Genetically predicted circulating concentrations of micronutrients and risk of colorectal cancer among individuals of European descent: a Mendelian randomization study

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ABSTRACT

Background: The literature on associations of circulating concentrations of minerals and vitamins with risk of colorectal cancer is limited and inconsistent. Evidence from randomized controlled trials (RCTs) to support the efficacy of dietary modification or nutrient supplementation for colorectal cancer prevention is also limited.

Objectives: To complement observational and RCT findings, we investigated associations of genetically predicted concentrations of 11 micronutrients (β-carotene, calcium, copper, folate, iron, magnesium, phosphorus, selenium, vitamin B-6, vitamin B-12, and zinc) with colorectal cancer risk using Mendelian randomization (MR).

Methods: Two-sample MR was conducted using 58,221 individuals with colorectal cancer and 67,694 controls from the Genetics and Epidemiology of Colorectal Cancer Consortium, Colorectal Cancer Transdisciplinary Study, and Colon Cancer Family Registry. Inverse variance-weighted MR analyses were performed with sensitivity analyses to assess the impact of potential violations of MR assumptions.

Results: Nominally significant associations were noted for genetically predicted iron concentration and higher risk of colon cancer [ORs per SD (OR_{SD}): 1.08; 95% CI: 1.00, 1.17; P value = 0.05] and similarly for proximal colon cancer, and for vitamin B-12 concentration and higher risk of colorectal cancer (OR_{SD}: 1.12; 95% CI: 1.03, 1.21; P value = 0.01) and similarly for colon cancer. A nominally significant association was also noted for genetically predicted selenium concentration and lower risk of colon cancer (OR_{SD}: 0.98; 95% CI: 0.96, 1.00; P value = 0.05) and similarly for distal colon cancer. These associations were robust to sensitivity analyses. Nominally significant inverse associations were observed for zinc and risk of colorectal and distal colon cancers, but sensitivity analyses could not be performed. None of these findings survived correction for multiple testing. Genetically predicted concentrations of β-carotene, calcium, copper, folate, magnesium, phosphorus, and vitamin B-6 were not associated with disease risk.

Conclusions: These results suggest possible causal associations of circulating iron and vitamin B-12 (positively) and selenium (inversely) with risk of colon cancer. *Am J Clin Nutr* 2021;113:1490–1502.

Keywords: Mendelian randomization, genes, nutrition, supplements, colorectal cancer

Introduction

Colorectal cancer was the third most common cancer worldwide in 2018 (1). Diet and nutrition have an important role in the development of colorectal cancer. A higher consumption of red and processed meat has been linked to a higher risk of colorectal cancer, whereas a higher intake of fiber, milk, and whole grains has been associated with a lower risk, with reasonable consistency in prospective cohort studies (2, 3).

Most of the evidence regarding the nutritional epidemiology of colorectal cancer comes from observational studies that often rely on food frequency questionnaires (FFQs) to measure the consumption of foods and nutrients. This approach is prone to measurement error, because it is based on participants' self-reports often provided at 1 point in time and the conversion of

foods consumed into nutrient intake based on food composition databases that might be inaccurate (4). Furthermore, individuals who follow different diets might also vary in other characteristics, which are not always adequately controlled for in statistical analyses. In addition, evidence from randomized controlled trials (RCTs) to support the efficacy of dietary modification or nutrient supplementation for colorectal cancer prevention is lacking because few adequately powered trials exist, and those that do exist have in general failed to support protective associations (5–8). The molecular epidemiology literature on associations of circulating concentrations of minerals and vitamins with risk of colorectal cancer is generally less extensive and inconsistent (2, 3).

Our aim was to complement findings from observational research and RCTs, and investigate whether circulating concentrations of micronutrients are associated with risk of colorectal cancer using Mendelian randomization (MR) to improve causal inference in observational epidemiology. MR uses genetic variables as instrumental variables to assess the association of the genetically predicted component of the micronutrient biomarkers with colorectal cancer (9). We estimated the relations of single nucleotide polymorphisms (SNPs) associated with circulating concentrations of 11 systematically selected micronutrients (β-carotene, calcium, copper, folate, iron, magnesium, phosphorus, selenium, vitamin B-6, vitamin B-12, and zinc) with the risk of colorectal cancer and its subsites (colon, rectum, and proximal and distal colon). We used summary genetic association data for colorectal cancer and its subsites from 3 consortia: the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO), the Colorectal Cancer Transdisciplinary Study (CORECT), and the Colon Cancer Family Registry (CCFR).

Methods

Data for the genetic epidemiology of circulating micronutrient concentrations

We initially identified 20 micronutrients—β-carotene, calcium, copper, folate, iron, magnesium, phosphorus, potassium, retinol, selenium, sodium, zinc, and vitamins B-1, B-2, B-6, B-12, C, D, E, and K—for which associations with colorectal cancer have been reported in the literature (3). We conducted a search of published genome-wide association studies (GWASs) performed among individuals of European ancestry on circulating concentrations of these minerals and vitamins in the GWAS catalog and PubMed (last search performed in October 2019). Vitamin D was subsequently excluded from our analysis because recently published MR studies have already investigated the role of circulating vitamin D concentrations in relation to risk of colorectal cancer (10, 11). Potassium, sodium, and vitamins B-1, B-2, C, and K were also excluded because either no GWAS has been conducted or no genome-wide significant results have been reported (12, 13). GWASs for circulating vitamin E and retinol concentrations were not used because they adjusted for BMI (14, 15), which may cause collider bias in GWAS and MR estimates (16). After exclusions, published GWASs for 11 micronutrients were retrieved: β-carotene, calcium, copper, folate, iron, magnesium, phosphorus, selenium, vitamins B-6 and B-12, and zinc (13, 17–25). Two separate GWASs were used to instrument calcium concentrations (24, 25), the more recent of which was conducted in UK Biobank samples and published on a preprint server in June 2019 (25). SNPs that were associated with the circulating concentrations of these micronutrients at a genome-wide significance level ($P < 5 \times 10^{-8}$) and were not in linkage disequilibrium (linkage disequilibrium $r^2 \leq 0.01$) were selected. We used summary estimates for 3 (rs1800562, rs1799945 and rs855791) out of the 5 (rs1800562, rs1799945, rs855791, rs7385804, and rs8177240) available genome-wide significant SNPs for serum iron, because these 3 SNPs showed a concordant effect on serum iron, ferritin, transferrin, and transferrin saturation, and have been associated with an overall increased systemic iron status (17, 26). Three SNPs with minor allele frequency (MAF) <5% (rs12272669, rs2336573, rs6859667) in the GWASs for selenium and vitamin B-12 were excluded because their association estimates with the micronutrients might be imprecise. In total, summary genetic association data for 253 common (MAF ≥ 0.05) SNPs robustly associated with the 11 micronutrient concentrations were obtained. The selected GWASs included data from 12 European countries and the United States and most of the participants were women, with percentages ranging from 55%

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Supplemental Tables 1–7 and Supplemental Figures 1–21 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: CCFR, Colon Cancer Family Registry; CORECT, Colorectal Cancer Transdisciplinary Study; GECCO, Genetics and Epidemiology of Colorectal Cancer Consortium; GWAS, genome-wide association study; *HFE*, hemochromatosis; IVW, inverse variance–weighted; MAF, minor allele frequency; MR, Mendelian randomization; MR-PRESSO, Mendelian randomization pleiotropy residual sum and outlier test; RCT, randomized controlled trial; SNP, single nucleotide polymorphism.

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to 69% of total sample size. **Supplemental Table 1** provides detailed information on the selected genetic variants.

Data for the genetic epidemiology of colorectal cancer

A recently published large GWAS of almost 126,000 participants of European ancestry from the GECCO, CORECT, and CCFR consortia provided the genetic effects of the selected instruments on risk of colorectal (58,221 cases and 67,694 controls), colon (31,083 cases), rectal (15,775 cases), proximal colon (13,857 cases), and distal colon (15,306 cases) cancer (27). These endpoints were predeclared and did not change during the analyses. The regression models were adjusted for age, sex, study, and genetic principal components to account for population structure. Out of the 253 SNPs, rs1550532 and rs780094 associated with calcium, rs855791 associated with iron, rs602662 and rs1801222 associated with vitamin B-12, and rs2120019 associated with zinc concentrations were also nominally statistically significantly associated with risk of colorectal cancer (P value range: 5.89×10^{-4} to 0.03). **Supplemental Table 2** provides detailed information on the association of the genetic instruments with risk of colorectal cancer and its subsites.

Statistical power

Power calculations were performed using an online tool available at http://cnsgenomics.com/shiny/mRnd/ (28). The statistical power to capture an OR for colorectal cancer of 1.10 or 0.90 per SD change in the circulating concentrations of the micronutrients ranged from 0.39 for folate to 0.99 for vitamin B-12, and the statistical power was >0.80 for 6 of the 11 instruments tested, namely calcium (UK Biobank), copper, iron, selenium, vitamin B-12, and zinc. **Supplemental Table 3** shows detailed power calculations for all outcomes and **Supplemental Table 4** shows the minimum detectable ORs for 80% power.

MR analysis

A 2-sample MR using summary association data from GWASs of circulating micronutrients (first sample) and colorectal cancer risk (second sample) was performed. MR uses genetic variants as instruments to measure the genetically predicted component of the micronutrient concentrations and estimates the association of this component with colorectal cancer risk (29). In the case of β-carotene, where only 1 SNP was available, the effect estimate was calculated as the ratio of the SNP-outcome divided by the SNP–nutrient association (29), whereas the fixed-effects inverse variance-weighted (IVW) method was implemented when the instruments consisted of multiple SNPs. The IVW analysis can be thought of as a meta-analysis of single SNP effects (30). The β estimates and SEs from the regressions for circulating concentrations of β-carotene, copper, selenium, vitamin B-6, and zinc were transformed from the logarithmic scale provided in the published GWAS to the natural scale using a published formula (31). All reported associations correspond to an OR for risk of colorectal cancer and its subsites per SD change in the genetically predicted circulating concentrations of the nutrients.

Methods to assess the robustness of MR findings

To produce valid results, the IVW method requires that all genetic instruments are associated with the micronutrient concentrations (relevance assumption), but not directly with colorectal cancer (only via the micronutrients; exclusion restriction), nor any confounders of the relation between the micronutrient concentrations and colorectal cancer (independence assumption) (9). The strength of each instrument in relation to the circulating concentrations of the micronutrients (relevance assumption) was measured using the F statistic with the formula: $F = R^2(n-2)/(1-R^2)$, where R^2 is the proportion of the variance of the micronutrient concentration explained by each genetic instrument and n is the sample size of the GWAS for the SNP–micronutrient association (32). The F statistics ranged from 16 to 2407 for all genetic instruments, implying an absence of weak instruments because all values were >10 (Supplemental Table 1) (32).

Descriptive and statistical analyses were performed to examine the robustness of the MR results to potential violation of the exclusion restriction and independence assumptions. We used diagnostic plots (scatter plots, forest plots, and funnel plots), the Cochran's Q statistical test for heterogeneity, and the I^2 statistic to evaluate the extent to which any differences in the individual effect sizes among the selected genetic instruments may be related to pleiotropic effects rather than chance (33). Horizontal pleiotropy is defined as: where 1 genetic variant has independent effects on multiple traits, and is the main reason for potential violation of the exclusion restriction MR assumption. We further evaluated whether the selected genetic instruments were associated with secondary phenotypes in PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/) and the GWAS catalog (34, 35). For valid instruments, little heterogeneity across the SNP instruments would be expected because they all estimate similar associations, resulting in uniform plots and small values for the statistical tests and metrics of heterogeneity. The presence and magnitude of any heterogeneity, identified through either visual inspection of the plots or high values for the Q test or the I^2 metric, may thus be used to estimate the presence and magnitude of horizontal pleiotropy that may be biasing the MR estimate. When there was evidence of such heterogeneity, we also performed a random-effects IVW MR analysis to account for the additional heterogeneity in the estimation of the SEs (36).

Where the number of genetic instruments was ≥3, robust MR analyses that allow for horizontal pleiotropy were performed, namely the MR-Egger regression, weighted median, and weighted mode methods. The intercept from MR-Egger regression is a statistical test for horizontal pleiotropy, whereas the slope can be interpreted as the circulating nutrient effect on colorectal cancer adjusted for horizontal pleiotropy (37). This method assumes, however, that the pleiotropic effects are independent of the instrument strength (InSIDE assumption). Another limitation is that the MR-Egger method is subject to low power, particularly when using a small number of SNPs (e.g., <10). The weighted median estimator provides a valid causal estimate when at least half of the instruments are valid (38). The estimate from the weighted mode analysis is valid when the largest group of instruments with consistent MR estimates is valid (39). The MR pleiotropy residual sum and outlier test

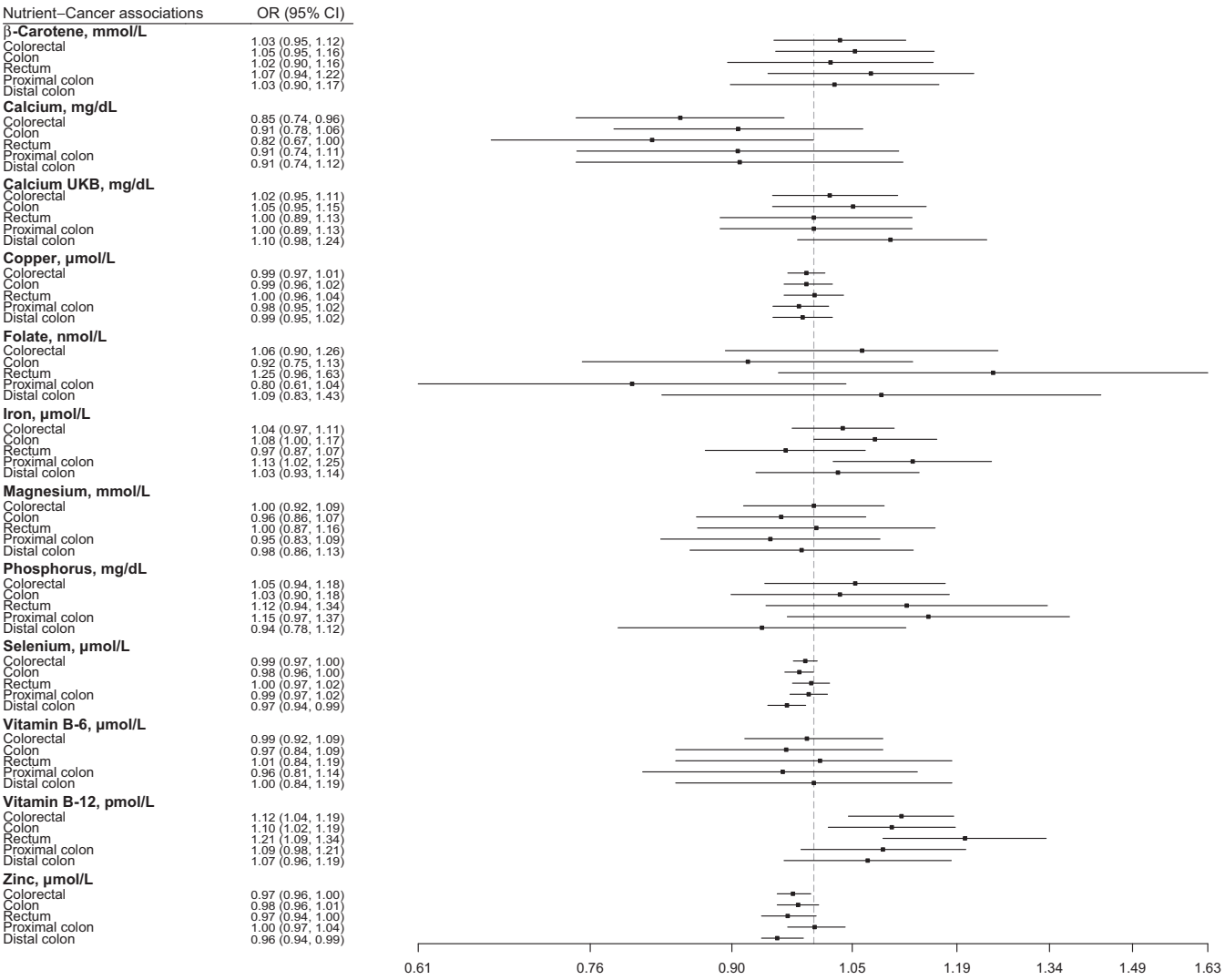

FIGURE 1 Fixed-effects inverse variance–weighted Mendelian randomization analyses of 11 micronutrient concentrations and risk of colorectal cancer and its subsites. UKB, UK Biobank.

(MR-PRESSO) was also implemented to identify outlying genetic variants and analyses were rerun after excluding these variants (40). *P* values < 0.05 were considered nominally significant, whereas high-confidence findings were those that survived multiple-testing adjustment with a Bonferroni-corrected threshold of 0.0045. All analyses were prespecified, and implemented in the statistical software R (R Core Team, 2020) version 3.4.3 using the MendelianRandomization package and in Stata (StataCorp. 2013) version 13 using the MRrobust package.

Results

Figure 1 shows all associations using the IVW fixed- and random-effects models. **Figures 2–4** depict associations using the MR sensitivity analyses methods for each of the 5 cancer sites studied (colorectum, colon, rectum, and proximal and distal colon). **Supplemental Tables 5–7** detail analyses using sensitivity analyses methods; **Supplemental Figures 1**A–**21**E show diagnostic plots for all associations.

There was evidence that genetically predicted circulating concentrations of iron, selenium, vitamin B-12, and zinc were associated with risk of colorectal cancer or its subsites (Figures 1–4) (presented in detail in what follows). There was little evidence that circulating concentrations of β-carotene, calcium, copper, folate, magnesium, phosphorus, and vitamin B-6 were associated with risk (Figures 1–4).

Iron and colorectal cancer

A positive nominally significant association was observed for a 1-SD (6.13-µmol/L) increase in genetically predicted iron concentration and risk of colon (OR: 1.08; 95% CI: 1.00, 1.17; *P* value = 0.05) and proximal colon (OR: 1.13; 95% CI: 1.02, 1.25; *P* value = 0.01) cancer in the IVW fixed-effects analysis, but there was little evidence of an association for rectal and distal colon cancer (Figure 1). These associations did not survive correction for multiple testing. No heterogeneity was detected in the individual SNPs instrumenting iron and risk of colon (I^2: 0%,

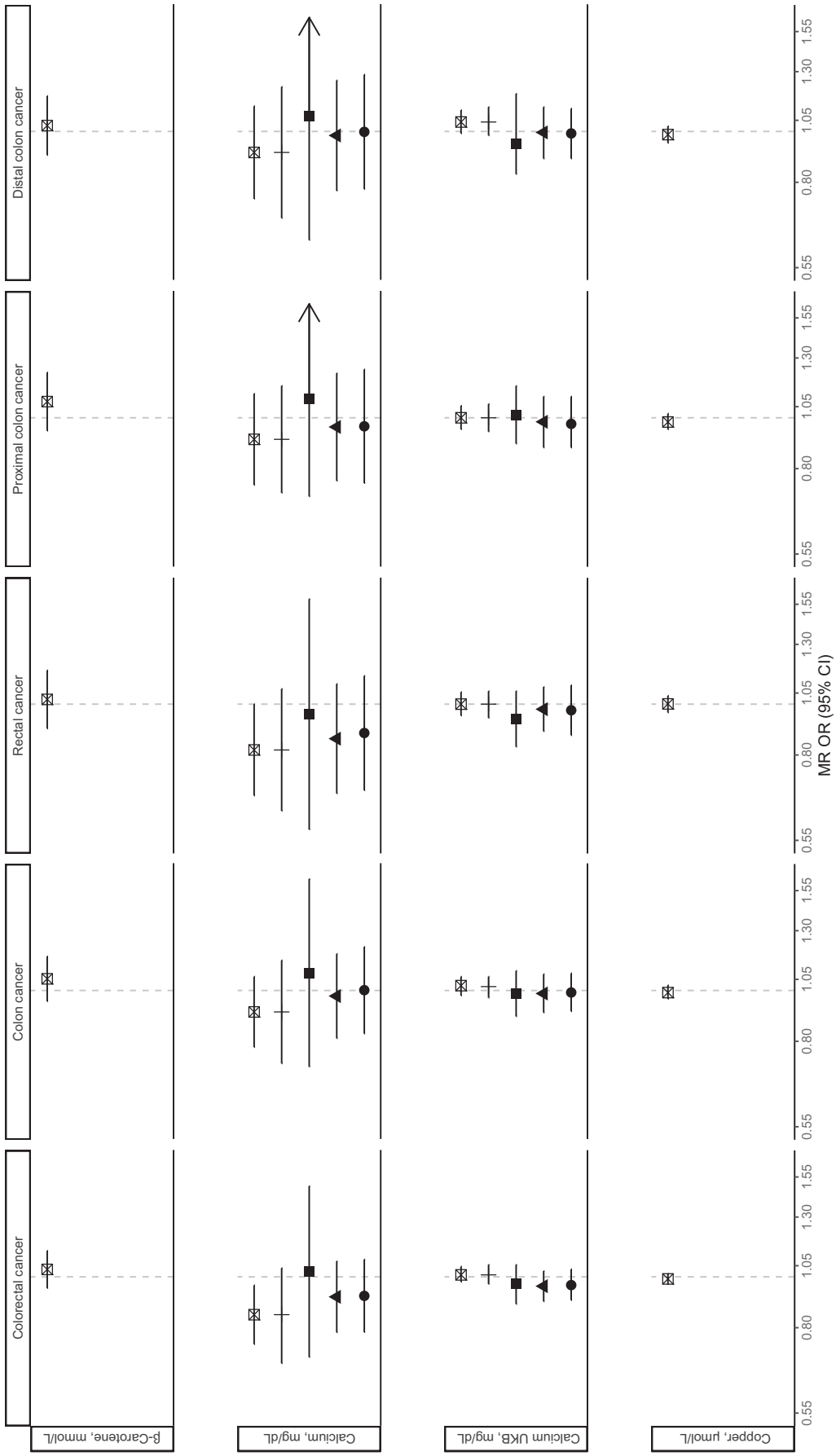

FIGURE 2 Associations of β-carotene, calcium, and copper with risk of colorectal cancer and its subtypes using main and sensitivity MR analyses. IVW, inverse variance–weighted; MR, Mendelian randomization; UKB, UK Biobank.

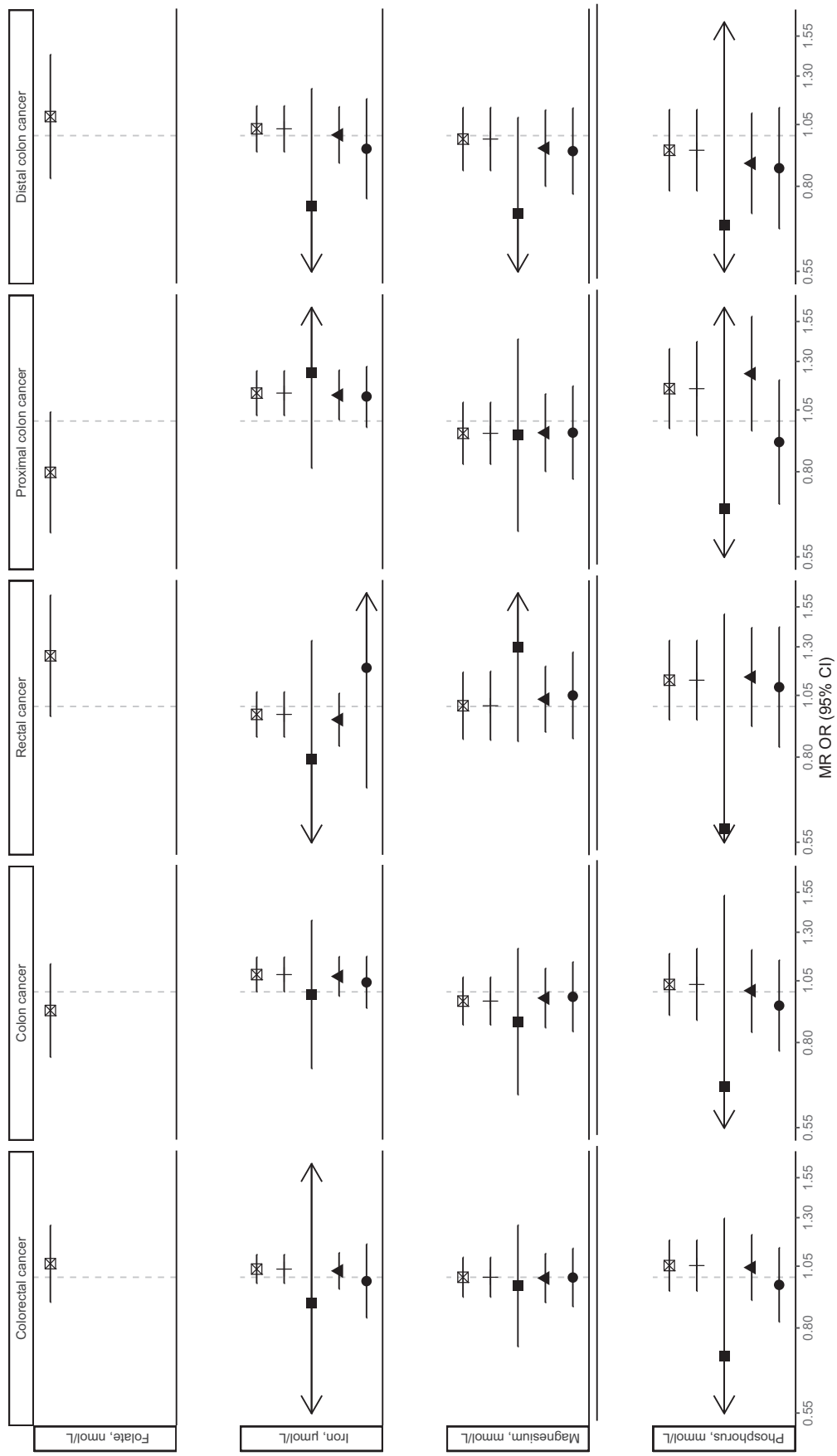

FIGURE 3 Associations of folate, iron, magnesium, and phosphorus with risk of colorectal cancer and its subtypes using main and sensitivity MR analyses. IVW, inverse variance–weighted; MR, Mendelian randomization.

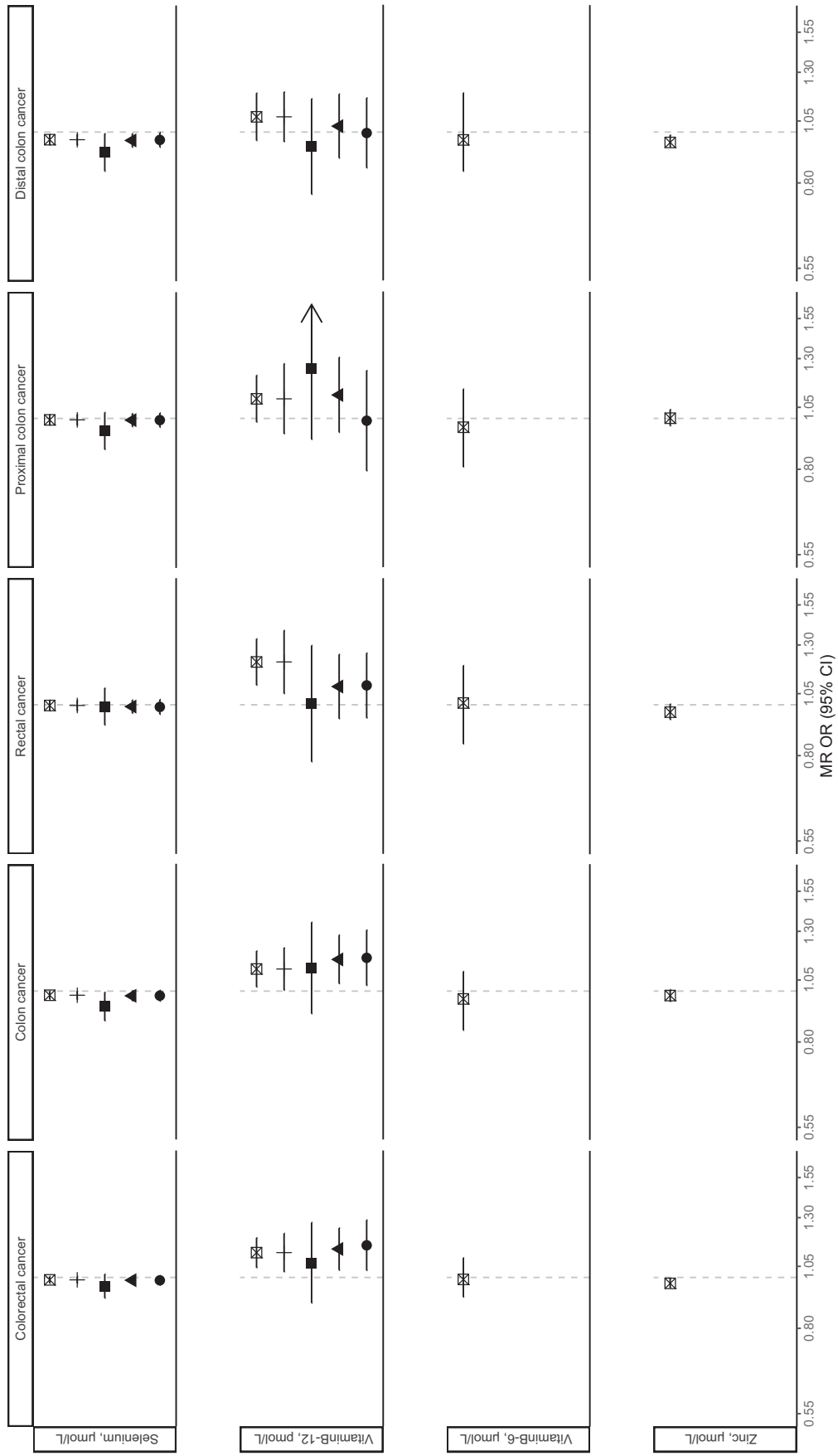

FIGURE 4 Associations of selenium, vitamin B-6, vitamin B-12, and zinc with risk of colorectal cancer and its subtypes using main and sensitivity MR analyses. IVW, inverse variance–weighted; MR, Mendelian randomization.

Cochran's Q test P value = 0.59) and proximal colon (I^2: 0%, P value = 0.91) cancer. There was no indication of horizontal pleiotropy based on the MR-Egger intercept test (Supplemental Table 5) (smallest P value = 0.19). Results based on MR-Egger regression were imprecisely estimated (i.e., wide CIs), but the weighted median and weighted mode estimates were consistent with the IVW MR analyses for colon and proximal colon cancer risk (Figures 2–4). The MR-PRESSO analysis did not reveal outlying SNPs (Supplemental Table 6).

Selenium and colorectal cancer

An inverse nominally significant association was observed for a 1-SD (0.53-μmol/L) increase in the genetically predicted selenium concentration and risk of colon (OR: 0.98; 95% CI: 0.96, 1.00; P value = 0.05) and distal colon (OR: 0.97; 95% CI: 0.94, 0.99; P value = 0.005) cancer in the IVW fixed-effects analysis, but there was little evidence of an association for rectal and proximal colon cancer (Figure 1). These associations did not survive correction for multiple testing. No heterogeneity was detected in the association of individual SNPs instrumenting selenium concentrations and risk of colon (I^2: 0%, Cochran's Q test P value = 0.38) and distal colon (I^2: 0%, P value = 0.54) cancer. There was no indication of horizontal pleiotropy based on the MR-Egger intercept test (Supplemental Table 5) (smallest P value = 0.10), and the associations remained consistent in the MR-Egger regression, the weighted median, and the weighted mode methods compared with the IVW MR results (Figures 2–4). The MR-PRESSO analysis did not reveal outlying SNPs (Supplemental Table 6).

Vitamin B-12 and colorectal cancer

Using the IVW fixed-effects method (Figure 1), a 1-SD (173-pmol/L) increase in the genetically predicted concentration of vitamin B-12 was associated with a 12% (OR: 1.12; 95% CI: 1.04, 1.19; P value = 0.001), 10% (OR: 1.10; 95% CI: 1.02, 1.19; P value = 0.02), and 21% (OR: 1.21; 95% CI: 1.09, 1.34; P value = 0.0003) higher risk of colorectal, colon, and rectal cancer, respectively, but not cancer in other subsites. Moderate heterogeneity was detected in the association of individual SNPs instrumenting vitamin B-12 concentrations with risk of colorectal (I^2: 44%, Cochran's Q test P value = 0.11), colon (I^2: 37%, P value = 0.19), and rectal (I^2: 35%, P value = 0.06) cancer. When the IVW random-effects MR analysis was performed, all associations were still observed (colorectal cancer OR: 1.12; 95% CI: 1.03, 1.21; P value = 0.01; colon cancer OR: 1.10; 95% CI: 1.00, 1.21; P value = 0.04; rectal cancer OR: 1.21; 95% CI: 1.05, 1.39; P value = 0.008), but none survived correction for multiple testing. There was no indication of horizontal pleiotropy based on the MR-Egger intercept test (Supplemental Table 5) (smallest P value = 0.11). The slope of the MR-Egger regression did not yield any associations, but the weighted median and weighted mode estimates were consistent with the IVW MR analyses for colorectal and colon cancer (Figures 2–4). For rectal cancer, all sensitivity analysis MR methods provided little evidence of any association. The MR-PRESSO analysis did not reveal outlying SNPs (Supplemental Table 6).

Zinc and colorectal cancer

Genetically predicted concentrations of zinc were inversely nominally significantly associated with risk of colorectal cancer overall (per SD: 65 μmol/L; OR: 0.97; 95% CI: 0.96, 1.00; P value = 0.02) and distal colon cancer (OR: 0.96; 95% CI: 0.94, 0.99; P value = 0.01), but not in other subsites using the IVW fixed-effects analysis (Figure 1). These associations did not survive correction for multiple testing. Only 2 SNPs were used as instruments for zinc concentrations; thus, sensitivity MR analyses were not performed, but these SNPs have not been associated in GWASs with phenotypes that may indicate horizontal pleiotropy in relation to colorectal cancer (Supplemental Table 7).

Calcium and colorectal cancer

Using the IVW fixed-effects method and the older GWAS for calcium concentrations (n = 7 instruments) (Figure 1) (24), a 1-SD (0.48-mg/dL) higher genetically predicted concentration of calcium was nominally significantly associated with a 15% (OR: 0.85; 95% CI: 0.74, 0.96; P value = 0.01) lower risk of colorectal cancer; similar associations were also found for colon and rectal cancer, but these associations did not survive correction for multiple testing. However, when the larger and more recent GWAS using UK Biobank samples was used (n = 207 instruments) (25), little evidence for an association was observed for colorectal cancer (OR per SD: 1.02; 95% CI: 0.95, 1.11; P value = 0.55) or its subsites. There was heterogeneity in the association of individual SNPs instrumenting calcium with risk of colorectal cancer outcomes in analyses using both GWASs (Supplemental Table 5). No indication of horizontal pleiotropy was found in the analyses based on the MR-Egger intercept test (Supplemental Table 5). The slope of the MR-Egger regression, the weighted median, and the weighted mode estimates suggested little evidence of any associations in analyses using both GWASs (Figures 2–4, Supplemental Table 6).

Discussion

Main findings and comparisons with the literature

In this comprehensive MR analysis of 11 circulating micronutrient concentrations and risk of colorectal cancer and its main anatomical subsites, we observed that genetically predicted concentrations of circulating iron and vitamin B-12 were associated with higher risk of colon cancer, whereas selenium concentrations were associated with lower risk of colon cancer. An inverse association was also observed for zinc and colorectal cancer risk, but sensitivity analyses could not be performed. These associations did not survive correction for multiple testing. We observed little evidence that circulating concentrations of any of the other micronutrients (i.e., β-carotene, calcium, copper, folate, magnesium, phosphorus, and vitamin B-6) were associated with risk of colorectal cancer or its subsites.

Iron and colorectal cancer

High iron load has been linked to increased cancer risk in animal models and human experiments (41). The most prominent

postulated underlying mechanism is the iron-induced formation of hydroxyl radicals leading to the generation of reactive oxygen species, oxidative tissue damage, and subsequent carcinogenesis (41). However, the epidemiological literature on iron intake and circulating iron biomarkers and colorectal cancer risk is mixed and inconclusive. Whereas heme iron intake, present mostly in red meat, has been positively associated with risk of colorectal cancer in several meta-analyses (42–44), findings for dietary and total iron intake have been mixed, and ferritin (a protein that stores iron) concentrations have been inversely associated with colorectal cancer risk (44). In the current MR study, genetically predicted concentrations of circulating iron were associated with higher risk of colon cancer, and these findings were robust to sensitivity MR methods. This finding was in agreement with another recently published MR study (45), but we used a sample more than double in size, estimated associations with greater precision, and studied associations in colorectal cancer subsites. Three loci were used as genetic instruments: rs1800562 and rs1799945 in the hemochromatosis (*HFE*) gene and rs855791 in the transmembrane protease serine 6 (*TMPRSS6*) gene, whose products have recognized roles in iron homeostasis (17). The rs1800562 in *HFE* has also shown associations with plasma lipids and lipoproteins (Supplemental Table 6) (17), which may indicate horizontal pleiotropy, but lipoprotein particles were not clearly associated with colorectal cancer risk in recent MR studies (46, 47).

Selenium and colorectal cancer

A protective effect of selenium on colorectal cancer has been supported by in vitro and animal studies, and selenium is hypothesized to reduce cancer risk by the antioxidative activity of selenoenzymes (48). However, the evidence from observational studies and RCTs is inconclusive. A meta-analysis of 10 observational studies showed an inverse association between circulating selenium concentrations and risk of colorectal neoplasia, but the association was only present in men (49). The association was no longer observed after excluding studies that measured selenium after cancer diagnosis. Selenium supplementation lowered colorectal cancer incidence by 61% (95% CI: 10%, 83%) in the secondary analysis of an RCT performed among patients with a history of nonmelanoma skin cancer that aimed to study recurrence of nonmelanoma skin cancer (50). In contrast, no such benefit was observed in a prespecified secondary analysis in the large SELECT (Selenium and Vitamin E Cancer Prevention Trial) study that was designed to investigate prostate cancer prevention (HR: 1.05; 95% CI: 0.66, 1.67), where selenium supplementation caused a median 114-μg/L increase in circulating selenium (7). The lower baseline selenium concentrations among participants of the first trial may have contributed to the observed benefit, and this phenomenon has also been shown in observational studies (51). In the current MR study, an increase of 114 μg/L in genetically predicted circulating selenium was associated with lower risk of colon cancer (OR: 0.94; 95% CI: 0.92, 1.00). This finding was in agreement with another recently published MR study (45), and may suggest that early-life effects of selenium play a role in the prevention of colorectal cancer because selenium is known to enhance the DNA damage repair response (52). These potentially early-life effects can be picked up in MR studies but are missed in RCTs.

Vitamin B-12 and colorectal cancer

B vitamins, including vitamin B-12, are essential for DNA methylation, synthesis, stability, and repair (53). Data from both in vitro and animal studies have suggested a protective effect of B vitamins against colorectal carcinogenesis (54), although the associations and mechanisms between the different cofactors of the one-carbon metabolism pathway are complex and have not yet been fully elucidated. No association was observed in the meta-analysis of epidemiological studies for circulating vitamin B-12 concentrations and colorectal cancer risk (per 150 pmol/L RR: 1.02; 95% CI: 0.88, 1.19). Long-term follow-up of participants ($n = 2524$) in the B-PROOF (B Vitamins for the Prevention of Osteoporotic Fractures) trial, a multicenter, double-blind, placebo-controlled RCT designed to assess the effect of 2–3 y daily supplementation with folic acid (400 mg) and vitamin B-12 (500 mg) compared with placebo on fracture incidence (55), showed that allocation to B vitamins was associated with a higher risk of colorectal cancer (43 compared with 25 cases; HR: 1.77; 95% CI: 1.08, 2.90). The dosage of vitamin B-12 was almost 200 times higher than the recommended intake, and the authors could not rule out that the high dosage of vitamin B-12 supplementation influenced the risk of colorectal cancer in their study. In the current MR study, genetically predicted concentrations of circulating vitamin B-12 were associated with higher risk of colorectal and colon cancer, and these findings were robust to sensitivity MR methods. This finding was in agreement with another recently published MR study (45) and was estimated with much greater precision in our study. Of the 9 loci associated with serum B-12 concentrations, most can be directly linked to the current understanding of B-12 metabolism such as absorption, transport, or enzymatic processes. One of them, *fucosyltransferase 2* (*FUT2*), functions in cell surface glycobiology, and has previously been associated with liver enzymes, cholesterol concentrations, and Crohn disease (Supplemental Table 6), which may indicate horizontal pleiotropy, but when this SNP (i.e., rs602662) was removed from the analyses the results remained very similar.

Other micronutrients and colorectal cancer

In the current MR study, we observed an inverse association between genetically predicted concentrations of zinc and risk of colorectal cancer, but sensitivity analyses could not be performed. There were only 2 genetic instruments available for zinc; thus, we cannot preclude the presence of a potential causal association. Larger GWASs are needed to better understand the genetic regulation of zinc and to better define instrumental variables for MR analysis. Little evidence was observed in the current MR study that genetically predicted concentrations of β-carotene, calcium, copper, folate, magnesium, phosphorus, and vitamin B-6 were associated with risk of colorectal cancer. The observational molecular epidemiology literature for these micronutrient concentrations and risk of colorectal cancer is sparse, but in general the results from the available prospective studies agree with the null results of the current MR study

(3, 56–58), with 2 potential exceptions for vitamin B-6 and calcium. A meta-analysis of 4 prospective studies ($n = 883$ total cases) showed an inverse association (OR: 0.52; 95% CI: 0.38, 0.71) comparing the highest with the lowest category of vitamin B-6 concentrations (59), but this result may be a chance finding given the relatively small sample size. RCTs of calcium supplementation showed a reduction in adenoma recurrence (60–62), but the Women's Health Initiative trial did not find any reduction in colorectal cancer incidence after a mean of 7 y of supplementation with calcium and vitamin D (63). In a reanalysis, a 17% nonsignificant reduction was observed among participants not already taking calcium or vitamin D at randomization (64). In the current MR study, genetically predicted concentrations of circulating calcium were not associated with risk of colorectal cancer or its subsites when the large GWAS on calcium from UK Biobank was used. This analysis used 207 genetic instruments for calcium and yielded good statistical power, but had a higher likelihood of horizontal pleiotropy owing to the large number of instruments. The pleiotropy-robust MR methods also suggested little evidence of association, but had point estimates below unity that fall within the potential effects suggested by the trials. However, the circulating concentrations of calcium are tightly regulated in the human body to maintain homeostasis, which means that large changes in intake will not lead to detectable changes in circulating concentrations; thus, MR estimates should not be interpreted as relevant to dietary intake.

Strengths and limitations

MR studies can be useful in nutritional epidemiology, because they can avoid biases that are commonly present in traditional observational literature (4). The main challenge of MR studies in this field is to identify genetic variants that are associated with exposures related to diet, specifically for blood concentrations of micronutrients in the current study. Minerals and vitamins are obtained from diet. However, genetic variation in absorption, metabolism, and storage can also be important in determining risk of deficiency and toxicity. MR estimates have a causal interpretation only if the assumptions of the instrumental variable approach hold. Although it is not possible to prove the validity of the assumptions, we performed several sensitivity analyses to detect potential violations. We have taken a conservative approach and only highlighted associations that were robust in sensitivity analyses.

Several limitations should be also considered in interpreting our findings. The summary-level data that we used did not allow for analyses stratified by covariates of interest, such as age, sex, alcohol consumption, dietary intakes, gut flora, or according to whether populations were deficient or not for specific elements. Furthermore, the currently known SNPs associated with folate and vitamin B-6 concentrations account for only a small amount of the variance explained, and the observed nonsignificant associations may be due to low power. In addition, the one-carbon metabolism is a complex web of biochemically interdependent reactions and it may be misleading to examine a single one-carbon nutrient (e.g., folate and vitamins B-6 and B-12) in isolation without considering the others. SNPs for micronutrients predict blood concentrations and genetic factors affecting concentrations in more clinically relevant tissues may differ. Future large pooling consortiums, larger single- and multitrait GWASs of micronutrient concentrations, and MR studies with individual-level data could address some of the latter issues.

Conclusion

In summary, using a comprehensive MR study, we found evidence for possible causal associations of higher circulating concentrations of iron and vitamin B-12 with higher risk of colon cancer, and higher selenium concentrations with lower risk of the disease. These results in combination with previous literature could open up new possibilities for chemoprevention of colorectal cancer using diet, supplements, or other means to modify circulating iron, vitamin B-12, and selenium concentrations.

The authors' responsibilities were as follows—KKT and MJG: conceived and designed the study; NP, ND, and KKT: analyzed the data; KKT: wrote the paper taking into account the comments and suggestions of all the coauthors and had primary responsibility for the final content; NP, ND, DG, SJL, RMM, NM, GM, VZ, AJC, KB, DSL, TJK, RCT, AP-C, DJH, FJBvD, DA, VA, SIB, SB, DTB, JB, HB, AB-H, PTC, GC, SC-B, ATC, JC-C, AdlC, JCF, SJG, GGG, PJG, AG, JH, HH, MH, MAJ, TOK, S-SK, SCL, LLM, CIL, LL, AL, V Martín, RLM, V Moreno, HN, RN, PAN, KO, PDPP, EAP, JDP, LQ, GR, LCS, CS, MLS, LS, JS, SNT, CMU, BVG, SH, KV, LV, HW, EW, AW, MOW, AHW, WZ, BB-d-M, M-CB-R, DJH, PJ, TK, DP, ER, ELG, BLB, SBG, UP, and MJG: commented on the analysis and interpretation of the findings; and all authors: read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

All data described in the article are provided within the article.

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