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Calcium Intake and Ion Transporter Genetic Polymorphisms Interact in Human Colorectal Neoplasia Risk in a 2-Phase Study[1–3]

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Abstract

Background: The kidney-specific sodium-potassium-chloride cotransporter (NKCC2) protein encoded by solute carrier family 12 member 1 (SLC12A1) is the direct downstream effector of the inward-rectifier potassium channel (ROMK) encoded by potassium inwardly-rectifying channel, subfamily J, member 1 (KCNJ1), both of which are critical for calcium reabsorption in the kidney.
Objective: We hypothesized that polymorphisms in *KCNJ1, SLC12A1*, and 7 other genes may modify the association between calcium intake and colorectal neoplasia risk.
Methods: We conducted a 2-phase study in 1336 cases and 2891 controls from the Tennessee Colorectal Polyp Study.
Results: In phase I, we identified 5 single-nucleotide polymorphisms (SNPs) that significantly interacted with calcium intake in adenoma risk. In phase II, rs2855798 in *KCNJ1* was replicated. In combined analysis of phases I and II, the *P* values for interactions between calcium intake and rs2855798 were 1×10^{-4} for all adenoma and 5×10^{-3} for multiple/advanced adenoma. The highest calcium intake was not associated with risk among those with no variant allele but was significantly associated with a 41% reduced adenoma risk among those who carried at least 1 variant allele in *KCNJ1*. The corresponding reduction in risk of multiple or advanced adenomas was 52% among those with at least 1 variant allele. The *P* values for interactions between calcium intake and combined SNPs from the *KCNJ1* and *SLC12A1* genes were 7.5×10^{-5} for adenoma and 9.9×10^{-5} for multiple/advanced adenoma. The highest calcium intake was not associated with risk among those with nonvariant alleles in 2 genes but was significantly associated with a 34% reduced adenoma risk among those who carried a variant allele in 1 of the genes. The corresponding reduction in risk of multiple or advanced adenomas was 64% among those with variant alleles in both genes.
Conclusion: These findings, if confirmed, will be critical for the development of personalized prevention strategies for colorectal cancer. J. Nutr. 144: 1734–1741, 2014.

Introduction

Colorectal cancer is believed to arise mostly from adenomatous polyps (1). Because of the rapidly increased use of colonoscopy or improved cancer treatments, both the incidence and mortality of colorectal cancer has decreased in recent years (2). However, colorectal cancer still remains the fourth most common incident cancer and the second most common cause of cancer death in the United States (3). Thus, it is critical to develop novel preventive strategies beyond standard screening practices. Cohort studies of colorectal cancer (4) and adenoma (5) and intervention trials of adenoma recurrence (6) and colorectal carcinogenesis biomarkers (7–11) indicate that high calcium consumption confers a reduction in colorectal cancer risk. However, the Women's Health Initiative, a large-scale randomized clinical trial, did not show an effect from supplementation with calcium plus vitamin D (12). Several possible explanations (13–16) have been proposed. These include low compliance, contamination of the placebo group, and low dose of vitamin D. Another possible explanation for the null finding is that, if the primary mechanism of vitamin D is inhibition of early

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[3] Supplemental Tables 1–4 are available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.
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carcinogenesis, the follow-up time in the Women's Health Initiative (7 y) may not have been sufficiently long to affect early carcinogenesis.

Previous studies identified substantial individual variability in calcium absorption (10–70%) (17). Women with both low fractional calcium absorption and a low calcium intake had a significantly increased risk of hip fracture (17). The differences in calcium absorption persist over time (18), and only a small portion of cases can be explained by age, smoking, dietary factors, intestinal transit, urinary excretion, vitamin D metabolites, and birth weight (18). It is possible that polymorphisms in related genes play an important role. Previous studies examined whether the association between calcium and risk of colorectal cancer or adenoma may be modified by genetic susceptibility in calcium absorption, or regulation. Although not concordant, some epidemiologic studies suggested that variants in the vitamin D receptor (VDR)[11] (19), the calcium-sensing receptor (CaSR) (20), and the transient receptor potential melastatin 7 (TRPM7) (13) may confer modifying effects. Ten grams of calcium are filtered daily, on average, of which 98% is reabsorbed in the kidneys. Each of these genes is involved in calcium reabsorption and thus are essential in calcium homeostasis.

In addition to *VDR, CaSR*, and *TRPM7*, there are other genes involved in calcium reabsorption. The kidney-specific sodium-potassium-chloride cotransporter (*NKCC2*), encoded by solute carrier family 12 member 1 (*SLC12A1* at chromosome 15q15), is the direct downstream effector of the inward-rectifier potassium channel (*ROMK*), encoded by potassium inwardly rectifying channel, subfamily J, member 1 (*KCNJ1* at chromosome 11q24) (21). Both *ROMK* and *NKCC2* serve as driving forces for reabsorption of calcium (21). Homozygous rare mutations in *SLC12A1* and *KCNJ1* cause type I and type II Bartter syndrome, respectively, both of which are severe neonatal syndromes characterized by marked hypercalciuria (calcium wasting) (21,22). One recent study found that heterozygous rare mutations in *KCNJ1* led to a later-onset Bartter syndrome (23).

To the best of our knowledge, no study has been reported to conduct tests of gene-calcium interactions on colorectal polyp risk using a multiple-phase design. We conducted a 2-phase study to investigate whether the associations between intake of calcium and risk of colorectal adenoma were modified by common polymorphisms in 9 candidate genes (e.g., *KCNJ1*, *SLC12A1)* involved in calcium (re)absorption and homeostasis.

Participants and Methods

The study was approved by the institutional review boards of Vanderbilt University and the Tennessee Valley Veterans Affairs Medical Center and by the Research and Development Committee of the Department of Veterans Affairs. Included in the study were participants of the Tennessee Colorectal Polyp Study (TCPS), a colonoscopy-based case-control study of colorectal adenoma, hyperplastic polyp, and polyp-free controls conducted in Nashville, TN, from 1 February 2003 to 29 October 2010. A total of 12,585 eligible participants aged 40 to 75 y were identified from patients scheduled for colonoscopy at the Vanderbilt University Gastroenterology Clinic and the Tennessee Valley Veterans Affairs Health System campus; of these, 7954 (63%) consented to participate in the TCPS. Excluded from our study were patients who had genetic or familial colorectal cancer syndromes (e.g., hereditary nonpolyposis colorectal cancer or familial adenomatous polyposis), inflammatory bowel disease, or a history of adenomatous polyps or any cancer other than nonmelanoma skin cancers. A detailed description of this study was reported elsewhere (13,24).

On the basis of the colonoscopy's results and pathological diagnosis, participants were classified as adenoma cases or polyp-free controls. To be assigned as a control, the participant must have been polyp-free at a complete colonoscopy to the cecum. Adenoma cases had at least 1 adenoma. Participants with at least 2 adenomas were considered to have multiple adenomas. Hyperplastic cases had at least 1 hyperplastic polyp and no adenomas. Advanced adenoma cases had at least 1 advanced adenoma defined as follows: *1*) size ≥1 cm, *2*) tubulovillous or villous, or *3*) high-grade dysplasia.

Data and sample collection and assessment. A total of 6482 (81%) participants completed a telephone interview on medication use, demographic characteristics, medical history, family history, reproductive history, anthropometry, and lifestyle. Participants were also asked to complete a semiquantitative 108-item FFQ, which was developed to capture diet in the southeastern United States (25). A total of 6485 participants (82%) completed the FFQ.

The usual dietary intakes of nutrients, including calcium, were calculated on the basis of frequency and usual portion size by using race- and sex-specific nutrient databases, which were constructed by using NHANES and USDA food composition tables (26). Supplemental calcium intakes from calcium and multivitamin supplements were also taken into account by estimating intake on the basis of the most common ingredients in calcium and multivitamin supplements (500 mg calcium per calcium supplement pill and 162 mg calcium per multivitamin pill) (13). One hundred seventy-three participants were excluded from the analyses due to >10 missing items on the FFQ or unreasonably high (4000 kcal for women and 7000 kcal for men) or low (<500 kcal) energy intakes.

Biologic samples. Participants recruited at colonoscopy were asked to provide blood, buccal cell, or saliva samples (collected by using the Oragene DNA kit, DNA Genotek, Inc.). Participants recruited after colonoscopy were asked to provide buccal cell or saliva samples. A total of 7443 (94%) participants donated DNA samples.

Study design and genotyping. This was a 2-phase design (discovery and replication) candidate-gene study to focus on investigating gene-nutrient (calcium) interactions between 2 independent samples of participants from the TCPS. A total of 4227 participants with genotyping and FFQ information were included in the analysis. The discovery phase was conducted among a sample of adenoma cases (n = 728) and controls (n = 756) from the TCPS. To improve the power in phase I, we oversampled multiple or advanced adenoma cases. The detailed descriptions of genotyping and quality control for phase I were reported elsewhere (27). Five independently funded candidate-gene studies based on the TCPS samples were coordinated to perform the Affymetrix Genome-Wide Human SNP Array 5.0 assays. Eighty tagging single-nucleotide polymorphisms (SNPs) in 9 genes, including 13 in *KCNJ1* and 6 in *SLC12A1*, were evaluated. In phase II, genotypes of selected SNPs with significant gene-nutrient interactions or direct associations in the first phase were assayed among another independent sample of participants from the TCPS (adenoma cases = 608, controls = 2135) by using Applied Biosystems' OpenArray or Sequenom MassARRAY genotyping assays. In phase I, the average concordance of genotypes within the duplicate quality control samples was 99.9%. SNPs were removed if missing >5%, or a minor allele frequency (MAF) of <1%, or Hardy-Weinberg equilibrium $P < 1 \times 10^{-6}$ (27). In phase II, these SNPs passed filters for consistency rates (>99%) among replicate quality control participants with <5% missing data, a Hardy-Weinberg equilibrium ≥0.05, and MAF agreement with phase I.

Statistical analysis. Chi-square tests (categorical variables) as well as t tests or generalized linear models (continuous variables) were used to

[11] Abbreviations used: *CaSR*, calcium-sensing receptor; *CLCNKB*, chloride channel Kb; COX, cyclooxygenase; DRI, dietary reference intakes; *KCNJ1*, potassium inwardly-rectifying channel, subfamily J, member 1; MAF, minor allele frequency; *NKCC2*, kidney-specific sodium-potassium-chloride cotransporter; NSAID, nonsteroidal anti-inflammatory drug; PGE_2, prostaglandin E_2; PGE-M, metabolite of prostaglandin E_2; *PTH*, parathyroid hormone; *PTH1R*, parathyroid hormone 1 receptor; ROMK, inward-rectifier potassium channel; *SLC12A1*, solute carrier family 12 member 1; SNP, single-nucleotide polymorphism; TCPS, Tennessee Colorectal Polyp Study; *TRPM7,* transient receptor potential melastatin 7; *TRPV,* transient receptor potential cation channel subfamily V; *VDR,* vitamin D receptor.

evaluate case-control differences in the distribution of potential confounding factors (**Supplemental Table 1**). Unconditional multivariable logistic regression models were used to calculate ORs and 95% CIs to measure the associations adjusting for potential confounders, such as age, sex, race, education, recruitment site, BMI, smoking status, alcohol consumption status, physical activity, and daily intakes of total energy, calcium, or magnesium, respectively. Tests for trend across tertile categories were performed in logistic regression models by assigning the score *j* to the *j*th level of the variable selected. Stratified analyses by the calcium Dietary Reference Intakes (DRI) or by genotype were conducted. Tests for multiplicative interactions between intakes of calcium with the gene polymorphisms in relation to colorectal adenoma risk were conducted in logistic regression models by likelihood ratio tests. In **Table 1**, we present the main results for the calcium-gene interactions in phase I, phase II, and phases I and II combined. Tests were 2-sided and the significance level was set at 0.05 for phase I analysis. As prespecified in our original design, 1-sided tests at $P \leq 0.05$ were conducted in phase II because the direction of the gene-nutrient interaction for a given gene variant was provided in phase I. Calcium intake was analyzed as a continuous variable and additive genetic models were used for genetic variants. Because NKCC2 encoded by *SLC12A1* is the direct downstream effector of ROMK encoded by *KCNJ1*, we conducted additional analysis for the interactions between calcium intake and the joint effect of *KCNJ1* with *SLC12A1* polymorphisms in relation to risk of adenoma and multiple or advanced adenomas. To increase power and because multiple or advanced adenomas were oversampled in phase I, we used the combined phase I and II data in these additional analyses. Statistical analyses were performed by using SAS statistical software (version 9.3; SAS Institute).

TABLE 1 Interaction of *KCNJ1* rs2855798, *SLC12A1* rs1531916, and intakes of calcium with colorectal adenoma risk: the Tennessee Colorectal Polyp Study[1]

Gene, SNP, and phase (all adenomas)	Cases/controls	MAF	*P*-interaction[2]
	n/n		
KCNJ1, rs2855798			
I	728/756	0.20/0.20	0.006
II	608/2135	0.20/0.20	0.03
I and II	1336/2891	0.20/0.20	0.0001
KCNJ1, rs4529890			
I	727/757	0.15/0.16	0.01
II	548/2063	0.14/0.14	0.06
I and II	1275/2820	0.15/0.15	0.001
KCNJ1, rs4937378			
I	728/757	0.23/0.23	0.01
II	547/2059	0.25/0.25	0.36
I and II	1275/2816	0.24/0.24	0.02
KCNJ1, rs6590354			
I	728/757	0.04/0.04	0.02
II	—	—	—
SLC12A1, rs1531916			
I	723/755	0.17/0.18	0.01
II	547/2061	0.22/0.21	0.72
I and II	1270/2816	0.19/0.19	0.08

[1] *KCNJ1*, potassium inwardly-rectifying channel, subfamily J, member 1; MAF, minor allele frequency; *SLC12A1*, solute carrier family 12 member 1; SNP, single-nucleotide polymorphism.

[2] Interactions between genetic polymorphism and calcium intake (continuous) by using a likelihood ratio test adjusting for age (continuous), sex, race (Caucasian, other), education (categorical), recruitment sites, BMI (categorical), smoking status (never, former, current), alcohol consumption status (never, former, current), physical activity (yes, no), and daily intakes of total energy and magnesium (continuous).

Results

Two-phase study. Overall, in phase I, we included a total of 80 tagging SNPs in 9 candidate genes [*CaSR*, chloride channel Kb (*CLCNKB*), *KCNJ1*, *SLC12A1*, parathyroid hormone (*PTH*), parathyroid hormone 1 receptor (*PTH1R*), transient receptor potential cation channel subfamily V (TRPV) member 5,6 (*TRPV5*, *TRPV6*), and *VDR*] involved in calcium (re) absorption/regulation. In phase I, we found 5 SNPs in 2 genes (*KCNJ1* and *SLC12A1*) that significantly interacted with intake of calcium in relation to risk of colorectal adenoma. In phase II, we replicated only 1 of the 5 SNPs (rs2855798 in *KCNJ1*; Table 1) as significantly interacting with intake of calcium in association with risk of colorectal adenoma. The MAF for rs2855798 was 19% (**Supplemental Table 2**). On the other hand, in phase I, we found that 1 tagging SNP (*KCNJ1* rs11221484) was significantly directly associated with adenoma risk, whereas it was not significantly related to risk in the phase II replication sample (data not shown).

Associations with SNPs by calcium intake. The frequency of homozygotes for the minor alleles at rs2855798 in *KCNJ1* was 4.97%, which was rare. Thus, we used a dominant mode of inheritance in the analysis. **Table 2** shows the associations between the *KCNJ1* genotype and risks of adenoma and multiple or advanced adenomas stratified by total calcium intake amount (above or below the DRI) by using combined data from phases I and II. Overall, we found that the genotype was not significantly related to risk of adenoma and multiple or advanced adenomas. In the stratified analysis, among those whose calcium intake was below the DRI, subjects who carried at least 1 variant allele had a significantly increased risk of adenoma (OR: 1.35; 95% CI: 1.08, 1.69), particularly multiple or advanced adenoma (OR: 1.72; 95% CI: 1.28, 2.32), compared with those with no variant allele. Conversely, among those whose calcium intake was above the DRI, risks of adenoma and multiple or advanced adenoma were reduced for those who carried at least 1 variant allele. The *P* values for interactions between calcium intake and rs2855798 were 1×10^{-4} and 5×10^{-3} for adenoma and multiple/advanced adenoma, respectively.

In addition, due to the strong biologic plausibility for a joint effect between *KCNJ1* and *SLC12A1*, we conducted a joint analysis of rs1531916 (*SLC12A1*) and rs2855798 (*KCNJ1*). The frequency of homozygotes for the minor alleles at rs1531916 was also rare (4.61%). Thus, we used a dominant mode of inheritance in the joint analysis. **Table 3** shows the associations between the number of genes carrying variant allele(s) and risks of adenoma and multiple or advanced adenomas stratified by total calcium intake. Again, we found that the number of genes with variant allele(s) was not related to risk of adenoma. In the stratified analysis, we found among subjects whose calcium intake was below the DRI that those who carried variant allele(s) in 2 genes had a significantly increased risk of adenoma (OR: 1.53; 95% CI: 1.07, 2.19; *P*-trend = 0.03), particularly multiple or advanced adenoma (OR: 2.20; 95% CI: 1.36, 3.54; *P*-trend = 0.001), compared with those with no variant allele. Conversely, among those whose calcium intake was above the DRI, risks of adenoma and multiple/advanced adenoma were significantly reduced for those who carried variant allele(s) in either gene, particularly for those with variants in both genes (OR: 0.64; 95% CI: 0.40, 1.02). The *P* values for interactions between calcium intake and combined SNPs were 7.5×10^{-5} and 9.9×10^{-5} for adenoma and multiple/advanced adenoma, respectively. A similar interaction pattern was observed for both Caucasians and African Americans (data not shown).

TABLE 2 ORs (95% CIs) for colorectal adenoma according to the rs2855798 polymorphism in *KCNJ1* stratified by calcium intake: the Tennessee Colorectal Polyp Study[1]

	Adenomas vs. controls			Multiple/advanced adenomas vs. controls		
	Cases/controls	OR (95% CI)	*P*	Cases/controls	OR (95% CI)	*P*
	n/n			*n/n*		
All subjects			0.84			0.06
GG	844/1847	1.00		350/1847	1.00	
GT/TT	492/1044	1.02 (0.88, 1.17)		240/1044	1.21 (0.99, 1.47)	
Calcium intake <1000 mg/d			0.008			0.0001
GG	345/716	1.00		145/716	1.00	
GT/TT	245/374	1.35 (1.08, 1.69)		127/374	1.72 (1.28, 2.32)	
Calcium intake ≥1000 mg/d			0.03			0.45
GG	499/1131	1.00		205/1131	1.00	
GT/TT	247/670	0.80 (0.66, 0.98)		113/670	0.90 (0.69, 1.18)	

[1] Phase I + phase II. Unconditional logistic regression models adjusted for age (continuous), sex, race (Caucasian, other), education (categorical), recruitment sites, BMI (categorical), smoking status (never, former, current), alcohol consumption status (never, former, current), physical activity (yes, no), and daily intakes of total energy and magnesium (continuous). *P* values for interactions between calcium intake (continuous) and the rs2555798 (*KCNJ1*) polymorphism were 0.0001 for all adenomas and 0.005 for multiple/advanced adenomas. *KCNJ1*, potassium inwardly-rectifying channel, subfamily J, member 1.

Associations with calcium intake by SNPs. The associations between total calcium intake and risk of colorectal adenoma stratified by *KCNJ1* genotype are presented in **Table 4**. High calcium intake was not associated with adenoma risk among those with no variant allele, but was associated with a reduced risk for those with at least 1 variant allele. The highest calcium intake tertile was associated with a 41% reduced risk of adenoma (*P*-trend = 0.005) among those with at least 1 variant allele. Risk was even more substantially reduced (by 52%) for multiple or advanced adenomas among those who carry at least 1 variant allele.

The associations between total calcium intake and risk of colorectal adenoma stratified by the number of genes carrying variant allele(s) are presented in **Table 5**. We observed that 45% of the study population carry variant allele(s) in 1 gene whereas 13% of the population carry variant alleles in both genes. High calcium intake was not associated with adenoma risk among those with no variant allele but was associated with a reduced risk for those with variant alleles. The highest calcium intake tertile was associated with a 34% reduced risk of adenoma (*P*-trend = 0.005) among those with a variant allele or alleles. Risk was even more substantially reduced (by 48%) for multiple or advanced adenomas among those who carry variant allele(s), particularly those with variant allele(s) in both genes (OR: 0.36; 95% CI: 0.14, 0.92). Similarly, we found that supplemental calcium intake was associated with a reduced risk of multiple/advanced adenomas only among those who carry variant allele(s) (*P*-trend = 0.02; **Supplemental Table 3**). Finally, we conducted sensitivity analysis by limiting the analysis to those who had colonoscopy as a true screening measure and found that the highest calcium

TABLE 3 ORs (95% CIs) for colorectal adenoma according to the rs2855798 polymorphism in *KCNJ1* and the rs1531916 polymorphism in *SLC12A1* stratified by calcium intake: the Tennessee Colorectal Polyp Study[1]

Number of genes with variant alleles	Adenomas vs. controls			Multiple/advanced adenomas vs. controls		
	Cases/controls	OR (95% CI)	*P*-trend	Cases/controls	OR (95% CI)	*P*-trend
	n/n			*n/n*		
All subjects			0.59			0.40
0	528/1131	1.00		215/1131	1.00	
1	560/1272	0.92 (0.79, 1.07)		250/1272	1.04 (0.84, 1.29)	
2	158/334	0.99 (0.78, 1.25)		73/334	1.15 (0.84, 1.59)	
Calcium intake <1000 mg/d			0.03			0.001
0	210/446	1.00		83/446	1.00	
1	245/466	1.12 (0.88, 1.43)		109/466	1.36 (0.96, 1.93)	
2	84/123	1.53 (1.07, 2.19)		45/123	2.20 (1.36, 3.54)	
Calcium intake ≥1000 mg/d			0.008			0.08
0	318/685	1.00		132/685	1.00	
1	315/806	0.81 (0.66, 0.99)		141/806	0.91 (0.69, 1.20)	
2	74/211	0.69 (0.50, 0.95)		28/211	0.64 (0.40, 1.02)	

[1] Phase I + phase II. Unconditional logistic regression models adjusted for age (continuous), sex, race (Caucasian, other), education (categorical), recruitment sites, BMI (categorical), smoking status (never, former, current), alcohol consumption status (never, former, current), physical activity (yes, no), and daily intakes of total energy and magnesium (continuous). *P* values for interactions between calcium intake (continuous) and combined single-nucleotide polymorphisms (rs2855798 in *KCNJ1* and rs1531916 in *SLC12A1*) were 7.5×10^{-5} for adenomas and 9.9×10^{-5} for multiple/advanced adenomas. *KCNJ1*, potassium inwardly-rectifying channel, subfamily J, member 1; *SLC12A1*, solute carrier family 12 member 1.

TABLE 4 Association of calcium intake with colorectal adenoma risk stratified by the rs2855798 polymorphism of *KCNJ1*: the Tennessee Colorectal Polyp Study[1]

	Calcium intake			
KCNJ1, rs2855798	<1000 mg/d	1000 mg/d ≤ calcium intake <1300 mg/d	≥1300 mg/d	*P*-trend
All adenomas vs. controls				
Total				0.01
Cases/controls, *n*/*n*	590/1090	244/578	502/1223	
OR (95% CI)	1.00	0.78 (0.64, 0.96)	0.75 (0.61, 0.92)	
GG				0.20
Cases/controls, *n*/*n*	345/716	164/374	335/757	
OR (95% CI)	1.00	0.90 (0.70, 1.16)	0.85 (0.65, 1.09)	
GT/TT				0.005
Cases/controls, *n*/*n*	245/374	80/204	167/466	
OR (95% CI)	1.00	0.60 (0.42, 0.86)	0.59 (0.41, 0.85)	
Multiple/advanced adenomas vs. controls				
Total				0.003
Cases/controls, *n*/*n*	272/1090	107/578	211/1223	
OR (95% CI)	1.00	0.75 (0.56, 0.99)	0.65 (0.49, 0.86)	
GG				0.15
Cases/controls, *n*/*n*	145/716	70/374	135/757	
OR (95% CI)	1.00	0.90 (0.63, 1.29)	0.77 (0.53, 1.10)	
GT/TT				0.002
Cases/controls, *n*/*n*	127/374	37/204	76/466	
OR (95% CI)	1.00	0.54 (0.33, 0.87)	0.48 (0.30, 0.78)	

[1] Phase I + phase II. Unconditional logistic regression models adjusted for age (continuous), sex, race (Caucasian, other), education (categorical), recruitment sites, BMI (categorical), smoking status (never, former, current), alcohol consumption status (never, former, current), physical activity (yes, no), and daily intakes of total energy and magnesium (continuous). *P* values for interactions between calcium intake (continuous) and rs2555798 (*KCNJ1*) polymorphism were 0.0001 for all adenomas and 0.005 for multiple/advanced adenomas. *KCNJ1*, potassium inwardly-rectifying channel, subfamily J, member 1.

intake was associated with an almost 90% reduced risk of multiple/advanced adenomas among those with variant allele(s) in both genes (**Supplemental Table 4**).

Discussion

In this 2-phase study that included 80 SNPs in 9 genes, we identified and replicated 1 SNP in *KCNJ1* that significantly interacted with intakes of calcium in association with colorectal adenoma risk. The highest calcium intake tertile was not associated with risk among those with no variant allele but was significantly associated with 41% reduced adenoma risk among those who carried at least 1 variant allele in *KCNJ1*. The corresponding reduction in risk of multiple or advanced adenomas was 52% among those with at least 1 variant allele. In joint analysis of *KCNJ1* and *SLC12A1*, which encodes a protein as an upstream effector of the protein encoded by *KCNJ1*, we observed that the highest calcium tertile intake was significantly associated with a 34% reduced risk of adenoma and a 48% reduced risk of multiple or advanced adenoma among those with variant allele(s) and a 64% reduced risk of multiple or advanced adenoma among those who carry variant allele(s) in both genes. We conducted separate analyses among Caucasians and African Americans and found the same interaction patterns.

On average, 10 g of calcium are filtered every day, and 98% of this is reabsorbed by the kidney (28,29). Potassium (K+) recycling through ROMK (encoded by *KCNJ1)* and NKCC2 (coded by *SLC12A1)* serve as driving forces for paracellular reabsorption of calcium in the kidney (21). Homozygous mutations in *SLC12A1* and *KCNJ1* cause type I and II Bartter syndrome, respectively, which are rare and severe recessive familial diseases in early infancy characterized by calcium wasting, high systemic concentrations of prostaglandin E_2 (PGE_2), hypokalemia, sodium wasting, and hypotension (21). However, unlike rare mutations, among those with calcium intake above the DRI the common SNPs in *KCNJ1* or *SLC12A1* alone may not lead to calcium deficiency. NKCC2 is the direct downstream effector of ROMK, whereas *SLC12A1* and *KCNJ1* are located on 2 different chromosomes and the r^2 between the 2 SNPs is zero. Although they are not physically linked, our joint analysis of the 2 SNPs indicates that high intake of calcium may only protect against colorectal adenomas, particularly multiple/advanced adenomas among those who carry variant allele(s) in 2 genes, and particularly for those who carry variant alleles in both genes.

Cyclooxygenase (COX), including COX-1 and COX-2, are the key enzymes responsible for the conversion of arachidonic acid to prostaglandins, particularly PGE_2, a critical mediator of inflammation (30). Previous studies consistently showed that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) and COX-2 inhibitors was associated with a reduced risk of colorectal cancer and adenoma recurrence (31). Studies also found that COX-2 expression was elevated in 50% and 85% of human adenoma and carcinoma cases, respectively (32), matching well with the progressive reduction in apoptosis index from adenoma to carcinoma (32). Compared with controls, concentrations of the urinary metabolite of PGE_2 (PGE-M) were significantly elevated in individuals with colorectal cancers or multiple or advanced adenomas (33,34), and baseline PGE-M was associated with a strong risk of subsequent diagnosis of colorectal cancer (35). We conducted a pilot study and found that PGE-M was significantly elevated among colorectal adenoma

TABLE 5 Association of calcium intake with colorectal adenoma risk stratified by number of genes (*KCNJ1* and *SLC12A1*) with variant alleles: the Tennessee Colorectal Polyp Study[1]

	Calcium intake			
Number of genes with variant alleles	<1000 mg/d	1000 mg/d ≤ calcium intake <1300 mg/d	≥1300 mg/d	*P*-trend
All adenomas vs. controls				
Total				0.02
Cases/controls, *n/n*	539/1035	231/549	476/1153	
OR (95% CI)	1.00	0.80 (0.65, 0.99)	0.77 (0.62, 0.96)	
0				0.64
Cases/controls, *n/n*	210/446	99/229	219/456	
OR (95% CI)	1.00	0.93 (0.67, 1.28)	0.92 (0.66, 1.27)	
≥1				0.005
Cases/controls, *n/n*	329/589	132/320	257/697	
OR (95% CI)	1.00	0.71 (0.53, 0.94)	0.66 (0.49, 0.88)	
1				0.01
Cases/controls, *n/n*	245/466	111/258	204/548	
OR (95% CI)	1.00	0.77 (0.56–1.05)	0.66 (0.48, 0.92)	
2				0.37
Cases/controls, *n/n*	84/123	21/62	53/149	
OR (95% CI)	1.00	0.53 (0.27, 1.05)	0.74 (0.39, 1.42)	
Multiple/advanced adenomas vs. controls				
Total				0.01
Cases/controls, *n/n*	237/1035	101/549	200/1153	
OR (95% CI)	1.00	0.79 (0.59, 1.06)	0.68 (0.51, 0.92)	
0				0.83
Cases/controls, *n/n*	83/446	39/229	93/456	
OR (95% CI)	1.00	0.95 (0.59, 1.52)	0.95 (0.59, 1.52)	
≥1				0.001
Cases/controls, *n/n*	154/589	62/320	107/697	
OR (95% CI)	1.00	0.68 (0.46, 0.99)	0.52 (0.35, 0.77)	
1				0.04
Cases/controls, *n/n*	109/466	53/258	88/548	
OR (95% CI)	1.00	0.82 (0.54, 1.25)	0.63 (0.40, 0.97)	
2				0.03
Cases/controls, *n/n*	45/123	9/62	19/149	
OR (95% CI)	1.00	0.39 (0.15, 1.01)	0.36 (0.14, 0.92)	

[1] Phase I + phase II. Unconditional logistic regression models adjusted for age (continuous), sex, race (Caucasian, other), education (categorical), recruitment sites, BMI (categorical), smoking status (never, former, current), alcohol consumption status (never, former, current), physical activity (yes, no), and daily intakes of total energy and magnesium (continuous). *P* values for interactions between calcium intake (continuous) and combined single-nucleotide polymorphisms (rs2855798 in *KCNJ1* and rs1531916 in *SLC12A1*) were 7.5×10^{-5} for adenomas and 9.9×10^{-5} for multiple /advanced adenomas. *KCNJ1*, potassium inwardly-rectifying channel, subfamily J, member 1; *SLC12A1*, solute carrier family 12 member 1.

cases in comparison with nonpolyp controls only among those with calcium intakes lower than the DRI and who had at least 1 variant allele in *KCNJ1* or *SLC12A1*. This finding is consistent with the fact that calcium wasting and elevated systemic concentrations of PGE_2 are caused by rare mutations in *KCNJ1* or *SLC12A1*. However, unlike rare mutations, among those with calcium intakes above the DRI the common SNPs in *KCNJ1* or *SLC12A1* alone may not lead to calcium deficiency. It is worth noting that a large clinical trial of calcium supplementation on adenoma recurrence (6) as well as both the Nurses' Health Study and the Health Professionals Follow-Up Study on colorectal cancer (36) found that high intakes of calcium or calcium supplementation were associated with reduced colon cancer risk or adenoma recurrence only among those who did not use aspirin or NSAIDs. NSAIDs decrease PGE-M concentrations (30). Thus, it is likely that high calcium intakes may be associated with reduced colorectal polyp risk only among those with a higher PGE-M concentration.

This study has several strengths. All of the controls in our study completed a full colonoscopy, and thus potential contamination of cases in the control group is not a major concern. Furthermore, virtually all participants provided a DNA sample.

This study also has limitations. As with all case-control studies, differential recall bias may exist. However, most participants were recruited before colonoscopy, and there were only very few cases identified with a malignant lesion; thus, differential recall bias may be minimized. Selection bias is another concern for case-control studies, but we found that age, sex, and reason for the colonoscopy for consenters and nonconsenters were similar (13). Moreover, most participants were recruited before the colonoscopy, which determines their case or control status, and thus controls are not any less likely to participate than cases. In our study, 57% of all cases had the colonoscopy as a true screening measure with no other indication for the examination other than age. We conducted sensitivity analyses among these subjects and found that the associations were even stronger. Despite this, cautious interpretation of our results is warranted, particularly regarding generalization of our findings from a high-risk population to the general public. The calcium content of drinking water could

not be included in the calculation of calcium intake; we also used the most common calcium quantity in calcium and multivitamin supplements to calculate the total intakes of calcium. These may lead to nondifferential misclassification of calcium intake, which usually biases associations toward the null. We adjusted for many potential confounding factors; however, this still may not eliminate the possibility that other residual confounding factors, or a related dietary pattern, may explain our results. However, it is unlikely in our study that gene-nutrient interactions solely depend on residual confounding factors because there is strong biologic support from previous studies and the interactions were remarkably consistent in both phases I and II as well as for multiple/advanced adenomas. Together with other studies (37), we defined participants with at least 2 adenomas as having multiple adenomas. However, we also considered the more common clinical definition of at least 3 adenomas and found very similar findings (data not shown). One weakness of the study is that the power was not enough to examine if vitamin D status affects calcium-gene interactions in relation to risk of colorectal neoplasia. Future studies are warranted to understand this complex mechanism (38).

Because we oversampled multiple or advanced adenoma cases in phase I, 1 possible explanation for the fact that we only replicated 1 of 5 calcium-SNP interactions is type II error. Although we found that the joint genotypes of *KCNJ1* and *SLC12A1* had a stronger interaction with calcium intake than did *KCNJ1* genotype alone, the interaction between calcium intake and *SLC12A1* genotype alone was not replicated in the phase II study. Future larger studies are needed to further examine the interaction.

Over the past several years, thousands of genetic variants have been successfully identified to be associated with complex diseases in genome-wide association studies. However, most SNPs have a small effect size (39) and account for a small proportion of heritability (40). It is possible that rare functional variants (40) and gene-environment interactions (41) can explain some portion of the missing trait variance. Our findings support the importance of gene-environment interactions in the development of colorectal neoplasia. Future studies, including intervention trials, are necessary to confirm our findings. These results, if confirmed, may provide a new avenue for the personalized prevention of colorectal adenoma and cancer.

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