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


Published in:
The American Journal of Clinical Nutrition

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

Publisher rights
Copyright 2021 The Authors. Published by Oxford University Press.
This work is made available online in accordance with the publisher’s policies. Please refer to any applicable terms of use of the publisher.

General rights
Copyright for the publications made accessible via the Queen's University Belfast Research Portal is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The Research Portal is Queen's institutional repository that provides access to Queen's research output. Every effort has been made to ensure that content in the Research Portal does not infringe any person's rights, or applicable UK laws. If you discover content in the Research Portal that you believe breaches copyright or violates any law, please contact openaccess@qub.ac.uk.

Open Access
This research has been made openly available by Queen's academics and its Open Research team. We would love to hear how access to this research benefits you. – Share your feedback with us: http://go.qub.ac.uk/oa-feedback
Genetically predicted circulating concentrations of micronutrients and risk of colorectal cancer among individuals of European descent: a Mendelian randomization study

1Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece
2Department of Epidemiology and Biostatistics, School of Public Health, Imperial College London, London, UK
3Section of Nutrition and Metabolism, International Agency for Research on Cancer, Lyon, France
4Department of Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK
5MRC Integrative Epidemiology Unit, Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK
6University Hospitals Bristol NHS Foundation Trust National Institute for Health Research Bristol Biomedical Research Centre, University of Bristol, Bristol, UK
7MRC Biostatistics Unit, School of Clinical Medicine, University of Cambridge, UK
8Department of Preventive Medicine and Community Health, The University of Texas Medical Branch, Galveston, TX, USA
9Nuffield Department of Population Health, University of Oxford, Oxford, UK
10Division of Human Nutrition and Health, Wageningen University & Research, Wageningen, The Netherlands
11Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA.
12Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany.
13Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA.
Service de Génétique Médicale, Centre Hospitalier Universitaire (CHU) Nantes, Nantes, France.

Leeds Institute of Cancer and Pathology, University of Leeds, Leeds, UK.

Huntsman Cancer Institute and Department of Population Health Sciences, University of Utah, Salt Lake City, Utah, USA.

Division of Preventive Oncology, German Cancer Research Center (DKFZ) and National Center for Tumor Diseases (NCT), Heidelberg, Germany.

German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany.

Institute for Health Research, Kaiser Permanente Colorado, Denver, Colorado, USA.

Behavioral and Epidemiology Research Group, American Cancer Society, Atlanta, Georgia, USA.

Center for Public Health Genomics, University of Virginia, Charlottesville, Virginia, USA.

Gastroenterology Department, Hospital Clinic, Institut d’Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas (CIBEREHD), University of Barcelona, Barcelona, Spain.

Division of Gastroenterology, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA.

Channing Division of Network Medicine, Brigham and Women’s Hospital and Harvard Medical School, Boston, Massachusetts, USA.

Clinical and Translational Epidemiology Unit, Massachusetts General Hospital and Harvard Medical School, Boston, Massachusetts, USA.

Broad Institute of Harvard and MIT, Cambridge, Massachusetts, USA.

Department of Epidemiology, Harvard T.H. Chan School of Public Health, Harvard University, Boston, Massachusetts, USA.
28Department of Immunology and Infectious Diseases, Harvard T.H. Chan School of Public Health, Harvard University, Boston, Massachusetts, USA.

29Division of Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg, Germany.

30University Medical Centre Hamburg-Eppendorf, University Cancer Centre Hamburg (UCCH), Hamburg, Germany.

31Department of Cancer Biology and Genetics and the Comprehensive Cancer Center, The Ohio State University, Columbus, Ohio, USA.

32Department of Medicine, Samuel Oschin Comprehensive Cancer Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA.

33Department of Preventive Medicine, Keck School of Medicine, University of Southern California, Los Angeles, California, USA.

34Lunenfeld Tanenbaum Research Institute, Mount Sinai Hospital, University of Toronto, Toronto, Ontario, Canada.

35Cancer Epidemiology Division, Cancer Council Victoria, Melbourne, Victoria, Australia.

36Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Victoria, Australia.

37Precision Medicine, School of Clinical Sciences at Monash Health, Monash University, Clayton, Victoria, Australia.

38SWOG Statistical Center, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA.

39Institute of Cancer Research, Department of Medicine I, Medical University Vienna, Vienna, Austria.

40Department of Medicine I, University Hospital Dresden, Technische Universität Dresden (TU Dresden), Dresden, Germany.
Division of Human Genetics, Department of Internal Medicine, The Ohio State University
Comprehensive Cancer Center, Columbus, Ohio, USA.

Center for Gastrointestinal Biology and Disease, University of North Carolina, Chapel Hill, North Carolina, USA.

Department of Preventive Medicine, Chonnam National University Medical School, Gwangju, Korea.

Jeonnam Regional Cancer Center, Chonnam National University Hwasun Hospital, Hwasun, Korea.

Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden.

University of Hawaii Cancer Center, Honolulu, Hawaii, USA.

Public Health Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA.

Department of Family Medicine, University of Virginia, Charlottesville, Virginia, USA.

Department of Clinical Genetics, Karolinska University Hospital, Stockholm, Sweden.

Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden.

CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain.

Biomedicine Institute (IBIOMED), University of León, León, Spain.

Oncology Data Analytics Program, Catalan Institute of Oncology-IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain.

Department of Clinical Sciences, Faculty of Medicine, University of Barcelona, Barcelona, Spain.

ONCOBEL Program, Bellvitge Biomedical Research Institute (IDIBELL), L'Hospitalet de Llobregat, Barcelona, Spain.

Department of Epidemiology, Richard M. Fairbanks School of Public Health, Indiana University, Indianapolis, Indiana, USA.
IU Melvin and Bren Simon Cancer Center, Indiana University, Indianapolis, Indiana, USA.

Department of Pathology, School of Medicine, Umm Al-Qura’a University, Saudi Arabia

School of Public Health, University of Washington, Seattle, Washington, USA.

Clinical Genetics Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, New York, USA.

Department of Medicine, Weill Cornell Medical College, New York, New York, USA.

Department of Public Health and Primary Care, University of Cambridge, Cambridge, UK.

Department of Epidemiology, Johns Hopkins Bloomberg School of Public Health, Baltimore, Maryland, USA.

Department of Public Health Sciences, School of Medicine, University of California Davis, Davis, California, USA.

Department of Community Medicine and Epidemiology, Lady Davis Carmel Medical Center, Haifa, Israel.

Ruth and Bruce Rappaport Faculty of Medicine, Technion-Israel Institute of Technology, Haifa, Israel.

Clalit National Cancer Control Center, Haifa, Israel.

Division of Research, Kaiser Permanente Northern California, Oakland, California, USA.

Department of General Surgery, University Hospital Rostock, Rostock, Germany.

Department of Internal Medicine, University of Utah, Salt Lake City, Utah, USA.

Department of Epidemiology, University of Iowa College of Public Health, Iowa City, Iowa, USA.

Division of Laboratory Genetics, Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota, USA.

Huntsman Cancer Institute and Department of Population Health Sciences, University of Utah, Salt Lake City, Utah, USA.
Department of Radiation Sciences, Oncology Unit, Umeå University, Umeå, Sweden.

Department of Molecular Biology of Cancer, Institute of Experimental Medicine of the Czech Academy of Sciences, Prague, Czech Republic.

Institute of Biology and Medical Genetics, First Faculty of Medicine, Charles University, Prague, Czech Republic.

Faculty of Medicine and Biomedical Center in Pilsen, Charles University, Pilsen, Czech Republic.

Department of Epidemiology, University of Washington, Seattle, Washington, USA

Memorial University of Newfoundland, Discipline of Genetics, St. John’s, Canada.

Division of Epidemiology, Department of Medicine, Vanderbilt-Ingram Cancer Center, Vanderbilt Epidemiology Center, Vanderbilt University School of Medicine, Nashville, Tennessee, USA.

Former senior scientist, Department for Determinants of Chronic Diseases (DCD), National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.

Faculty of Medicine, CESP, University of Paris-Sud, Faculty of Medicine UVSQ, INSERM, University of Paris-Saclay, Villejuif, France.

Centre for Research in Epidemiology and Population Health (CESP), Gustave Roussy, Villejuif, France.

Cancer Biology and Therapeutics Group, UCD Conway Institute, School of Biomolecular and Biomedical Science, University College Dublin, Dublin, Ireland.

Unit of Nutrition and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology - IDIBELL, L'Hospitalet de Llobregat, Barcelona, Spain.

Facultat Ciències Salut Blanquerna, Universitat Ramon Llull, Barcelona, Spain.

Cancer Risk Factors and Life-Style Epidemiology Unit, Institute for Cancer Research, Prevention and Clinical Network - ISPRO, Florence, Italy.
Department of Nutrition, Harvard T.H. Chan School of Public Health, Harvard University, Boston, Massachusetts, USA.

USC Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA, USA.

**Corresponding author:**

Konstantinos Tsilidis, PhD

Department of Epidemiology and Biostatistics

Imperial College London

St’ Mary’s Campus, W2 1PG, London, United Kingdom

Tel: +44 (0) 2075942623

Email: k.tsilidis@imperial.ac.uk

**Short title:** Circulating micronutrients and colorectal cancer

**Data accessibility:** All data described in the manuscript are provided within the manuscript.

**Disclosure of potential conflicts of interest:** The authors declare no potential conflicts of interest.

**Funding:** This work was supported by the World Cancer Research Fund International Regular Grant Programme (WCRF 2014/1180 to Konstantinos K. Tsilidis). The study sponsor had no role in the design and conduct of the study; collection, management, analysis and interpretation of the data; preparation, review or approval of the article; and decision to submit the article for publication. Funding statements for the GECCO, CORECT and CCFR
consortia are shown in the supplement. DG is supported by the Wellcome 4i Programme at Imperial College London. RMM was supported by a Cancer Research UK (C18281/A19169) programme grant (the Integrative Cancer Epidemiology Programme) and is part of the Medical Research Council Integrative Epidemiology Unit at the University of Bristol supported by the Medical Research Council (MC_UU_12013/1, MC_UU_12013/2, and MC_UU_12013/3) and the University of Bristol. RMM is also supported by the National Institute for Health Research (NIHR) Bristol Biomedical Research Centre which is funded by the National Institute for Health Research (NIHR) and is a partnership between University Hospitals Bristol NHS Foundation Trust and the University of Bristol. LV was supported by Czech Science Foundation 18-09709S and 17-16857S.

Department of Health and Social Care disclaimer: The views expressed are those of the author(s) and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care.

IARC disclaimer: The authors alone are responsible for the views expressed in this article and they do not necessarily represent the decisions, policy or views of the International Agency for Research on Cancer / World Health Organization.

**Abbreviations:** Mendelian randomization, MR; standard deviation, SD; odds ratio, OR; confidence interval, CI; food frequency questionnaire, FFQ; randomised controlled trials, RCT; single nucleotide polymorphisms, SNPs; Genetic and Epidemiology of Colorectal Cancer Consortium, GECCO; Colorectal Cancer Transdisciplinary Study, CORECT; Colon Cancer Family Registry, CCFR; genome-wide association studies, GWAS; body mass index, BMI; inverse variance weighted, IVW; MR pleiotropy residual sum and outlier test, MR-PRESSO; minor allele frequency, MAF.
Abstract

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

Objective: To complement observational and RCT findings, we investigated associations of genetically predicted concentrations of 11 micronutrients (beta-carotene, calcium, copper, folate, iron, magnesium, phosphorus, selenium, vitamin B6, vitamin B12 and zinc) with colorectal cancer risk using Mendelian randomization (MR).

Design: Two-sample MR was conducted using 58,221 individuals with colorectal cancer and 67,694 controls from the GECCO, CORECT and CCFR consortia. 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 (odds ratios per standard deviation [ORSD]: 1.08, 95% confidence interval [CI]: 1.00, 1.17, P-value: 0.05) and similarly for proximal colon cancer, and for vitamin B12 concentration and higher risk of colorectal (ORSD: 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 (ORSD: 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. A nominally significant inverse association was observed for zinc and risk of colorectal and distal colon cancer, but sensitivity analyses could not be performed. None of these findings survived correction for multiple testing.
Genetically predicted concentrations of beta-carotene, calcium, copper, folate, magnesium, phosphorus and vitamin B6 were not associated with disease risk.

**Conclusions:** These results suggest possible causal associations of circulating iron and vitamin B12 (positively) and selenium (inversely) with risk of colon cancer.

**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 fibre, 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 (FFQ) 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 one 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 randomised controlled trials (RCT) 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 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 by assessing risk associations of the genetically predicted component of the micronutrient biomarkers [9]. We estimated associations of single nucleotide polymorphisms (SNPs) associated with circulating concentrations of 11 systematically selected micronutrients (beta-carotene, calcium, copper, folate, iron, magnesium, phosphorus, selenium, vitamin B6, vitamin B12 and zinc) with risk of colorectal cancer and its subsites (colon, rectum, proximal and distal colon). We used summary genetic association data for colorectal cancer and its subsites from three consortia: the Genetic and Epidemiology of Colorectal Cancer Consortium (GECCO), the Colorectal Cancer Transdisciplinary Study (CORECT), and the Colon Cancer Family Registry (CCFR).

Subjects and Methods

Data for the genetic epidemiology of circulating micronutrient concentrations

We initially identified 20 micronutrients: beta-carotene, calcium, copper, folate, iron, magnesium, phosphorus, potassium, retinol, selenium, sodium, zinc and vitamins B1, B2, B6, B12, C, D, E, and K, for which associations for 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 recent published MR studies have already investigated the role of circulating vitamin D concentrations and risk of colorectal cancer [10, 11]. Potassium, sodium, vitamins B1, B2, C and K were also excluded because either no GWAS has been conducted or no genome-wide significant results have been reported [12, 13]. GWAS for circulating vitamin E and retinol
concentrations were not used because they adjusted for body mass index (BMI) [14, 15], which may cause collider bias in GWAS and MR estimates [16]. After exclusions, published GWAS for 11 micronutrients were retrieved: beta-carotene, calcium, copper, folate, iron, magnesium, phosphorus, selenium, vitamins B6, B12 and zinc [13, 17-25]. Two separate GWAS were used to instrument calcium concentrations [24, 25], the more recent of which was conducted in UK Biobank samples and published on a pre-print 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 (LD $r^2 \leq 0.01$) were selected. We used summary estimates for three (rs1800562, rs1799945 and rs855791) out of the five (rs1800562, rs1799945 and rs855791, rs7385804, rs8177240) available genome-wide significant SNPs for serum iron, because these three 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) smaller than 5% (rs12272669, rs2336573, rs6859667) in the GWAS for selenium and vitamin B12 were excluded because their association estimates with the micronutrients might be imprecise. In total, summary genetic association data for 253 common (MAF $\geq 0.05$) SNPs robustly associated with the 11 micronutrient concentrations were obtained. The selected GWASs included data from 12 European countries and the United States of America and most of the participants were women with percentages ranging from 55% to 69% of total sample size. Detailed information on the selected genetic variants is provided in supplemental Table 1.

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 pre-declared 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 B12, and rs2120019 associated with zinc concentrations, were also nominally statistically significantly associated with risk of colorectal cancer (P-value range: 5.89E-4 to 0.03). Detailed information on the association of the genetic instruments with risk of colorectal cancer and its subsites is provided on supplemental Table 2.

**Statistical power**

Power calculations were performed using an online tool available at http://cnsgenomics.com/shiny/mRnd/ [28]. The statistical power to capture an odds ratio (OR) for colorectal cancer of 1.10 or 0.90 per standard deviation (SD) change in the circulating concentrations of the micronutrients ranged from 0.39 for folate to 0.99 for vitamin B12, and the statistical power was larger than 0.80 for six of the 11 instruments tested, namely calcium (UK Biobank), copper, iron, selenium, vitamin B12 and zinc. Detailed power calculations for all outcomes are shown in supplemental Table 3 and minimum detectable ORs for 80% power are shown in supplemental Table 4.
Mendelian randomization analysis

A two-sample MR using summary association data from GWASs of circulating micronutrients (1st sample) and colorectal cancer (2nd sample) risk 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. In the case of beta-carotene, where only one 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 beta estimates and standard errors from the regressions for circulating concentrations of beta-carotene, copper, selenium, vitamin B6, 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 circulating concentrations of the nutrients.

Methods to assess the robustness of Mendelian randomization 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 relationship 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 explained variance of the micronutrient concentration by each genetic instrument and $N$ the sample size of the GWAS for the SNP-micronutrient association [32]. The F statistics ranged from 16 to 2,407 for all genetic instruments implying absence of weak instruments as all values were above 10 (supplemental Table 1).

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 where one 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 GWAS catalog [34, 35]. For valid instruments, little heterogeneity would be expected in their individual MR estimates, as they will 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, either through 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 standard errors [36].
Where the number of genetic instruments was $\geq 3$, 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 (MR-PRESSO) was also implemented to identify outlying genetic variants and analyses were re-run after excluding these variants [40]. P-values less than 0.05 were considered nominally significant, whereas high-confidence findings were those that survived multiple testing adjustment with Bonferroni-corrected threshold of 0.0045. All analyses were pre-specified, and implemented in the statistical software R version 3.4.3 using the package MendelianRandomization and in Stata version 13 using the MRrobust package.

**Results**

All associations using the IVW fixed- and random-effects models are shown in Figure 1. Figures 2 to 4 depict associations using the MR sensitivity analyses methods for each of the five cancer sites studied (colorectum, colon, rectum, proximal and distal colon). Analyses
using sensitivity analyses methods are detailed in supplemental Tables 5 to 7, and
diagnostic plots for all associations are shown in supplemental Figures 1a to 21e.

There was evidence that genetically predicted circulating concentrations of iron, selenium, vitamin B12 and zinc were associated with risk of colorectal cancer or its subsites (Figures 1 to 4) (presented in detail below). There was little evidence that circulating concentrations of beta-carotene, calcium, copper, folate, magnesium, phosphorus and vitamin B6 were associated with risk (Figures 1 to 4).

Iron and colorectal cancer

A positive nominally significant association was observed for each one SD (6.13 μmol/L) increment 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%, 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 confidence intervals), but the weighted median and weighted mode estimates were consistent with the IVW MR analyses for colon and proximal colon cancer risk (Figures 2 to 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 each one SD (0.53 μmol/L)
higher 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 to the IVW MR results (Figures 2 to 4). The MR-PRESSO analysis did not reveal outlying SNPs (supplemental Table 6).

**Vitamin B12 and colorectal cancer**

Using the IVW fixed-effects method (Figure 1), each one SD (173 pmol/L) higher genetically predicted concentration of vitamin B12 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 in other subsites. Moderate heterogeneity was detected in the association of individual SNPs instrumenting vitamin B12 concentrations and 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 to 4). For rectal cancer, all sensitivity 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 two SNPs were used as instruments for zinc concentrations; thus, sensitivity MR analyses were not performed, but these SNPs have not been associated in GWAS 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 one 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; with similar associations found for colon and rectal cancer, but these associations did not survive correction for multiple testing. However, when the larger more recent GWAS using UK Biobank samples was used (n=207 instruments) [25], little evidence for an association was observed for colorectal cancer.
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 and risk of
colorectal cancer outcomes in analyses using both GWAS (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 GWAS (Figures 2 to  and supplemental Table 6).

Discussion

Main findings and comparisons with the literature

In this comprehensive MR analysis of 11 circulating micronutrient concentrations with risk
of colorectal cancer and its main anatomical subsites, we observed that genetically predicted
concentrations of circulating iron and vitamin B12 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 (e.g. beta-carotene, calcium, copper, folate, magnesium, phosphorus and
vitamin B6) 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
of iron intake and circulating iron biomarkers and colorectal cancer risk is mixed and
inconclusive. While 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 more than double
sample size and 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 showed 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 anti-oxidative
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 levels 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 a RCT performed
among patients with a history of non-melanoma skin cancer [50]. In contrast, no such benefit
was observed in a pre-specified secondary analysis in the large SELECT trial (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 levels among participants of the first
trial may have contributed to the observed benefit, and this phenomenon has been also
showed in observational studies [51]. In the current MR study, 114 μg/L higher 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 B12 and colorectal cancer**

B vitamins, including vitamin B12, 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 B12 levels and colorectal cancer
risk (per 150 pmol/L RR: 1.02; 95% CI: 0.88, 1.19). Long-term follow-up of the B-PROOF
trial participants (N=2,524), a multicenter, double-blind placebo-controlled RCT designed to
assess the effect of 2 to 3 years daily supplementation with folic acid (400 mg) and vitamin
B12 (500 mg) versus placebo on fracture incidence [55], showed that allocation to B vitamins was associated with a higher risk of colorectal cancer (43 vs. 25 cases; hazard ratio: 1.77; 95% CI: 1.08, 2.90). The dosage of vitamin B12 was almost 200 times higher than the recommended intake, and authors could not rule out that the high dosage of vitamin B12 supplementation influenced the risk of colorectal cancer in their study. In the current MR study, genetically predicted concentrations of circulating vitamin B12 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 in much greater precision in our study. Of the nine loci that associate with serum B12 concentrations, most can be directly linked to the current understanding of B12 metabolism such as absorption, transport or enzymatic processes. One of them, FUT2, functions in cell surface glycobiology, and has previously been associated with liver enzymes, cholesterol concentrations and Crohn’s 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 with risk of colorectal cancer, but sensitivity analyses could not be performed. There were only two genetic instruments available for zinc; thus, we cannot preclude presence of a potential causal association. Larger GWAS 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 beta-carotene, calcium, copper, folate, magnesium, phosphorus and vitamin B6 concentrations 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 two potential exceptions for vitamin B6 and calcium. A meta-analysis of four prospective studies (N=883 total cases) showed an inverse association (OR: 0.52, 95% CI: 0.38, 0.71) comparing the highest to lowest category of vitamin B6 concentrations [59], but this analysis may be underpowered. 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 seven years of supplementation with calcium and vitamin D [63]. In a re-analysis, a 17% non-significant 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 higher likelihood for horizontal pleiotropy due 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, as 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 levels 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. Though 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 stratified analyses 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 B6 concentrations account for only a small amount of the variance
explained, and observed non-significant associations may be due to low power. In addition,
the one carbon metabolism is a complex web of biochemically inter-dependent reactions and
it may be misleading to examine a single one carbon nutrient (e.g. folate and vitamins B6 and
B12) 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 multi-trait GWAS 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 between higher circulating concentrations of iron and vitamin B12 with higher risk of colon cancer, and lower 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 B12 and selenium concentrations.
Author contributions: The study was conceived and designed by KKT and MJG. NP, ND and KKT analyzed the data. The article was written by KKT taking into account the comments and suggestions of all the co-authors. All co-authors commented on the analysis and interpretation of the findings and approved the final version for publication. KKT had primary responsibility for final content.
References


Legends for figures

Figure 1. Fixed-effects IVW MR analyses of 11 micronutrient concentrations and risk of colorectal cancer and subsites (Abbreviations: confidence interval, CI; odds ratio, OR)

Figure 2. Associations of beta-carotene, calcium and copper and risk of colorectal cancer and subtypes using main and sensitivity MR analyses (Abbreviations: confidence interval, CI; Mendelian randomization, MR; odds ratio, OR; UK Biobank, UKB)

Figure 3. Associations of folate, iron, magnesium and phosphorus and risk of colorectal cancer and subtypes using main and sensitivity MR analyses (Abbreviations: confidence interval, CI; Mendelian randomization, MR; odds ratio, OR)

Figure 4. Associations of selenium, vitamin B6, B12 and zinc and risk of colorectal cancer and subtypes using main and sensitivity MR analyses (Abbreviations: confidence interval, CI; Mendelian randomization, MR; odds ratio, OR)