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Calcium/magnesium intake ratio, but not magnesium intake, interacts with genetic polymorphism in relation to colorectal neoplasia in a two-phase study

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Abstract

Background—Some studies suggest that the calcium to magnesium ratio intakes modifies the associations of calcium or magnesium with risk of colorectal adenoma, adenoma recurrence and cancer. Parathyroid hormone (PTH) plays a key role in the regulation of homeostasis for both calcium and magnesium. We hypothesized that polymorphisms in *PTH* and 13 other genes may modify the association between the calcium/magnesium intake ratio and colorectal neoplasia risk.

Methods—We conducted a two-phase study including 1,336 cases and 2,891 controls from the Tennessee Colorectal Polyp Study.

Results—In Phase I, we identified 19 SNPs that significantly interacted with the calcium/magnesium intake ratio in adenoma risk. In Phase II, rs11022858 in *PTH* was replicated. In

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Competing interests

The authors declare that they have no competing interests.

Authors' contribution

X.Z., Q.D. designed and drafted the manuscript. X.Z. carried out the statistical analysis. M.J.S., T.L.E., Z. C., R.M.N., W.E.S. and W.Z. contributed to acquisition of data and human specimens. Q.C., J.L., G.L. contributed to the genotyping. All authors contributed to data interpretation, and the critical review of the final manuscript. All authors read and approved the final version of the manuscript.

combined analysis of phases I and II, we found high calcium/magnesium intake ratio tended to be associated with a reduced risk of colorectal adenoma (p for trend, 0.040) among those who carried the TT genotype in rs11022858. In stratified analyses, calcium intake (>1000 mg/day) was significantly associated with 64% reduced adenoma risk (OR=0.36 (95% CI: 0.18–0.74)) among those homozygous for the minor allele (TT genotype) (p for trend, 0.012), but not associated with risk in other genotypes (CC/TC). Conversely, we found highest magnesium intake was significantly associated with 27% reduced risk (OR=0.73 (95% CI: 0.54–0.97)) of colorectal adenoma (p for trend, 0.026) among those who possessed the CC/TC genotypes, particularly among those with the TC genotype; whereas magnesium intake was not linked to risk among those with the TT genotype.

Conclusions—These findings, if confirmed, will help for the development of personalized prevention strategies for colorectal cancer.

Keywords

PTH; colorectal neoplasia; gene-nutrient interaction; calcium; magnesium

BACKGROUND

Colorectal cancer remains the fourth most common incident cancer and the second most common cause of cancer death in the US (1). Thus, development of novel preventive strategies is critically needed. It has been postulated that intake of calcium may neutralize the toxic effect of free fatty acids and bile acids (2) and reduce proliferation of human rectal epithelial cells (3) or inhibit colorectal tumors through the calcium sensing receptor (4). Magnesium, the most abundant intracellular divalent cation in the body, plays an essential role in over 300 biological activities (5), including inflammatory responses related to colorectal carcinogenesis (6–10). Several studies have linked high intake of calcium (11–13), magnesium (14–18) to a reduced risk of colorectal cancer or polyps. Nevertheless, results have not been consistent (19–21).

Adequate body magnesium status is not only determined by magnesium intake, but also intestinal absorption and renal reabsorption (22). Magnesium and calcium have similar structures because they belong to the same family in the periodic table and share the homeostatic regulating system (23) and both respond to calcium sensing receptor (24). Moreover, although the changes in blood or colon lumen concentrations of ionized calcium and magnesium are monitored by the same mechanism—the calcium-sensing receptor—the potency of ionized magnesium in binding to the receptor is only one-half to one-third that of calcium (14). Thus, calcium and magnesium may directly or indirectly compete for (re) absorption (24). One previous study found high calcium intake reduced the absorption of calcium and magnesium in the jejunum and ileum. The ileum is more sensitive than the jejunum to variations in dietary calcium intake (25).

Some (24–26), but not all (27) previous human studies indicate that high calcium intake may affect the absorption rate of magnesium. It is known that over 80% of plasma magnesium is ultrafiltrated and reabsorbed in the kidneys. Thus, kidney reabsorption plays a key role in regulating magnesium homeostasis (22). Likewise, ten grams of calcium are filtered daily on

average, of which 98% is reabsorbed in the kidneys (28,29). Thus far, clinical trials consistently found high calcium intake leads to significantly increased excretion of magnesium in the urine (22,30–33). Furthermore, some previous reports suggest that the ratio of calcium to magnesium intakes (calcium/magnesium intake ratio) modifies the associations of calcium or magnesium with risk of colorectal adenoma, adenoma recurrence and cancer (14,34,35).

We previously reported that the calcium/magnesium intake ratio interacted with a functional polymorphism in the gene of transient receptor potential melastatin 7 (TRPM7), a transporter essential for magnesium absorption and homeostasis (22,36), in relation to risk of both adenoma and hyperplastic polyps (14). In this two-phase study, we examined whether intake of magnesium and the calcium/magnesium intake ratio interacted with common tagging single nucleotide polymorphisms (SNPs) in fourteen candidate genes critically related to the (re)absorption and homeostasis for both calcium and magnesium.

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 polyps and polyp-free controls conducted in Nashville, TN during February 1, 2003, to October 29, 2010. 12,585 eligible participants aged 40 to 75 years 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 TCPS. The detailed description of this study is available elsewhere (14,37).

Based on 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 one adenoma.

Data and Sample Collection and Assessment

6,482 (81%) 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 specifically developed to capture diet in the Southeastern United States (38,39). 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. (14). 6,485 participants (82%) completed the FFQ.

The usual dietary intakes of nutrients, including calcium, magnesium were calculated based on frequency and usual portion size by using race- and sex-specific nutrient databases which

were constructed based on National Health and Nutrition Examination Survey and US Department of Agriculture food composition tables (39). Supplemental calcium, magnesium intake from calcium and multivitamin supplements has been calculated (40).

Biological Samples

Participants recruited at colonoscopy were asked to provide blood, buccal cell or saliva samples (using Oragene™ DNA kit, DNA Genotek, Inc.). Participants recruited following colonoscopy were asked to provide buccal cell or saliva samples. 7,443 (94%) participants donated DNA samples.

Study Design and Genotyping

This is a two-phase design (discovery and replication) candidate-gene study to focus on investigating gene-nutrient interactions among two independent samples of participants from the TCPS. A total of 4,227 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. The detailed descriptions of sample selection, genotyping and quality control for Phase I are available elsewhere (41). The candidate-gene studies based on the TCPS samples were coordinated to perform the Affymetrix Genome-Wide Human SNP Array 5.0 (Affymetrix, Inc.) assays. 126 tagging SNPs in 14 genes, including *TRPM7* (22), *TRPM6* (42), parathyroid hormone (PTH) (43), parathyroid hormone receptor 1 (PTH1R) (43), calcium sensing receptor (CaSR) (23), vitamin D receptor (VDR) (43–46), claudin-16 (CLDN16) (22), FXYD domain containing ion transport regulator 2 (FXYD2) (22), chloride channel KB (CLCNKB), transient receptor potential cation channel subfamily V (TRPV) member 5, 6 (TRPV5, TRPV6), potassium inwardly rectifying channel, subfamily J, member 1 (KCNJ1) (40), and solute carrier family 12 (sodium-chloride transporters), member 1,3 (SLC12A1, SLC12A3) (22) were evaluated. In Phase II, genotypes of selected SNPs with statistically 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) 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%, a minor allele frequency (MAF) of < 1%, Hardy-Weinberg equilibrium $p < 1 \times 10^{-6}$ (41). In Phase II, these SNPs passed filters for consistency rates (>99%) among replicate quality controls participants, missing data less than 5%, 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 evaluate case-control differences in the distribution of potential confounding factors (Table 1).

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, including age, sex, race, education, recruitment site, body mass index, smoking status, alcohol drinking status, physical activity, and daily intakes of total energy,

calcium, magnesium because they significantly differed by case-control status in Table 1. Tests for trend across tertiles were performed in logistic regression models by including a variable with an assigned score of 1, 2, or 3 to the 1st, 2nd, and 3rd tertile, respectively. Stratified analyses by genotype were conducted. Tests for interactions between intakes of calcium, magnesium and calcium/magnesium ratio with the gene polymorphisms in relation to colorectal adenomas risk were conducted in logistic regression models by likelihood ratio tests. In Table 2, we present the main results for the nutrients-*PTH* interactions in Phase I, Phase II, phases I and II combined. To control for multiple comparisons, we have used the two-phase design. Among two SNPs in the *PTH* interacting with calcium/magnesium intake ratio in phase I, we have selected one tagging SNP and examined its interaction with calcium/magnesium ratio in the phase II. Thus, it remained significant after considering multiple comparisons. Tests were two-sided and statistical significance level was 0.05 for Phase I analysis. As pre-specified in our original design, one-sided tests at $p = 0.05$ were conducted in Phase II because the direction of the gene-nutrient interaction for a given gene variant is provided in Phase I. Calcium, magnesium and the calcium to magnesium ratio intake were analyzed as continuous variables and additive genetic models were used for genetic variants. In addition to the separate analyses in Phase I and Phase II, we have conducted analysis using the combined data of phases I and II. We have also conducted analysis to show the joint effects of intakes of calcium, magnesium and rs11022858 genotype of *PTH*. In the regression model, we have excluded those with missing data. Statistical analyses were performed by using SAS statistical software (version 9.3; SAS Institute, Cary, NC).

RESULTS

Covariates by cases and controls

Selected demographic characteristics and potential confounding factors were compared between cases and controls in both discovery and replication phases (Table 1). Compared with controls, cases were older and were more likely to be male, smokers and alcohol drinkers, and to have lower educational attainment, higher BMI (body mass index), higher intake of total energy, and lower intakes of calcium and magnesium, and were less likely to be physical active in the past ten years.

Two-phase Study—A total of 126 tagging SNPs in fourteen candidate genes critically involved in the (re)absorption and homeostasis for both calcium and magnesium were included in the study (Figure 1). In Phase I discovery, 5 SNPs were found directly associated with the risk of colorectal adenoma, 5 SNPs were found to significantly interact with calcium intake, 3 SNPs interact with magnesium intake, and 19 SNPs were found to significantly interact with the calcium/magnesium ratio intake in relation to risk of colorectal adenoma (Supplemental Table 1). In Phase II replication, one SNP (rs11022858) in *PTH* (Table 2) significantly interacted with the calcium/magnesium intake ratio and another SNP (rs2855798) in *KCNJ1* significantly interacted with calcium intake (40) in association with risk of colorectal adenoma (Figure 1). On other hand, no SNP directly associated with colorectal adenoma, no SNP interacting with magnesium intake was replicated.

In the combined analysis of discovery and replication phases, the significant interaction still remained with the tagging SNP (rs11022858) in *PTH*. Thus, only rs11022858 in *PTH* was included in subsequent analyses.

Associations with Nutrient Intake by Genotype—Presented in Table 3 are the associations between total calcium intake and risk of colorectal adenoma stratified by *PTH* genotype (rs11022858) in the combined analysis of discovery and replication phases. It showed that a high calcium/magnesium intake ratio tended to be associated with a reduced risk of colorectal adenoma (*p* for trend, 0.040) among those who carried the TT genotype in rs11022858 of the *PTH* gene, but was not related to risk among those carrying the CC/TC genotypes. In additional stratified analyses, intake of calcium (i.e. 1000 mg/day) was significantly associated with a 64% reduced risk of adenoma among those homozygous for the minor allele (TT) (*p* for trend, 0.012). However, high intake of calcium was not related to risk among those with other genotypes (CC/TC). Conversely, high intake of magnesium, particularly intake levels 320 mg/day was significantly associated with 27% reduced risk of colorectal adenoma (*p* for trend, 0.026) among those who possessed the CC/TC genotypes, particularly among those with the TC genotype (38% reduced risk, *p* for trend, 0.013); whereas high intake of magnesium was not linked to risk among those with the TT genotype. We also conducted analyses using a different reference category. The results were stable. Similar results were found with risk of distal adenomas, but not proximal adenomas. The joint associations of intakes of calcium, magnesium, calcium/magnesium ratio and *PTH* genotype were shown in Supplemental Table 2. In addition, we found that higher intake of magnesium from dietary source was associated with a reduced risk of adenomas only among those who carry CC/TT genotypes in *PTH* (*P*-trend = 0.004; Supplemental Table 3).

In the current study, we did not find *PTH* polymorphism significantly modified the association between intakes of calcium, magnesium and calcium/magnesium intake ratio and risk of hyperplastic polyp, advanced or multiple adenomas (data not shown).

DISCUSSION

In this two-phase study including 126 SNPs in 14 genes, we identified and replicated one SNP in the *PTH* (rs11022858) gene that significantly interacted with the calcium/magnesium intake ratio in association with colorectal adenoma. Furthermore, we found high calcium intake was significantly associated with 64% reduced risk of colorectal adenoma only among those who carried the TT genotype in the *PTH* gene while high magnesium intake was significantly related to 27% reduced risk of adenoma solely among those with the CC/TC genotype of *PTH*. A similar pattern was observed for both Caucasians and African Americans (data not shown). We did not replicate any SNP interacting with magnesium intake.

Calcium sensing receptor (CaSR) senses minute variations in extracellular concentrations of Ca^{2+} ion and functions to tightly regulate systemic Ca^{2+} and modulate *PTH* secretion (47,48). Furthermore, CaSR also senses the magnesium concentrations, although with two to three-fold less potency (23). Mutations in *PTH* and *CaSR* result in a lower set point for plasma ionized calcium and magnesium. This, in turn, leads to reduced *PTH* secretion and

renal reabsorption, and increased urinary excretion of calcium and magnesium (22). In addition to the effect of PTH on magnesium and calcium homeostasis, it was observed in previous studies that magnesium deficiency leads to impaired PTH response, reduced concentrations of 1,25 (OH)₂ vitamin D (the active form of vitamin D) and Ca²⁺ (43). Thus, these previous findings indicate in collaboration with *CaSR* or others, *PTH* modulates not only magnesium and calcium, but also their balance. Our findings of the interaction between the calcium/magnesium intake ratio and PTH polymorphism have strong biological plausibility. However, unlike rare mutations, common polymorphisms alone may not lead to increased risk of colorectal adenoma. Only those who carried susceptible alleles and had low intake of calcium or magnesium are at increased risk. It is also possible that among subjects who carry the TT genotype in *PTH*, a higher calcium intake is needed whereas among those with CC/TT genotypes, a higher magnesium intake is required.

In addition to a PTH polymorphism, we previously reported an interaction between the calcium/magnesium intake ratio and a functional SNP (rs8042919) in the *TRPM7* gene in relation to risk of colorectal adenoma and hyperplastic polyp (14). The findings from the current study provide additional support for the interaction. It was only recently that Mg²⁺ was thought to share ion channels with Ca²⁺ (22). Genetic studies in recent years identified *TRPM7*, an ubiquitously expressed constitutive ion channel with a higher affinity for Mg²⁺ than Ca²⁺ (22), as a more specific ion channel for Mg²⁺ homeostasis (36). *TRPM7* was found to form a functional ion channel complex with *TRPM6*, contributing essentially to epithelial magnesium (re)absorption (42). Therefore, our findings are not likely to have occurred by chance, but have strong support from biological evidence. Our findings indicate that *PTH* and *TRPM7* play key roles in maintaining the balance of calcium to magnesium, including in relation to colorectal carcinogenesis.

Virtually all participants have provided a DNA sample. Furthermore, all controls in our study completed a full colonoscopy, and, thus, our control group is not likely to be contaminated by cases. However, this study has limitations. As with all case-control studies, differential recall bias may exist. However, most participants were recruited prior to colonoscopy and there were only very few cases identified with a malignant lesion (excluded from the analysis in the current study), thus, 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 (14). The prevalence of adenoma was slightly higher in one of the medical centers in comparison to the other medical center. In stratified analysis by study site, the findings did not differ by site. Moreover, most participants were recruited prior to the colonoscopy which determines their case or control status, and, thus, potential selection bias by diagnosis is minimized. 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 have conducted sensitivity analyses among these subjects and found similar associations. The calcium or magnesium content of drinking water could not be included; also we have used the most common quantity in calcium and multivitamin supplements to calculate the total intakes of calcium or magnesium. These may lead to non-differential misclassification of calcium intake, which usually biases associations toward the null. We have adjusted for many potential confounding factors. 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 a strong biological support from previous studies for both interactions with the PTH and TRPM7 polymorphisms and the interactions were remarkably consistent in both phases I and II.

Rs11022858 has not been reported in any previous studies. It does not locate in coding region and functional significance is not clear. Further, the current study is the first to examine the potential interaction of this SNP with magnesium, calcium or calcium/magnesium ratio. Thus, future functional studies or fine mapping studies are needed to understand the functional significance or identify the underlying functional SNPs in LD with rs11022858.

Our findings indicate *PTH* together with *TRPM7* play essential roles in regulating the calcium/magnesium intake ratio and, thus, the calcium/magnesium balance. Also, our findings support the importance of gene (*PTH* and *TRPM7*)-nutrient interactions in the development of colorectal neoplasia. Future studies, including intervention trials, are necessary to confirm our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CaSR	Ca-sensing receptor
CLCNKB	chloride channel KB
CLDN16	claudin-16
FFQ	food frequency questionnaire
FXYP2	FXYP domain containing ion transport regulator 2
GWAS	genome-wide association studies
KCNJ1	potassium inwardly rectifying channel, subfamily J, member 1

MAF	minor allele frequency
PTH	parathyroid hormone
PTH1R	parathyroid hormone receptor 1
SLC12A1, SLC12A3	solute carrier family 12 member 1, 3
SNP	single-nucleotide polymorphism
TCPS	the Tennessee Colorectal Polyp Study
TRPM6, TRPM7	Transient receptor potential cation channel, subfamily M, member 6, 7
TRPV5, TRPV6	transient receptor potential cation channel subfamily V (TRPV) member 5, 6
VDR	vitamin D receptor

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Figure 1.

Phase I to II for 126 tagging SNPs in 14 genes directly or interacted with Ca, Mg, and/or Ca/Mg intakes associations with colorectal adenoma risk. *Presented in Table S1.

Table 1

Comparison of cases and controls by selected descriptive characteristics, the Tennessee Colorectal Polyp Study¹

Characteristic	Adenoma Cases (N=1336)	Controls (N=2891)	P-value
Age (years), Mean $\pm$ SD	59.5 $\pm$ 7.2	57.6 $\pm$ 7.6	<0.001
Sex, %			<0.001
Man	997 (74.7)	1619 (56.0)	
Race, %			0.27
White	1181 (88.5)	2590 (89.6)	
Recruitment sites, %			<0.001
Vanderbilt Medical Center	780 (58.4)	2159 (74.7)	
Education, %			<0.001
High school or below	426 (33.2)	620 (22.3)	
Some college	373 (29.0)	777 (27.9)	
College graduates	235 (18.3)	611 (22.0)	
Graduates	251 (19.5)	772 (27.8)	
Smoking status, %			<0.001
Never smoker	464 (36.0)	1517 (54.4)	
Former smoker	481 (37.3)	975 (34.9)	
Current smoker	343 (26.6)	298 (10.7)	
Alcohol consumption status, %			<0.001
Never drinker	633 (49.2)	1672 (60.0)	
Former drinker	370 (28.7)	568 (20.4)	
Current drinker	284 (22.1)	546 (19.6)	
Physically active past 10 years, % ²	665 (51.6)	1633 (58.5)	<0.001
BMI (kg/m ²), Mean $\pm$ SD	28.6 $\pm$ 5.5	27.9 $\pm$ 5.7	<0.001
Daily nutrients intake, Mean $\pm$ SD			
Total energy (kcal) ³	1827 $\pm$ 47	1791 $\pm$ 44	0.15
Total calcium (mg) ⁴	969 $\pm$ 24	1054 $\pm$ 26	<0.0001
Dietary calcium (mg) ⁴	766 $\pm$ 16	824 $\pm$ 17	<0.0001
Supplement calcium (mg) ³	262 $\pm$ 24	268 $\pm$ 23	0.59
Total magnesium (mg) ⁴	341 $\pm$ 6	363 $\pm$ 7	<0.0001
Dietary magnesium (mg) ⁴	301 $\pm$ 5	318 $\pm$ 5	<0.0001
Supplement magnesium(mg) ³	75 $\pm$ 5	79 $\pm$ 5	0.09

¹Phase I +Phase II

²Regularly engage in exercise activities over the past 10 years: at least two hours per week continuously for at least six months in a row, exclude activities which were part of your job or house work

³Least square mean value $\pm$ SE, adjusting for age (year), site, sex, and race;

⁴Least square mean value $\pm$ SE, adjusting for age (year), site, sex, race and total energy

Table 2

Interaction of rs11022858 of *PTH* and the calcium, magnesium or calcium/magnesium intake ratio with colorectal adenoma risk, the Tennessee Colorectal Polyp Study

Adenomas	Phase	Cases/Controls	MAF	<i>P</i> for interaction [†]		
				calcium	magnesium	calcium/magnesium
<i>PTH</i> , rs11022858	I	728/757	0.367/0.367	0.469	0.905	0.040
	II	546/2062	0.411/0.386	0.340	0.117	0.050
	I & II	1274/2819	0.386/0.380	0.239	0.415	0.050
<i>PTH</i> , rs11600801	I	725/751	0.162/0.151	0.839	0.707	0.603
	II	–	–	–	–	–
<i>PTH</i> , rs10766086	I	728/757	0.368/0.367	0.433	0.915	0.036
	II	–	–	–	–	–

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

Odds ratios (95% CI) for colorectal adenoma according to intakes of calcium and magnesium stratified by rs11022858 genotype of *PTH*, the Tennessee Colorectal Polyp Study¹⁻³

Table 3

<i>PTH</i> genotype (rs11022858)		All adenomas vs controls			Distal adenomas vs controls		
		<1.7	1.7-2.6	2.6	<1.7	1.7-2.6	2.6
Calcium/magnesium							
All	Cases/Controls	67/127	544/993	663/1699	42/127	321/993	396/1699
	OR (95% CI)	1.00	1.11(0.80-1.55)	0.96(0.69-1.33)	1.00	1.04(0.71-1.53)	0.93(0.63-1.37)
CC/TC	Cases/Controls	54/111	460/840	571/1448	33/111	277/840	340/1448
	OR (95% CI)	1.00	1.23(0.85-1.79)	1.09(0.75-1.59)	1.00	1.26(0.67-2.37)	1.12(0.59-2.11)
CC	Cases/Controls	29/55	199/388	251/648	19/55	125/388	153/648
	OR (95% CI)	1.00	1.19(0.70-2.02)	1.09(0.64-1.85)	1.00	1.36(0.71-2.62)	1.17(0.60-2.25)
TC	Cases/Controls	25/56	261/452	320/800	14/56	152/452	187/800
	OR (95% CI)	1.00	1.30(0.76-2.23)	1.13(0.66-1.93)	1.00	1.14(0.54-2.41)	1.08(0.51-2.28)
TT	Cases/Controls	13/16	84/153	92/251	9/16	44/153	56/251
	OR (95% CI)	1.00	0.74(0.31-1.74)	0.52(0.22-1.23)	1.00	0.61(0.23-1.63)	0.53(0.20-1.41)
Total calcium intake (mg/d)							
All	Cases/Controls	185/362	367/708	722/1749	111/362	220/708	428/1749
	OR (95% CI)	1.00	0.95(0.74-1.22)	0.74(0.56-0.98)	1.00	0.94(0.70-1.27)	0.71(0.51-1.00)
CC/TC	Cases/Controls	141/303	322/592	622/1504	85/303	191/592	374/1504
	OR (95% CI)	1.00	1.14(0.86-1.52)	0.88(0.64-1.21)	1.00	1.11(0.79-1.57)	0.83(0.56-1.23)
CC	Cases/Controls	60/146	132/260	287/685	35/146	85/260	177/685
	OR (95% CI)	1.00	1.19(0.78-1.83)	1.01(0.63-1.62)	1.00	1.30(0.77-2.19)	1.05(0.59-1.89)
TC	Cases/Controls	81/157	190/332	335/819	50/157	106/332	197/819
	OR (95% CI)	1.00	1.09(0.74-1.61)	0.81(0.52-1.26)	1.00	1.00(0.63-1.59)	0.73(0.43-1.25)
TT	Cases/Controls	44/59	45/116	100/245	26/59	29/116	54/245
	OR (95% CI)	1.00	0.43(0.23-0.80)	0.36(0.18-0.74)	1.00	0.49(0.23-1.05)	0.38(0.16-0.91)
Total magnesium intake (mg/d)							
All	Cases/Controls	219/492	192/434	863/1893	126/492	116/434	517/1893
	OR (95% CI)	1.00	0.96(0.74-1.23)	0.80(0.62-1.04)	1.00	1.01(0.74-1.38)	0.83(0.60-1.14)
CC/TC	Cases/Controls	184/409	161/361	740/1629	106/409	96/361	448/1629
	OR (95% CI)	1.00	0.96(0.74-1.23)	0.80(0.62-1.04)	1.00	1.01(0.74-1.38)	0.83(0.60-1.14)

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PTH genotype (rs11022858)		All adenomas vs controls				Distal adenomas vs controls			
CC	OR (95% CI)	1.00	0.89(0.67–1.19)	0.73(0.54–0.97)	0.026	1.00	0.96(0.68–1.36)	0.79(0.55–1.13)	0.168
	Cases/Controls	68/183	73/178	338/730		37/183	47/178	213/730	
TC	OR (95% CI)	1.00	0.96(0.62–1.50)	0.91(0.57–1.43)	0.670	1.00	1.24(0.72–2.15)	1.20(0.68–2.12)	0.595
	Cases/Controls	116/226	88/183	402/899		69/226	49/183	235/889	
TT	OR (95% CI)	1.00	0.86(0.59–1.27)	0.62(0.42–0.92)	0.013	1.00	0.83(0.52–1.33)	0.60(0.38–0.97)	0.032
	Cases/Controls	35/83	31/73	123/264		20/83	20/73	69/264	
	OR (95% CI)	1.00	1.56(0.78–3.10)	1.65(0.78–3.49)	0.213	1.00	1.65(0.71–3.81)	1.43(0.58–3.51)	0.505

¹Phase I + Phase II

²Unconditional 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)

³The P values for the interactions between calcium/magnesium intake ratio, calcium and magnesium intake (continuous) with rs11022858 genotype were 0.050, 0.239, 0.415 for all adenomas and 0.121, 0.209, 0.377 for distal adenomas.