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Journal of Nutritional Biochemistry 47 (2017) 35–40

Journal of Nutritional Biochemistry

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

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Received 11 November 2016; received in revised form 17 April 2017; accepted 25 April 2017

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 that 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 association 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 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 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* values for interactions between calcium:magnesium intake ratio and rs2720574 were .002 for all adenomas and <.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].

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 Polyps Study.

☆ Funding: This study was supported through the National Center for Complementary and Alternative Medicine grant R01AT004660 and Office of Dietary Supplements, National Cancer Institute grants R01 CA149633 (to Q.D.) and U01 CA182364 (to Q.D. and T.L.E.) as well as parent studies P50CA95103 (to W.Z.) and R01CA121060 (to W.Z.) and the American Institute for Cancer Research grant #08A074 (to Q.D.). The survey and sample processing for the Tennessee Colorectal Polyps Study were conducted by the Survey and Biospecimen Shared Resource supported in part by the Vanderbilt-Ingram Cancer Center (P30 CA68485). Additional support was provided by the Vanderbilt Clinical and Translational Research Scholar award (5KL2RR024975) to T.L.E.

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http://dx.doi.org/10.1016/j.jnutbio.2017.04.016
0955-2863/

However, it still ranks as 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 semiessential 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 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 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 that 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 hormone* (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 association 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, 7954 (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 nonmelanoma 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 at least one adenomatous polyp. Participants with at least two adenomas were considered to have multiple adenomas. Hyperplastic cases had at least one hyperplastic polyp and no adenomas. Advanced adenoma cases met at least one of the following criteria: (1) size ≥1 cm, (2) tubulovillous or villous or (3) 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 semiquantitative 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-h dietary recall data in National Health and Nutrition Examination Survey III for Southerners aged 45 and older. We found that intakes of energy and major nutrients are not different [19]. A total of 6485 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. A total of 7443 (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 oversampled 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, USA) to assay single-nucleotide polymorphisms (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 30-kb flanking sequences with r^2=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

χ^2 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 (ORs) 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 first, second and third 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 .05 for the first phase analysis. As prespecified in our original design, one-sided tests at $P\leq.05$ (which practically has a significance level of .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