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Genetic variation in *SLC7A2* interacts with calcium and magnesium intakes in modulating the risk of colorectal polyps

Pin Sun[1,**], **Xiangzhu Zhu**[2,3,**], **Martha J Shrubsole**[2,3], **Reid M Ness**[4], **Elizabeth A Hibler**[2], **Qiuyin Cai**[2], **Jirong Long**[2], **Zhi Chen**[2], **Guoliang Li**[2], **Lifang Hou**[5], **Walter E Smalley**[3,4], **Todd L. Edwards**[2,3], **Edward Giovannucci**[6], **Wei Zheng**[1,2], and **Qi Dai**[1,2,*]

[1]Department of Occupational health and Toxicology, School of Public Health, Fudan University, Shanghai, China 200032

[2]Division of Epidemiology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37203

[3]Geriatric, Research, Education and Clinical Center (GRECC), Department of Veterans Affairs, Tennessee Valley Healthcare System, Nashville, TN 37212

[4]Division of Gastroenterology, Department of Medicine, Vanderbilt University School of Medicine, Nashville, TN 37203

[5]Institute for Public Health and Medicine, Northwestern University, Chicago, IL

[6]Departments of Nutrition and Epidemiology, Harvard School of Public Health, Boston, MA

Abstract

Solute carrier family 7, member 2 (*SLC7A2*) gene encodes a protein called cationic amino acid transporter 2, which mediates the transport of arginine, lysine and ornithine. L-arginine is necessary for cancer development and progression, including an important role in colorectal cancer pathogenesis. Furthermore, previous studies found both calcium and magnesium inhibit the transport of arginine. Thus, calcium, magnesium or calcium:magnesium intake ratio may interact with polymorphisms in the *SLC7A2* gene in associations with colorectal cancer. We conducted a two-phase case-control study within the Tennessee Colorectal Polyps Study. In the first phase, 23 tagging single-nucleotide polymorphisms (SNPs) in the *SLC7A2* gene were included for 725 colorectal adenoma cases and 755 controls. In the second phase conducted in an independent set of 607 cases and 2113 controls, we replicated the significant findings in the first phase. We observed that rs2720574 significantly interacted with calcium:magnesium intake ratio in association with odds of adenoma, particularly multiple/advanced adenoma. In the combined analysis, among those with a calcium:magnesium intake ratio below 2.78, individuals who carried

[*]Correspondence to: Qi Dai, M.D., Ph.D., Vanderbilt Epidemiology Center, Institute for Medicine and Public Health, 2525 West End Avenue, Suite 800, Nashville, TN 37203-1738, Phone: (615) 936-0707, Fax: (615) 343-5938, qi.dai@vanderbilt.edu.

[**]These authors contributed equally to this work.

Disclosure

No author has a potential conflict to disclose.

GC/CC genotypes demonstrated higher odds of adenoma OR (95% CI):1.36(1.11–1.68) and multiple/advanced adenoma OR (95% CI):1.68(1.28, 2.20) than those who carried the GG genotype. The *P* for interactions between calcium:magnesium intake ratio and rs2720574 were 0.002 for all adenoma and <0.001 for multiple/advanced adenoma. Among those with the GG genotype, a high calcium:magnesium ratio was associated with increased odds of colorectal adenoma OR (95%CI): 1.73(1.27–2.36) and advanced/multiple adenomas [1.62(1.05–2.50)]. Whereas, among those with the GC/CC genotypes, high calcium:magnesium ratio was related to reduced odds of colorectal adenoma [0.64(0.42–0.99)] and advanced/multiple adenomas [0.55(0.31–1.00)].

Keywords

SLC7A2; colorectal polyps; gene-nutrient interaction; calcium; magnesium

1. Introduction

Colorectal cancer remains the third most common cancer in men and the second in women worldwide[1]. So far, the molecular mechanism of carcinogenesis and development of colorectal cancer is not fully understood. Attributable to changes in colorectal cancer related risks and the introduction of screening, the incidence and mortality have declined over the past 20 years in the United States[2]. However, it still ranks the second leading cause of cancer death for developed countries and the fourth for developing countries[1]. Thus, novel preventive strategies for colorectal cancer are critically needed.

L-arginine, a semi-essential amino acid, is a substrate for protein biosynthesis and a precursor for nitric oxide and polyamines, which play a crucial role in regulation of cell proliferation and differentiation[3]. Several studies showed that L-arginine was associated with for cancer development and progression[4–9], including colorectal cancer[10]. Recently, an epidemiologic study demonstrated that the concentration of L-arginine and L-citrulline decreased in sera, but accumulated in tumor tissues, from colorectal cancer patients[11]. The evidence suggests that relevant transporters might regulate colorectal cancer development and progression. L-arginine transport into the cell is enabled primarily by cationic amino acid transporters (CATs). There are four confirmed transport proteins for cationic amino acids, and CAT2, encoded by the *Solute carrier family 7, member 2* (*SLC7A2*) gene, is important for transport of L-arginine, lysine and ornithine. However, it is not known whether genetic polymorphisms in the *SLC7A2* gene are associated colorectal cancer development and progression.

A study suggests that both calcium and magnesium inhibit the transport of arginine[12]. Furthermore, observational studies and randomized trials have linked high intake of calcium[13–15] and magnesium[16–19] to a reduced odds and risk of colorectal cancer or polyps respectively. However, results have not been consistent[20–24]. On the one hand, magnesium and calcium have similar structures because they belong to the same family in the periodic table and both respond to calcium sensing receptor[25]. On the other hand, calcium and magnesium may directly or indirectly compete for (re) absorption[26]. Clinical

trials consistently found that high calcium intake leads to significantly increased excretion of magnesium in the urine[27–31]. One previous study found high calcium intake reduced the absorption of calcium and magnesium in the jejunum and ileum[32]. Our previous reports suggest that the calcium:magnesium intake ratio modifies the associations of calcium or magnesium with risk of colorectal adenoma, adenoma recurrence, and cancer [19, 33, 34]. Further, we reported that the calcium:magnesium intake ratio, but not magnesium intake alone interacted with transient receptor potential cation channel, subfamily M, member 7 (TRPM7) gene and *parathyroid horme* (PTH) gene in odds of colorectal neoplasia[19, 35].

However, no studies evaluated potential interactions between dietary intake of calcium, magnesium, and particularly calcium:magnesium intake ratio, and interactions between polymorphisms in *SLC7A2* in associations with colorectal neoplasia. To test this hypothesis, we conducted a two-phase case-control study within the Tennessee Colorectal Polyps Study (TCPS).

2. Materials 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 TCPS, a colonoscopy-based case-control study of colorectal adenoma, hyperplastic polyps and polyp-free controls conducted in Nashville, TN during February 1, 2003, and October 29, 2010. Eligible participants aged 40 to 75 years (n=12,585) were identified from patients scheduled for colonoscopy at the Vanderbilt University Gastroenterology Clinic and the Tennessee Valley Veterans Affairs Health System campus; of them, 7,954 (63%) consented to participate in the TCPS. Excluded from our study were patients who had genetic 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 non-melanoma skin cancers. The detailed description of this study, in addition to case and control definitions, was reported elsewhere [19, 36].

Based on the colonoscopy's results and pathological diagnosis, participants were classified as adenomas or polyp-free controls. To be assigned as a control, the participant must have been polyp free at a complete colonoscopy. Adenoma cases were defined as ≥1 adenomatous polyp. Participants with at least 2 adenomas were considered to have multiple adenomas. Hyperplastic cases had ≥1 hyperplastic polyp and no adenomas. Advanced adenoma cases met at least one of the following criteria: (i) size ≥ 1 cm, (ii) tubulovillous or villous, or (iii) high-grade dysplasia.

2.1 Data and Sample Collection and Assessment

Participants completed a telephone interview on medication use, demographics, medical history, family history, reproductive history, anthropometry, and lifestyle. Participants were also asked to complete a semi-quantitative 108-item food frequency questionnaire (FFQ) which was developed to capture diet in the Southeastern United States[37, 38]. We

compared daily nutrient between the FFQ in the current study and 24 hour dietary recall data in NHANES III for Southerners aged 45 and older. We found intakes of energy and major nutrients are not different [19]. A total of 6,485 participants (82%) completed both the telephone interview and FFQ. The usual dietary intakes of nutrients, including calcium and magnesium, were calculated based on frequency and usual portion size by using race- and sex- specific nutrient databases which were constructed on National Health and Nutrition Examination Survey and US Department of Agriculture food composition tables[38]. Total calcium and magnesium intakes from diet 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 and 100 mg magnesium per multivitamin pill)[19]. We excluded 173 participants from the analyses with more than 10 missing items in the FFQ or unreasonably high (4000 kcal for women and 7000 kcal for men) or low energy intake (less than 500 kcal).

2.2 Biological Samples

Participants recruited at colonoscopy were asked to provide blood, buccal cell or saliva samples (collected by Oragene™ DNA kit, DNA Genotek, Inc). Participants recruited following colonoscopy were also asked to provide buccal cell or saliva samples. 7,443 (98%) participants donated DNA samples.

2.3 Study Design and Genotyping

This is a two-phase design (discovery and replication) candidate-gene study to focus on investigating gene-nutrient (calcium-) interactions among two independent samples of participants from the TCPS. A total of 4200 participants with genotyping and FFQ information were included in the analysis. The discovery phase was conducted among a sample of adenoma cases (N=725) and controls (N=755) from the TCPS. To improve the power in the first phase, we have over-sampled advanced or multiple adenoma cases. The detailed descriptions of genotyping and quality control for the first phase were reported elsewhere[39]. Briefly, Initial genotyping was performed using the Affymetrix Genome-Wide Human SNP Array 5.0 (Affymetrix, Inc., Santa Clara, CA) to assay SNPs in *SLC7A2* gene. Based on our SNP quality control, SNPs were removed if they were missing in greater than 5% of participants, or if the minor allele frequency (MAF) in the samples that passed sample QC was less than 1%. After related and admixed participants were removed, SNPs were removed for major deviations from Hardy-Weinberg equilibrium (HWE) $p<1\times10^{-6}$. Additionally, array SNPs were forced into the Tagster algorithm on the SNPinfo web server (https://snpinfo.niehs.nih.gov/), and additional tag SNPs for the CEU HapMap population were genotyped using the Sequenom Mass Array system in order to tag the gene region and 30kb flanking sequences with r2 = 0.8 and MAF > 0.05. Finally, 23 SNPs was included in our study. In the second phase genotypes of selected SNPs with significant gene-nutrient interactions or direct association in the first-phase were assayed among another independent sample of participants from the TCPS (adenoma cases=607, controls=2113) using Applied Biosystems' OpenArray or Sequenom MassARRAY genotyping assays. These SNPs passed filters for consistency rates (>99%) among replicate QC participants, missing data less than 5%, HWE<0.05 and MAF agreement with the first phase. In addition, we also evaluated interactions by odds of advanced and multiple adenomas.

2.4 Statistical Analysis

Chi-square tests (categorical variables) as well as t-tests or generalized linear models (continuous variables) were used to evaluate case-control differences in the distribution of potential confounding factors. Unconditional multivariable logistic regression models were used to calculate odds ratios (OR) and 95% confidence intervals (95% CIs) to measure the associations adjusting for potential confounders, such as age, sex, race, education, recruitment site, body mass index, smoking status, alcohol drinking 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 1, 2, or 3 to the 1st, 2nd, and 3rd tertile, respectively. Stratified analyses by the calcium:magnesium intake ratio or by genotype were conducted. Tests for interactions between the calcium:magnesium intake ratio and gene polymorphisms in relation to colorectal adenomas risk were evaluated by likelihood ratio tests in logistic regression models. Tests were two-sided and statistical significance level was 0.05 for the first phase analysis. As pre-specified in our original design, one-sided tests at $P \leq 0.05$ (which practically has a significance level of 0.10) were conducted in the second phase because the direction of the gene-nutrient interaction for a given gene variant is provided in the first phase. Secondary analyses were performed to examine whether the polymorphisms modify the associations between calcium and magnesium intakes and the odds of advanced adenoma and multiple adenomas. In addition to the separate analyses in the first and second phase, we have conducted analysis using the combined data of the first and second phase. In the regression model, we have excluded those with missing data. Statistical analyses were performed by using SAS statistical software (version 9.1; SAS Institute, Cary, NC).

3. RESULTS

3.1 Demographic characteristics

Selected demographic characteristics and potential confounding factors were compared between adenoma cases and controls (Supplemental table 1). Overall, compared with controls, cases were older, more likely to be male, smokers and alcohol drinkers, and to have lower educational attainment and physical activity. Additionally, cases were more likely to be overweight or obesity and to have a higher daily intake of total energy, and a lower intake of total calcium as well as magnesium.

3.2 Two-phase Study

Overall, in the first phase, a total of 23 tagging SNPs in *SLC7A2* was included (Supplemental Table 2). We found 6 SNPs significantly interact with calcium intake, 5 SNPs significantly interact with magnesium intake, and 6 SNPs significantly interact with calcium:magnesium intake ratio in relation to risk of colorectal adenoma. In the second phase, we selected 2 SNPs (rs2188021 and rs2720574) to replicate. As it showed in Table 1, only rs2720574 significantly interacted with calcium:magnesium intake ratio in association with odds of adenoma, particularly advanced or multiple colorectal adenomas. On the other hand, no SNP interacting with calcium or magnesium intake in relation to odds of colorectal adenoma was replicated.

3.3 Association with *SLC7A2* Genotype by Calcium/Magnesium Intake Ratio

Shown in Table 2 are the associations between rs2720574 and odds of adenoma stratified by calcium:magnesium intake ratio using combined data from the first and second phase. Overall, *SLC7A2* polymorphism was not significantly associated with odds of adenoma or multiple/advanced adenoma. However, among those whose calcium:magnesium intake ratio was below 2.78, subjects who carried variant allele(s) had a significantly higher odds of adenoma [OR (95% CI) =1.36(1.11–1.68); *P* for trend, 0.01], particularly multiple or advanced adenoma [OR (95% CI) =1.68(1.28, 2.20), *P* for trend, 0.001], compared with those with GG genotype. Conversely, among those whose calcium:magnesium intake ratio was above 2.78, odds of adenoma and multiple/advanced adenoma tended to be lower for those who carried variant allele(s). Overall, our results indicated calcium:magnesium intake ratio significantly interacted with *SLC7A2* polymorphism in relation to odds of adenoma (*P* for interaction, 0.002) and multiple/advanced adenomas (*P* for interaction, 0.0001).

3.4 Association with Calcium/Magnesium Intake Ratio by *SLC7A2* Genotype

Presented in Table 3 is the associations between calcium:magnesium intake ratio and odds of colorectal adenoma stratified by the number of *SLC7A2* gene carrying variant allele. Overall, high calcium:magnesium intake ratio was not associated with odds of adenoma. However, the ratio was associated with higher odds among those with GG genotype and with a reduced odds among with GC/CC genotypes; compared with the lowest tertile, middle and the highest tertile of calcium:magnesium intake ratio were associated with 30% and 73% increased odds of adenoma (*P* for trend, 0.005). Among those with GC/CC genotypes, middle and the highest tertile of ratio were related to 28% and 36% lower odds of adenoma, respectively (*P* for trend, 0.03). Similar associations were observed for multiple or advanced adenomas.

4. Discussion

In this two-phase study, we observed that no SNPs in *SLC7A2* were directly associated with the odds of colorectal adenoma. However, we identified and replicated that rs2720574 polymorphism significantly interacted with calcium:magnesium intake ratio in association with odds of colorectal adenoma, particularly multiple/advanced adenoma. Among those with a calcium:magnesium intake ratio below 2.78, individuals who carried GC/CC genotypes were at a higher odds of adenoma and multiple/advanced adenoma than those who carried the GG genotype. Among those with the GG genotype, a high calcium:magnesium ratio was associated with higher odds of colorectal adenoma and advanced/multiple adenomas whereas, among those with the GC/CC genotypes, high calcium:magnesium ratio was related to lower odds of colorectal adenoma and advanced/multiple adenomas.

CAT2, encoded by *SLC7A2* gene, has two transcript variants encoding different isoforms (i.e. CAT2A and CAT2B). The expression of CAT2B is observed in many different cell types under inflammatory conditions, and CAT2A exhibit a 10-fold lower substrate affinity [40]. Although CAT2 is important for the transport of L-arginine through the cell membrane, a recent epidemiologic study reported that the expression of *SLC7A1*, not *SLC7A2*, was

significantly elevated in colorectal cancer tissues among colorectal cancer patients[11]. This may provide a possible interpretation that no direct association was observed between *SLC7A2* polymorphism and the odds of colorectal adenoma overall. To our knowledge, this is first study to investigate the relationship between *SLC7A2* polymorphisms and colorectal adenomas.

Similar to our previous findings with *TRPM7* and *PTH*[19, 35], we found the calcium:magnesium ratio, instead of magnesium alone, interacted with *SLC7A2* rs2720574 polymorphism in associations with colorectal neoplasia in the present study. The rs2720574 C>G polymorphisms is downstream of the flanking sequence of *SLC7A2* gene. If non-coding SNPs reside within the microRNA-binding site, the SNPs may strengthen or weaken binding to target genes, thus influencing the risk of cancer[41]. This may be one plausible interpretation for our novel finding. Although the precise biological function of this SNP is unclear, the chromosomal location of *SLC7A2* (8p22) is commonly deleted in sporadic colorectal cancer, hepatocellular carcinoma, breast cancer, and non-small cell lung cancers[42]. This suggests that SLC7A2 may play a critical role in cancer progression. Our findings further suggest that for those with the GG genotype (i.e. majority of populations), higher calcium:magnesium ratios are detrimental, whereas among a smaller group of individuals with variant allele(s) (i.e. GC/CC genotypes), higher ratios may be beneficial.

Dietary factors, such as calcium, or magnesium intake are believed to play an important role in colorectal cancer. Earlier epidemiological studies and clinic trials have found high calcium intake[13–15] were associated with a reduced risk of adenoma recurrence and colorectal cancer. However, two recent trials found calcium supplementation or calcium supplementation plus vitamin D did not reduce risk of colorectal cancer or adenoma recurrence[21, 43]. The associations between magnesium intake[16–19] and risk of colorectal cancer or adenoma are also inconsistent[20–24]. One plausible interpretation for the inconsistency is that the interaction between calcium and magnesium was not considered. Consistent with our previous studies[19, 33–35], this study suggested that the calcium:magnesium ratio might modify the association between risk factors and odds of colorectal adenoma, which gave a new evidence to support that calcium:magnesium ratio have an important role in the colorectal cancer.

In addition, we note that the rs2720574 is also located in the first intron of Platelet-derived growth factor receptor-like gene (*PDGFRL*). But previous evidence indicated *SLC7A2,* not *PDGFRL,* is associated with the calcium/magnesium related pathway. Using data from the discovery phase, we found that none of the 34 SNPs in *PDGFRL* significantly interacted with intake of calcium, magnesium or the calcium:magnesium ratio (data not shown). Thus, these findings, together with previous literature, suggests that the rs2720574 interacts with the calcium:magnesium ratio so the link with risk of colorectal adenoma might be associated with SLC7A2, not PDGFRL.

The present study has several strengths. All participants in our study completed a full colonoscopy, which decreases the likelihood of misclassification of disease status. Furthermore, virtually all participants provided a DNA sample. However, this study also has several limitations. As with all case-control studies, differential recall bias may exist. Yet

most participants were recruited prior to diagnosis, and only a few cases were identified as having a malignant lesion; thus, non-differential recall bias may be minimized. Selection bias is another concern for case-control studies, but we have found that age, sex, and reason for the colonoscopy for consenters versus non-consenters are similar[19]. Moreover, most participants are recruited prior to the colonoscopy, which determines their case or control status, and, thus, controls are not any less likely to participate than cases. Despite this, cautious interpretation of our results is warranted, particularly regarding generalization of our findings from high-risk population to general populations. The criterion used for calcium and magnesium intakes is another limitation of our study. We have used the most common calcium or magnesium ingredients in the calcium and multivitamin supplements to calculate the total intakes of calcium and magnesium. The magnesium content of drinking water could not be included in the calculation of magnesium intake. This may lead to non-differential misclassification of calcium and magnesium intakes, which usually biases associations toward the null. We have adjusted for many potential confounding factors, but that may not eliminate the possibility that other residual confounding factors, or a related dietary pattern, could explain our results. In addition, we included 23 tagging SNPs in the *SLC7A2* in our investigation. However, an early study indicated that tagging SNPs may not be efficient when the allele frequency at the marker locus is much different from the allele frequency at the disease locus [44]. Thus, further studies are warranted to understand the functional significance of the SNP and fine-map the underlying functional SNPs.

In summary, we demonstrated that calcium:magnesium ratio, instead of magnesium alone, interacted with *SLC7A2* polymorphism in associations with colorectal neoplasia. Our study is pertinent to colorectal adenoma, but colorectal cancer is believed to arise from adenomatous polyps via the well-established adenoma-carcinoma sequence [45]. Thus this finding, together with our previous finding on *TRPM7* and *PTH* [19, 35], indicate that the calcium:magnesium ratio may play a more important role, compared to magnesium alone, in colorectal carcinogenesis. Future studies, including functional researches about the genes located at the 8p22 chromosomal region, are necessary to confirm our findings. These results, if confirmed, may provide a new avenue for the prevention of colorectal cancer, particularly in Western populations with high calcium:magnesium intake ratios.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CATs	cationic amino acid transporters
FFQ	food frequency questionnaire
MAF	minor allele frequency
SLC7A2	Solute carrier family 7, member 2
SNP	single-nucleotide polymorphism
TCPS	the Tennessee Colorectal Polyp Study

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Table 1

Interaction of *SLC7A2* and calcium/magnesium intake ratio (continuous) with colorectal adenoma risk, the Tennessee Colorectal Polyp Study

SLC7A2 genotype	Phase	Cases/Controls	MAF	*P* for interaction[a]		
				calcium	magnesium	calcium/magnesium
All adenomas						
rs2720574	I	725/755	0.18/0.17	**0.040**	**0.019**	**0.030**
	II	607/2113	0.23/0.22	0.622	0.477	0.090
	I &II	1332/2868	0.21/0.20	0.172	0.210	**0.002**
rs2188021	I	725/755	0.35/0.36	0.096	**0.032**	0.817
	II	548/2061	0.37/0.38	0.055	0.563	**0.033**
	I &II	1276/2818	0.36/0.38	0.181	0.181	0.263
Advanced or multiple adenomas						
rs2720574	I	359/755	0.19/0.17	0.022	0.281	**0.009**
	II	230/2113	0.25/0.22	0.114	0.588	**0.010**
	I &II	589/2868	0.21/0.20	0.023	0.567	**<0.001**
rs2188021	I	360/755	0.35/0.36	0.336	0.068	0.773
	II	186/2061	0.37/0.38	0.224	0.113	0.091
	I &II	546/2818	0.36/0.38	0.127	0.065	0.032

[a]Interactions between genetic polymorphism and calcium/magnesium intake ratio (continuous) using likelihood ratio test adjusting for age (continuous), sex, race (white, others), education (categorical), recruitment sites, body mass index (categorical), smoking status (former, current, never), alcohol drinking status (former, current, never), physical activity (yes, no), and daily intakes of total energy, magnesium or calcium (continuous).

Table 2

Odds ratios (ORs) (and 95% CIs) for colorectal adenoma according to rs2720574 polymorphism stratified by calcium/magnesium intake ratio, the Tennessee Colorectal Polyp Study[a,b]

SLC7A2	Adenomas vs Controls			Multiple/advanced adenomas vs Controls		
rs2720574	**Cases/Controls**	**OR(95% CI)**	***P* for trend**	**Cases/Controls**	**OR(95% CI)**	***P* for trend**
All subjects						
GG	851/1900	1.00		365/1900	1.00	
GC/CC	481/968	1.09(0.94,1.26)		224/968	1.20(0.98,1.46)	
GC	408/835	1.10(0.94,1.28)		189/835	1.22(0.99,1.50)	
CC	73/133	1.16(0.83,1.62)	0.26	35/133	1.39(0.90,2.15)	0.07
Calcium/magnesium intake ratio ≤ 2.78						
GG	451/909	1.00		190/909	1.00	
GC/CC	297/435	1.36(1.11,1.68)		146/435	1.68(1.28,2.20)	
GC	254/369	1.37(1.11,1.69)		127/369	1.71(1.29,2.26)	
CC	43/66	1.24(0.78,1.97)	0.01	19/66	1.57(0.85,2.92)	0.001
Calcium/magnesium intake ratio > 2.78						
GG	400/991	1.00		175/991	1.00	
GC/CC	184/533	0.84(0.67,1.04)		78/533	0.78(0.57,1.06)	
GC	154/466	0.85(0.67,1.06)		62/466	0.76(0.54,1.05)	
CC	30/67	1.12(0.69,1.82)	0.25	16/67	1.34(0.72,2.49)	0.38
P for interaction[c]			0.002			0.0001

[a] Phase I +Phase II

[b] Unconditional logistic regression models adjusting for age (continuous), sex, race (white, others), education (categorical), recruitment sites, body mass index (categorical), smoking status (former, current, never), alcohol drinking status (former, current, never), physical activity (yes, no), daily intakes of total energy, calcium and magnesium (continuous).

[c] Interactions for genetic polymorphism with calcium/magnesium intakes ratio (continuous).

Table 3

Odds ratios for colorectal adenoma according to intakes of calcium and magnesium stratified by rs2720574 genotype of *SLC7A2*, the Tennessee Colorectal Polyp Study[a,b]

***SLC7A2* genotype (rs2720574)**		**Tertile Calcium/Magnesium Intake ratio**			
		T1(<2.45)	**T2(2.45–3.37)**	**T3(≥3.37)**	***P* for trend**
All adenomas vs controls					
All subjects	Cases/Controls	540/957	450/969	342/942	
	OR (95% CI)	1.00	1.05(0.88–1.26)	1.21(0.95–1.55)	0.14
GG	Cases/Controls	316/650	293/641	242/609	
	OR (95% CI)	1.00	1.30(1.03–1.64)	1.73(1.27–2.36)	0.0005
GC/CC	Cases/Controls	224/307	157/328	100/333	
	OR (95% CI)	1.00	0.72(0.53–0.98)	0.64(0.42–0.99)	0.03
p for interaction			0.002		
Multiple/advanced adenomas vs controls					
All subjects	Cases/Controls	237/957	213/969	139/942	
	OR (95% CI)	1.00	1.14(0.89–1.45)	1.12(0.80–1.58)	0.44
GG	Cases/Controls	131/650	135/641	99/609	
	OR (95% CI)	1.00	1.43(1.04–1.97)	1.62(1.05–2.50)	0.02
GC/CC	Cases/Controls	106/307	78/328	40/333	
	OR (95% CI)	1.00	0.78(0.53–1.17)	0.55(0.31–1.00)	0.05
p for interaction			0.0001		

[a] Phase I +Phase II

[b] Unconditional logistic regression models adjusting for age (continuous), sex, race (white, others), education (categorical), recruitment sites, body mass index (categorical), smoking status (former, current, never), alcohol drinking status (former, current, never), physical activity (yes, no), daily intakes of total energy, magnesium or calcium (continuous)

[c] Interactions for genetic polymorphism with calcium/magnesium intake ratio (continuous)