LG, et al. Molecular cloning and functional expression of human parathyroid calcium receptor cDNAs. *J Biol Chem* 1995;270:12919–25.
 50. Miyata T, Miyazawa S, Yasunaga T. Two types of amino acid substitutions in protein evolution. *J Mol Evol* 1979;12:219–36.
 51. Speer G, Cseh K, Fuszek P, et al. The role of estrogen receptor, vitamin D receptor and calcium receptor genotypes in the pathogenesis of colorectal cancer. *Orv Hetil* 2001;142:947–51.
 52. Speer G, Cseh K, Mucsi K, et al. Calcium-sensing receptor A986S polymorphism in human rectal cancer. *Int J Colorectal Dis* 2002;17:20–4.
 53. Lamprecht SA, Lipkin M. Cellular mechanisms of calcium and vitamin D in the inhibition of colorectal carcinogenesis. *Ann N Y Acad Sci* 2001;952:73–87.
 54. Hofstad B, Almendingen K, Vatn M, et al. Growth and recurrence of colorectal polyps: a double-blind 3-year intervention with calcium and antioxidants. *Digestion* 1998;59:148–56.
 55. Cole DE, Peltekova VD, Rubin LA, et al. A986S polymorphism of the calcium-sensing receptor and circulating calcium concentrations. *Lancet* 1999;353:112–5.
 56. Lorentzon M, Lorentzon R, Lerner UH, Nordstrom P. Calcium sensing receptor gene polymorphism, circulating calcium concentrations and bone mineral density in healthy adolescent girls. *Eur J Endocrinol* 2001;144:257–61.
 57. Cole DE, Vieth R, Trang HM, Wong BY, Hendy GN, Rubin LA. Association between total serum calcium and the A986S polymorphism of the calcium-sensing receptor gene. *Mol Genet Metab* 2001;72:168–74.
 58. Bollerslev J, Wilson SG, Dick IM, Devine A, Dhaliwal SS, Prince RL. Calcium-sensing receptor gene polymorphism A986S does not predict serum calcium level, bone mineral density, calcaneal ultrasound indices, or fracture rate in a large cohort of elderly women. *Calcif Tissue Int* 2004 Jan;74:12–7.
 59. Cetani F, Borsari S, Vignali E, et al. Calcium-sensing receptor gene polymorphisms in primary hyperparathyroidism. *J Endocrinol Invest* 2002;25:614–9.
 60. Miedlich S, Lamesch P, Mueller A, Paschke R. Frequency of the calcium-sensing receptor variant A986S in patients with primary hyperparathyroidism. *Eur J Endocrinol* 2001;145:421–7.
 61. McCullough ML, Robertson AS, Rodriguez C, et al. Calcium, vitamin D, dairy products, and risk of colorectal cancer in the Cancer Prevention Study II Nutrition Cohort (United States). *Cancer Causes Control* 2003;14:1–12.
 62. Xu CF, Lewis K, Cantone KL, et al. Effectiveness of computational methods in haplotype prediction. *Hum Genet* 2002;110:148–56.

Association of Genetic Variants in the Calcium-Sensing Receptor with Risk of Colorectal Adenoma

Ulrike Peters, Nilanjan Chatterjee, Meredith Yeager, et al.

Cancer Epidemiol Biomarkers Prev 2004;13:2181-2186.

Updated version Access the most recent version of this article at:
<http://cebp.aacrjournals.org/content/13/12/2181>

Cited articles This article cites 47 articles, 13 of which you can access for free at:
<http://cebp.aacrjournals.org/content/13/12/2181.full#ref-list-1>

Citing articles This article has been cited by 5 HighWire-hosted articles. Access the articles at:
<http://cebp.aacrjournals.org/content/13/12/2181.full#related-urls>

E-mail alerts [Sign up to receive free email-alerts](#) related to this article or journal.

Reprints and Subscriptions To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions To request permission to re-use all or part of this article, use this link
<http://cebp.aacrjournals.org/content/13/12/2181>.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.