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No evidence of gene-calcium interactions from genome-wide analysis of colorectal cancer risk

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Abstract

Background—Calcium intake may reduce risk of colorectal cancer (CRC), but the mechanisms remain unclear. Studies of interaction between calcium intake and single nucleotide polymorphisms (SNPs) in calcium-related pathways have yielded inconsistent results.

Methods—To identify gene-calcium interactions, we tested interactions between ~2.7 million SNPs across the genome with self-reported calcium intake (from dietary or supplemental sources) in 9,006 CRC cases and 9,503 controls of European ancestry. To test for multiplicative interactions, we used multivariable logistic regression and defined statistical significance using the conventional genome-wide α=5E-08.

Results—After accounting for multiple comparisons, there were no statistically significant SNP-interactions with total, dietary, or supplemental calcium intake.

Conclusions—We found no evidence of SNP-interactions with calcium intake for CRC risk in a large population of 18,509 individuals.

Impact—These results suggest that in genome-wide analysis common genetic variants do not strongly modify the association between calcium intake and CRC in European populations.

Observational studies suggest higher calcium intake may reduce risk of colorectal cancer (CRC) (1, 2); however, the underlying mechanisms remain unclear (2). Gene-environment interaction (GxE) analysis can provide insight into disease pathways (3, 4). Studies of gene-calcium interactions for CRC have focused on single nucleotide polymorphisms (SNPs) in calcium-related pathways with limited success (5, 6).

The availability of genome-wide SNP data (7) now enables hypothesis-free GxE searches. This method recently identified a novel gene-processed meat interaction for CRC (3)—highlighting the potential of this approach to provide clues into disease etiology. Here, we tested interactions between ~2.7 million SNPs across the genome and calcium intake in 9,006 CRC cases and 9,503 controls.

MATERIAL AND METHODS

We included 9,006 individuals with confirmed colorectal adenocarcinomas and 9,503 controls from the Colon Cancer Family Registry (CCFR) and the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) (3, 4, 7). We excluded participants missing all genotype or calcium data, or those of non-European ancestry. Participants gave informed written consent and studies were approved by their respective Institutional Review Boards.

Genotyping, quality control, and imputation procedures have been described (4, 7). Imputation to HapMap CEU was conducted using IMPUTE, BEAGLE, or MACH. In each

study, SNPs were restricted based on MAF>20/# samples and imputation accuracy (Rsq>0.3). We tested ~2.7 million SNPs.

Data collection/harmonization procedures have been described (3, 4). Calcium intake at the reference time was assessed using food frequency questionnaires (FFQs) or diet history (DALS); intake (mg/day) was determined from calcium in foods (i.e., dietary) or supplements (single+multivitamins+antacids) when available. Total intake was calculated as dietary+supplemental calcium. For studies that entered supplement data as regular- vs. non-user (CCFR, OFCCR, PMH-CCFR), regular use was assigned generic doses (1) of 500 mg/day, 500 mg/single pill, or 130 mg/multivitamin pill.

Multivariable logistic regression was used to estimate study-specific odds ratios (ORs) and 95% confidence intervals (CIs) for the association between calcium and CRC risk; study-specific estimates were combined using fixed-effects meta-analysis. We tested multiplicative GxE in each study using SNPxcalcium interaction terms, adjusting for age, sex, study center, total energy consumption, first 3 principal components of genetic ancestry, and SNP and calcium main effects. This was followed by meta-analysis across studies. Statistical significance was determined using the conventional genome-wide 2-sided α=5E-08 (3, 7). Heterogeneity was assessed using Woolf's test. We further explored interactions using the potentially more powerful Cocktail method (3). Statistical analyses used R, Version 2.15.1 or SAS, Version 9.3; power was estimated using Quanto, Version 1.2.4 (biostats.usc.edu/software).

RESULTS

Higher total calcium intake was associated with reduced CRC risk (OR per quartile=0.87, 95% CI: 0.84-0.89) (**Figure 1A**). Dietary and supplemental calcium were similarly associated with reduced risk (**Figure 1B-C**). Total calcium results were similar after excluding studies that entered calcium data from only diet (DALS, DACHS, PHS) or supplements (CCFR, OFCCR, PMH-CCFR) (OR=0.88, 95% CI: 0.84-0.92). Estimates were unchanged for right- vs. left-sided cancers (data not shown). Supporting the validity of our calcium data, 2q21.3/*MCM6*/rs4988235 aka 13910 T>C, a marker of preserved lactase levels used in genetic tests of lactose intolerance (8), was associated with dietary (*P*=1.1E-13), but not supplemental (*P*= 5.2E-01), intake.

There were no statistically significant SNP interactions with total, dietary, or supplemental calcium intake for CRC risk (**Table 1**). The strongest evidence of interaction was between 4q34.3/rs1028166 and supplemental calcium intake (OR-interaction=1.49, 95% CI: 1.27-1.74, *P*-interaction=7.3E-07). However, rs1028166 was located 669-kb from the nearest protein-coding gene (*TENM3*) and showed little evidence of interaction with total or dietary intake. The Cocktail approach (3) did not identify statistically significant interactions (data not shown).

DISCUSSION

In this large study, there were no statistically significant SNP-interactions with total, dietary, or supplemental calcium intake. Candidate-gene studies of interaction between SNPs in

calcium-related genes (e.g., *CASR*, *VDR*) and calcium intake have not reported consistent interactions (5, 6). Figueiredo et al. (2011) (9) investigated genome-wide SNP-calcium interactions for microsatellite stable/microsatellite-instability low CRC. Consistent with our findings, they reported no statistically significant interactions in 1,191 cases and 990 controls.

Strengths of this study include the large sample size, comprehensive genetic data, and harmonization of calcium intake across 13 studies. However, misclassification of calcium intake may have attenuated associations, although calcium assessed by FFQ is reasonably accurate compared to diet records/24-hour recalls [correlations=0.48-0.70 (1)], and we detected CRC-associations with magnitudes comparable to previous studies (1, 2). For total calcium quartiles, at α=5E-08, our study had >80% power to detect interaction ORs ≥1.33, 1.23, and 1.17 for SNPs with MAFs of 0.05, 0.10, and 0.20, respectively. We thus had adequate statistical power to detect modest interactions with common variants.

In summary, we did not observe evidence of SNP-interactions with calcium intake. This suggests that individual common genetic variants do not strongly modify the association between calcium and CRC risk in European populations. Large studies with sequence data are needed to investigate interactions involving rare variants.

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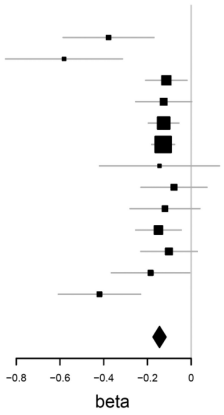
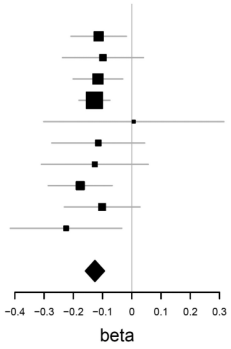
A) Total calcium intake (*P* for heterogeneity=0.01)

Study	Design	Case	Control	OR	Lower	Upper
CCFR	CC	934	966	0.69	0.56	0.84
OFCCR	CC	541	517	0.56	0.43	0.73
DALS	CC	1116	1174	0.89	0.81	0.98
PLCO	Cohort	434	684	0.88	0.78	1.00
WHI	Cohort	1462	1528	0.88	0.82	0.95
DACHS	CC	2358	2194	0.88	0.83	0.93
COLO2&3	CC	87	122	0.87	0.66	1.14
HPFS	Cohort	359	356	0.92	0.79	1.08
MEC	Cohort	314	334	0.89	0.76	1.04
NHS	Cohort	497	854	0.86	0.78	0.96
PHS	Cohort	373	389	0.90	0.79	1.03
VITAL	Cohort	254	263	0.83	0.69	0.99
PMH−CCFR	CC	277	122	0.66	0.54	0.79
Meta−analysis		**9006**	**9503**	**0.87**	**0.84**	**0.89**



B) Dietary calcium intake (*P* for heterogeneity=0.44)

Study	Case	Control	OR	Lower	Upper
DALS	1116	1174	0.89	0.81	0.98
PLCO	434	684	0.91	0.79	1.04
WHI	1462	1528	0.89	0.82	0.97
DACHS	2358	2194	0.88	0.83	0.93
COLO2&3	87	122	1.01	0.74	1.37
HPFS	359	356	0.89	0.76	1.05
MEC	314	334	0.88	0.73	1.06
NHS	497	852	0.84	0.75	0.94
PHS	373	389	0.90	0.79	1.03
VITAL	254	263	0.80	0.66	0.97
Meta−analysis	**7254**	**7896**	**0.88**	**0.85**	**0.91**



C) Supplemental calcium intake (*P* for heterogeneity=0.22)

Study	Case	Control	OR	Lower	Upper
CCFR	934	966	0.56	0.42	0.76
OFCCR	541	517	0.62	0.43	0.88
PLCO	434	684	0.95	0.68	1.33
WHI	1461	1528	0.77	0.66	0.90
COLO2&3	51	87	0.48	0.20	1.16
HPFS	359	356	1.02	0.63	1.64
MEC	314	334	0.77	0.51	1.14
NHS	497	854	0.75	0.59	0.95
VITAL	253	261	0.97	0.64	1.47
PMH−CCFR	277	122	0.45	0.29	0.70
Meta−analysis	**5121**	**5709**	**0.73**	**0.67**	**0.81**

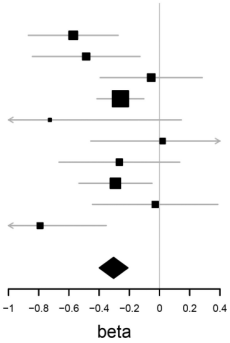


Figure 1.

Association between calcium intake and risk of CRC. Estimates correspond to each quartile increase in mg/day (total, dietary) or ≥500 vs. <500 mg/day (supplemental). Total and dietary calcium intake were coded as sex- and study-specific quartiles based on cut points in controls, and modeled as an ordinal variable. Estimates adjusted for age (continuous), sex (F/M), study center (indicators), and energy consumption (continuous). Abbreviations: CC, case-control; OR, odds ratio; Lower/Upper, lower and upper bounds of 95% confidence interval.

Table 1

SNP with smallest *P* for interaction with total, dietary, or supplemental calcium for CRC risk.

Calcium analysis	SNP with smallest *P* for interaction	Locus	Position (bp)[a]	Function class	Genetic region	Minor allele	Alt allele	MAF	Mean Rsq	Interaction results[b,c]			
										Variable	OR-int (95% CI)	*P*-value	*P*-het
Total	rs1933755	6q23.1	130925767	Intergenic	*TMEM200A/EPB41L2*	C	T	0.10	0.93	**Total**	0.84 (0.78-0.90)	1.5E-06	4.1E-01
										Dietary	0.86 (0.80-0.92)	5.2E-05	3.2E-01
										Suppl	0.81 (0.65-1.02)	7.4E-02	5.5E-01
Dietary	rs6855885	4q22.1	92039007	Intronic	*FAM190A*	A	G	0.50	0.94	Total	1.09 (1.04-1.13)	9.0E-05	2.5E-01
										Dietary	1.11 (1.06-1.16)	1.9E-06	5.5E-01
										Suppl	0.93 (0.81-1.07)	3.0E-01	2.4E-01
Supplemental	rs1028166	4q34.3	182813298	Intergenic	*AGA/TENM3*	G	A	0.31	0.85	Total	1.07 (1.02-1.12)	6.7E-03	3.8E-01
										Dietary	1.02 (0.97-1.07)	5.2E-01	7.4E-01
										Suppl	1.49 (1.27-1.74)	7.3E-07	2.9E-01

Abbreviations: SNP, single nucleotide polymorphism; Alt, alternate; MAF, minor allele frequency; Rsq, imputation Rsq; OR-int, odds ratio for interaction; CI, confidence interval; *P*-het, *P* for heterogeneity across studies

[a] Based on NCBI build 37 data.

[b] Corresponds to each additional copy of the minor allele (i.e., assuming additive genetic effects) and each quartile increase in calcium intake (total, dietary) or ≥500 vs. <500 mg/day (supplemental). Genotyped SNPs were modeled as 0, 1, or 2 copies of the minor allele; imputed SNPs were modeled as the expected number of copies of the minor allele (the genotype "dosage") (3, 7).

[c] Based on multivariable logistic regression adjusted for age (continuous), sex (F/M), study center (indicators), energy consumption (continuous), first 3 principal components of genetic ancestry (continuous), SNP main effect, and calcium main effect.