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Antioxidants, Minerals, and Vitamins in Relation to Crohn's disease and Ulcerative Colitis

Antioxidants, Minerals, and Vitamins in Relation to Crohn's disease and Ulcerative Colitis: A Mendelian Randomization Study

Authors: Jie Chen, PhD [1,2,3*], Xixian Ruan, MBBS [1*], Shuai Yuan, B.Med, MMedSc [4], Minzi Deng, PhD [1], Han Zhang, PhD [3], Jing Sun, PhD [3], Lili Yu, PhD [3], Jack Satsangi, MD, PhD [5], Susanna C. Larsson, PhD [4,6], Evropi Therdoratou, PhD [7,8], Xiaoyan Wang, MD, PhD [1#], Xue Li, PhD [3#]

[1] Department of Gastroenterology, The Third Xiangya Hospital, Central South University, Changsha, China

[2] Centre for Global Health, Zhejiang University School of Medicine, Hangzhou, China

[3] Department of Big Data in Health Science, School of Public Health and The Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, China.

[4] Unit of Cardiovascular and Nutritional Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

[5] Translational Gastroenterology Unit, Nuffield Department of Medicine, Experimental Medicine Division, University of Oxford, John Radcliffe Hospital, Oxford, UK

[6] Unit of Medical Epidemiology, Department of Surgical Sciences, Uppsala University, Uppsala, Sweden

[7] Centre for Global Health, Usher Institute, University of Edinburgh, Edinburgh, UK

[8] Cancer Research UK Edinburgh Centre, Medical Research Council Institute of Genetics and Cancer, University of Edinburgh, Edinburgh, UK

*Joint first authors

#Joint corresponding authors

Correspondence to:

Xue Li, PhD, School of Public Health and the Second Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, China; E-mail: xueli157@zju.edu.cn

Xiaoyan Wang, MD, PhD, Department of Gastroenterology, The Third Xiangya Hospital, Central South University, Changsha, China. E-mail: wangxiaoyan@csu.edu.cn

Author Contributions:

All authors made critical revisions of the manuscript for important intellectual content. All authors have read and approved the final version of the manuscript.

Jie Chen (Conceptualization: Equal; Methodology: Equal; Formal analysis: Equal; Data curation: Equal; and Writing – original draft: Equal)

Xixian Ruan (Conceptualization: Equal; Methodology: Equal; Formal analysis: Equal; and Writing – original draft: Equal)

Shuai Yuan (Conceptualization: Supporting; Methodology: Supporting; Writing - review & editing: Supporting)

Minzi Deng (Conceptualization: Supporting; Writing - review & editing: Supporting)

Han Zhang (Conceptualization: Supporting; Writing - review & editing: Supporting)

Jing Sun (Conceptualization: Supporting; Writing - review & editing: Supporting)

Lili Yu (Conceptualization: Supporting; Writing - review & editing: Supporting)

Jack Satsangi (Conceptualization: Supporting; Methodology: Supporting; Writing - review & editing: Supporting)

Susanna C. Larsson (Conceptualization: Equal; Methodology: Equal; Data curation: Equal; and Writing - review & editing: Leading)

Evropi Therdoratou (Conceptualization: Equal; Methodology: Equal; Writing - review & editing: Leading)

Xiaoyan Wang (Conceptualization: Leading; Data curation: Equal; and Funding acquisition: Leading; Writing - review & editing: Equal)

Xue Li (Conceptualization: Equal; Data curation: Equal; Funding acquisition: Equal; and Writing - review & editing: Leading)

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Abstract

Background: Evidence for antioxidants, minerals, and vitamins in relation to the risk of Crohn's disease (CD) and ulcerative colitis (UC) is limited and inconsistent. This mendelian randomization (MR) study aimed to examine the causal associations of circulating levels of antioxidants, minerals, and vitamins with CD and UC.

Methods: Single-nucleotide polymorphisms associated with antioxidants (beta-carotene, lycopene, and uric acid), minerals (copper, calcium, iron, magnesium, phosphorus, zinc, and selenium), and vitamins (folate, vitamin A, B6, B12, C, D, E, and K1) were employed as instrumental variables. Genetic associations with CD and UC were extracted from the UK Biobank, the FinnGen study, and the International Inflammatory Bowel Disease Genetics Consortium. The inverse variance weighted method and sensitivity analyses were performed.

Results: Genetically predicted higher lycopene (OR=0.94, 95% CI: 0.91-0.97), vitamin D (OR=0.65, 95% CI: 0.54-0.79), and vitamin K1 (OR=0.93, 95% CI: 0.90-0.97) levels were inversely associated with CD risk, whereas genetically predicted higher magnesium (OR=1.53, 95% CI: 1.23-1.90) levels were positively associated with CD risk. Higher levels of genetically predicted lycopene (OR=0.91, 95%CI: 0.88-0.95), phosphorus (OR=0.69, 95%CI: 0.58-0.82), selenium (OR=0.91, 95%CI: 0.85-0.97), zinc (OR=0.91, 95%CI: 0.89-0.94), folate (OR=0.71, 95% CI: 0.56-0.92) and vitamin E (OR=0.78, 95%CI: 0.69-0.88) were associated with reduced UC risk, whereas genetically predicted high levels of calcium (OR=1.46, 95% CI: 1.22-1.76) and magnesium (OR=1.24, 95% CI: 1.03-1.49) were associated with increased risk of UC.

Conclusions: Our study provided evidence that circulating levels of antioxidants, minerals, and vitamins might be causally linked to the development of IBD.

Key Words: Crohn's disease, Ulcerative colitis, Antioxidants, Minerals, Vitamins, Mendelian randomization analysis

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Introduction

Inflammatory bowel disease (IBD), including Crohn's Disease (CD) and ulcerative colitis (UC), is a chronic inflammatory disorder of the gastrointestinal tract with a rising incidence in populations.[1] Although the precise etiology of IBD remains unclear, accumulating evidence from observational studies suggests that dietary nutrients especially antioxidants, minerals, and vitamins contribute to the pathogenesis of IBD.[2, 3] Unfortunately, published research on the associations between dietary nutrients, especially antioxidants, minerals, and vitamins and IBD is scarce and inconclusive. An umbrella review of meta-analyses found that high vitamin D levels decreased the risk of CD and UC;[4] however, a randomized controlled trial (RCT) examining the effect of supplemental vitamin D did not show a significant clinical benefit.[5] Large prospective cohort studies have reported that dietary zinc intake was inversely associated with incident CD,[6, 7] whereas in a small RCT, zinc supplementation seemed to play little part in restraining inflammation in patients with IBD.[8] Potential explanations for these inconsistent results may relate to the substantial biases (residual confounding and reverse causation) in observational studies as well as low adherence to treatment, low treatment doses, short trial duration, and low statistical power in RCTs. The causal role of antioxidants, minerals, and vitamins in the development of CD or UC remains unclear.

Mendelian randomization (MR) utilizes genetic variants as instruments to make inferences in causal relationships between risk factors and disease outcomes.[9] As germline genetic variants are randomly allocated at meiosis, MR design minimizes confounding and is not

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influenced by environmental or self-adopted factors and therefore strengthens causal inference. Construction of a validated MR association relies on three key assumptions: 1) genetic variant is associated with the exposure; 2) the genetic variant is not related to confounding; 3) the genetic variant has no effect on outcome directly.[10] A previous MR study including 25,042 IBD cases indicated no association of genetically predicted vitamin D levels with CD or UC risk.[11] Still, the MR associations of antioxidants, minerals, and vitamins with CD or UC risk have not been systematically evaluated. Here, we conducted an MR investigation to comprehensively explore the causal associations of antioxidants, minerals, and vitamins with CD and UC.

Methods

Study design

This MR study design is depicted in Figure 1. The present study was based on publicly available data from UK biobank,[12] the FinnGen study,[13] the International Inflammatory Bowel Disease Genetics Consortium (IIBDGC),[14] and published genome-wide association studies (GWASs). All exposure-specific MR analyses were conducted separately in UK Biobank, FinnGen study, and IIBDGC, and individual results were subsequently meta-analyzed to pool estimates for each exposure on CD or UC risk. Included studies had been approved by corresponding institutional review boards and ethical committees.

Instrumental variable selection

We conducted a search of the latest published GWASs performed among European individuals on diet-related antioxidants, minerals, and vitamins at the circulating level in the NHGRI-EBI GWAS catalog and PubMed (from inception to 1st March 2022). After the searching, published GWASs for 20 exposures were initially identified: beta-carotene,[15] lycopene,[16] uric acid,[17] calcium,[18] copper,[19] magnesium,[20] sodium,[20] potassium,[20] copper,[19] zinc,[19] iron,[21] selenium,[22] phosphorus,[23] folate,[24] vitamins A,[25] B6,[26] B12,[24] C,[27] D,[28] E,[29] and K1 [30] (Table S1). For some circulating nutrients such as sulfur and vitamin B1, no available GWASs were found, so these were not considered in the current study. Even though more recent GWASs on calcium, phosphorus, and vitamin D were conducted in UK Biobank samples in which hundreds of SNPs were identified,[31] we decided not to apply this option due to the overlap of the study population.

To comprehensively evaluate the effects of circulating antioxidants, minerals, and vitamins on disease and obtain suitable instrumental variables (IVs), we selected eligible genetic instruments based on the following criteria: 1) SNPs should be associated with these circulating nutrients at a genome-wide significance level ($P < 5 \times 10^{-8}$); for those traits instrumented by less than 2 SNPs, suggestive significant genome-wide association significant ($P < 1 \times 10^{-5}$) or validated SNPs were included if available; 2) SNPs should be associated with exposure independently—that is, not in linkage disequilibrium (defined as $r^2 < 0.01$) with other genetic instruments for the same exposure; 3) the selected genetic instruments should explain at least 0.1% of the variance of exposure to ensure the strength of genetic IVs to be sufficient to evaluate a causal effect.[32] Potassium and sodium were excluded because the criteria were not met and therefore 18 circulating nutrients were

included in the analysis.

Detailed information of genetic instruments used for each nutrient is shown in Table S2. Notably, SNPs associated with circulating lycopene passing the threshold of $P < 1 \times 10^{-6}$ were identified from a small GWAS conducted in 441 individuals of European ancestry, explaining a substantial part (30.1%) of the total variance of circulating lycopene levels.[16, 33] SNPs for vitamin K1 in five loci passing the threshold of $P < 1 \times 10^{-6}$ were reported in a GWAS of 2,138 European individuals. Four SNPs with the strongest association with vitamin K1 levels in each locus were utilized explaining approximately 6.0% of genetic variance, and one SNP that was strongly associated with triglyceride and cholesterol was removed to minimize pleiotropy.[30] The GWAS for lycopene, vitamin A, Vitamin E, Vitamin D levels were adjusted for body mass index. For each exposure, instrumental variables were harmonized to omit ambiguous SNPs with non-concordant alleles and palindromic SNPs with ambiguous MAF (above 0.42 and less than 0.58). When an exposure-associated SNP was not available in the outcome GWAS, a proxy SNP identified in high linkage disequilibrium ($r^2 > 0.8$) was used instead (using the LDlink tool and the European 1000 Genomes data [34]). The variance explained by genetic instruments for each nutrient ranged from 0.2% to 30.1% (Table S3).

Outcome data sources

Summary-level GWAS data for CD and UC were available in UK Biobank,[12] the FinnGen study,[13] and IIBDGC.[14] The UK Biobank study is a large multicenter cohort study that recruited more than 500,000 European participants across the United Kingdom between

2006 and 2010.[12] In this study, summary statistics of genetic associations in UK Biobank were extracted from GWAS conducted by Lee lab.[35] Crohn's disease (1,743 cases and 334,783 controls) was defined according to the International Classification of Diseases 9th Revision (ICD-9) (555) and ICD-10 (K50); Ulcerative Colitis (3,195 cases and 334,783 controls) was defined according to ICD-9 (556.9) and ICD-10 (K51). The genetic-disease association estimates were obtained by logistic regression with adjustment for the genetic principal components, sex, and birth year. Summary-level estimates of genetic associations with CD and UC were also obtained in the last publicly available R6 data release of the FinnGen study. The FinnGen study is a large nationwide cohort study launched in 2017, which combined genetic data from Finnish biobanks and digital health record data from Finnish health registries.[13] A CD diagnosis (2,532 cases and 249,705 controls) was coded according to the ICD-8 (5630), ICD-9 (555), ICD-10 (K50); a UC diagnosis (5,349 cases and 249,705 controls) was coded according to the ICD-8 (5631, 569), ICD-9 (556), ICD-10 (K51). Genome-wide association analyses for each trait were adjusted for sex, age, genetic components, and genotyping batch. IIBDGC brings together genome-wide genotyping data and whole-genome sequencing data for over 75,000 patients with IBD.[14] Diagnosis of IBD in IIBDGC was based on accepted radiologic, endoscopic, and histopathologic evaluations. The genetic associations were obtained from logistic regression adjusted for age, sex, and genetic principal components. We employed European ancestry summary-level statistics including data for CD (5,956 cases and 14,927 controls) and UC (6,968 cases and 20,464 controls).

Statistical analysis

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The primary MR analyses were conducted by utilizing the inverse-variance weighted method. For exposures with more than 3 SNPs, the estimates for variants were then pooled using the random-multiplicative effects inverse-variance weighted method. For exposures instrumented by only 2 SNPs, the fixed-effects inverse-variance weighted method was employed. The inverse-variance weighted method provides the most precise estimates but assumes that all SNPs are valid instruments and any pleiotropy is balanced.[36] Heterogeneity among estimates based on individual SNPs was assessed with Cochran's Q value. Wald ratio method was performed if there was only 1 SNP for the exposure, in which the SNP-outcome association estimate was divided by its SNP-exposure association estimate to obtain the causal relationship.

To examine if there was any violation of the assumptions of MR or any other potential biases for exposure with 3 or more genetic instruments, the weighted median,[36] MR-egger regression,[37] and Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) [38] methods were additionally performed as sensitivity analyses. The weighted median model can provide consistent estimates if at least 50% of the weight comes from valid instrumental variables.[36] The intercept test of MR-Egger regression can be used as an indicator of horizontal pleiotropy albeit with low, precise estimates.[37] MR-PRESSO methods can identify horizontal pleiotropic outliers of SNPs and provide results identical to those from IVW in the absence of outliers.[38]

Fixed-effects meta-analysis was conducted to combine MR estimates from different data sources. The F-statistic was estimated to quantify instrument strength for each exposure

and an F-statistic > 10 suggests a sufficiently strong instrument. We conducted power analysis by using the online web tool mRnd (https://cnsgenomics.shinyapps.io/mRnd/) [32](Table S3). The Benjamini-Hochberg correction that controls the false discovery rate (FDR) was applied to correct for the multiple testing separately for CD and UC, and associations with a Benjamini–Hochberg adjusted *P*-value <0.05 were regarded as significant. All analyses were two-sided and performed using the TwoSampleMR,[39] and MRPRESSO R package [38] in R software 4.1.2.

Results

The causal role of antioxidants, minerals, and vitamins in CD

Genetically predicted higher circulating lycopene levels were associated with reduced risk of CD in IIBDGC, and the associations were directionally consistent in the other two databases (Figure 2). In the meta-analysis, the odds ratio (OR) of CD was 0.94 (95% CI: 0.91-0.97; *P*<0.001) for a 1μg/dl increase in genetically predicted lycopene (Table S4). Genetically predicted circulating magnesium levels were associated with CD risk in UK Biobank, and the associations were directionally consistent in the other two databases (Figure 2). For one SD increase in genetically predicted circulating magnesium levels, the combined OR of CD was 1.53 (95% CI: 1.23-1.90; *P* <0.001). Genetically predicted vitamin D (OR=0.65, 95% CI: 0.54-0.79; *P*<0.001, per SD increase) and Vitamin K1 (OR=0.93, 95% CI: 0.90-0.97; *P*=0.001, per 1-unit increase in natural logarithm-transformed) levels were inversely associated with the disease in the meta-analyses (Figure 2).

Associations across these sensitivity analyses were generally directionally consistent.

Heterogeneity was detected in the analysis of magnesium, vitamin B12, and Vitamin D (Table S5). The MR-Egger regression intercept suggested evidence of horizontal pleiotropy in the analysis of magnesium in IIBDGC. A few outliers were detected in the MR-PRESSO analysis of magnesium and vitamin B12, and the removal of these outliers did not change the direction of the associations.

The causal role of antioxidants, minerals, and vitamins in UC

Genetically predicted higher lycopene levels were associated with reduced risk of UC in IIBDGC, and the associations were directionally consistent in UK Biobank (Figure 3). For a 1µg/dl increase in the genetically predicted lycopene levels, the combined OR of UC was 0.91 (95% CI: 0.88-0.95; P<0.001). Higher genetically predicted phosphorus, zinc, and selenium levels were statistically associated with a decreased risk of UC, whereas genetically predicted calcium and magnesium levels were positively associated with the disease (Figure 3). The combined ORs per 1-SD increase in genetically predicted circulating levels of these minerals were 0.69 (95% CI: 0.58–0.82; P<0.004) for phosphorus, 0.91 (95% CI: 0.85–0.97; P=0.006) for selenium, 1.46 (95% CI: 1.22–1.76; P=0.022) for calcium and 1.24 (95% CI: 1.03–1.49; P=0.022) for magnesium. Genetically predicted folate (OR=0.71, 95% CI: 0.56–0.92; P=0.009, per SD increase), and vitamin E (OR=0.64, 95% CI: 0.46-0.89; P<0.001) levels were inversely associated with risk of UC in the meta-analyses (Figure 3); (Table S4).

Results from the sensitivity analyses were generally consistent with the primary analysis, though they did not always reach a significant level. Associations between SNPs on

magnesium and risk of UC showed evidence of heterogeneity in IIBDGC, and the direction of the association did not alter after removing one outlier in MR-PRESSO analysis (Table S6).

Discussion

In this MR analysis, we provided evidence that genetically predicted higher circulating lycopene, vitamin D, and vitamin K1 levels were associated with reduced risk of CD, whereas the genetically predicted high magnesium levels were associated with elevated CD risk. Our study also found that genetically predicted circulating levels of lycopene, phosphorus, selenium, zinc, folate, and vitamin E were inversely associated with UC risk, and genetically predicted higher calcium and magnesium levels were associated with increased risk of UC.

The protective role of antioxidants on IBD risk has been explored in previous observational studies.[40][41, 42]An inverse association of lycopene levels with CD and UC was detected in our MR analysis, which was in line with previous studies.[40, 41] Results from a cross-sectional study with 37 nonsmoking CD patients showed that plasma lycopene was significantly lower in CD patients than in controls;[40] another cross-sectional study of 56 UC patients in remission showed that higher lycopene intakes were associated with lower fecal blood, mucus, and pus among participants.[42] Evidence from laboratory studies indicated several underlying mechanisms. Lycopene is one of the free radical scavengers, which neutralizes free radicals by donating electrons [43] and thus delays the process of oxidative stress in IBD. A recent experimental animal study demonstrated that lycopene plays a

preventive role in dextran sulfate sodium-induced colitis mice by regulating the TLR4/TRIF/NF-κB signaling pathway.[44] Published studies also examined the associations between circulating levels of β-carotene, retinol, and ascorbate and the risk of CD and UC, but reported inconsistent findings.[40, 42, 45] In this MR investigation, we observed limited evidence in support of causal associations for genetically predicted beta-carotene, retinol (Vitamin A), and ascorbate (Vitamin C) in relation to IBD risk. The discrepancy between the observational studies and our MR study might be attributed to the residual confounding and reverse causation bias, or insufficient statistical power. In the case of lycopene, limited studies made the supplementation recommendations vague and dispensable. Taken together, our study supports that lycopene is one of the promising antioxidants with the potential for reducing both CD and UC risk.

Data on the associations of minerals with IBD are scarce. Inconsistent with our results, most cross-sectional studies observed that circulating calcium levels were lower in IBD patients.[46, 47] A most likely explanation for this discrepancy is that gastrointestinal damage as a result of ongoing inflammation leads to the malabsorption of calcium after the onset of IBD. Interestingly, we observed that genetic predisposition to higher circulating calcium and genetic predisposition to lower circulating phosphorus was associated with elevated UC risk with validated genetic instruments, which indicated that the disorders of calcium and phosphorus metabolism may be involved in the pathological process of UC onset. Considering the low variance explained by the genetic instrument for calcium (0.8%) and phosphorus (0.2%), we cannot rule out that weak associations between these two minerals and CD were overlooked. Although the mechanistic explanations for the association

between calcium and UC remain unclear, it has been reported that serum calcium might contribute to the inflammation by activating the inflammasome through the calcium-sensing receptor.[48] Besides, animal models have provided evidence that calcium/calmodulin-dependent protein kinase IV activation contributes to the pathogenesis of experimental colitis via inhibition of intestinal epithelial cell proliferation.[49]

We also observed that genetic predisposition to higher zinc and selenium levels were associated with a decreased risk of UC. Evidence from two large prospective cohorts of women based on semi-quantitative food frequency questionnaires showed that intake of zinc was inversely associated with risk of CD but not UC.[6] Another prospective cohort based on 24-hour dietary records reported that dietary zinc intake was inversely associated with incident CD.[7] We found a statistically significant positive association of circulating magnesium with CD and UC, which are novel and therefore require confirmation in further studies. Several biological mechanisms have been suggested for explaining the protective roles of several minerals, such as the anti-inflammatory effect mediated by selenium [50] and the regulation of immune function by zinc.[51] As for magnesium, a previous MR study uncovered that genetically predicted magnesium is associated with an 8.74-fold increased risk of rheumatoid arthritis;[52] however, definitive proof for mechanisms of magnesium in the regulation of immune diseases is still lacking.

The role of vitamin D in CD risk has been noted previously. A prospective cohort study with 122 incident cases of CD and 123 cases of UC in the Nurses' Health Study found that higher predicted plasma levels of 25(OH)D were associated with a 46% lower risk of

incident CD.[53] A previous MR study on vitamin D including approximately 2,000 IBD cases found no causal association with either CD or UC risk.[54] Another MR study including 25,042 IBD cases of mixed ancestry, provided limited evidence for the association of vitamin D with CD.[11] On the contrary, our MR study identified significant MR associations between vitamin D levels and the risk of CD and UC. These conflicting findings might be caused by the enhanced power of the current MR study, in which we used 3 databases to estimate the association with greater precision. In addition, the reliability of the results was significantly improved due to an increasing number of genetic instruments. We also observed the inverse association between vitamin K1 and CD. It has been reported that circulating vitamin K was insufficient in patients with CD, which was suggested to be associated with inflammatory processes.[55] Lacking prospective evidence, more studies are warranted to confirm this observed association. We also observed inverse associations of circulating folate and vitamin E with UC risk. In line with our results, a meta-analysis including 12 studies indicated that the average serum folate concentration in patients with UC was significantly lower than that in controls.[56] The inverse association between vitamin E and UC has been uncovered in some previous observational studies [57, 58] but not all.[59] Detailed pathophysiological mechanisms behind the association between vitamins and IBD remained elusive but several hypotheses are supporting such relationships. It is recognized that vitamin D is an essential anti-inflammatory nutrient in regulating gut mucosal immunity.[60] Studies suggested that vitamin D may affect intestinal epithelium integrity and innate immune barrier function in the involvement of IBD.[60] Emerging studies support that Vitamin K1 is involved in immune response and anti-inflammation [61] and is

associated with the protective and promoting role in the intestine.[62] It has been proved that folate plays a role in regulating reactive oxygen species production and reducing oxidative stress by acting directly as an antioxidant.[63] Vitamin E reduces lipid peroxidation and thus has a significant role in membrane stabilization [64] which may mitigate the oxidative stress in UC. The different vitamins in relation to CD and UC highlight distinctive pathological pathways in UC and CD. The majority of the literature on vitamin deficiencies in IBD centers around deficiencies in folate, vitamin D and B12 and the vitamin E and K were relatively overlooked. Deficiencies on vitamin E and K were usually underappreciated in the IBD patients with steatorrhea and the pre-morbid state was easily neglected.[65] Findings on protective role of vitamin E and K1 provided new insight into the pathogenesis of UC and CD.

Current European Society for Clinical Nutrition and Metabolism (ESPEN) recommendations suggest that micronutrient levels need to be assessed annually in IBD patients, with the correction of any deficit with supplementation.[66] Similarly, literature on supplementation of other micronutrients as therapy is limited to a few, small scale studies that only target specific subgroups of patients. Limited evidence makes specific recommendations on nutrient supplementation difficult. Findings from this study will complement the current evidence from observational studies supporting that circulating micronutrients of antioxidants, minerals, and vitamins are causally linked to the development of IBD, which would contribute to the research field of nutrient prevention for IBD.

The main strength of this study is the MR design that incorporated data from large consortia to provide solid genetic evidence for the reported associations. The MR study with data from populations of European ancestry reduces the risk of confounding and reverse causality and also minimizes bias caused by population stratification. In addition, three large databases with independent populations comprising 10,231 CD and 15,383 UC cases for gene-outcome associations were meta-analyzed in the present study. The results from these three data sources were generally consistent, making it less likely that the observed associations were caused by chance.

Limitations of this MR investigation also merit consideration. Genetic instruments used in this study collectively explained only a small proportion of the variance in blood levels of calcium, phosphorus, and folate, which may result in insufficient power to detect small or moderate associations. However, the large sample sizes in our outcomes datasets also alleviate the relatively inadequate statistical power. In addition, the small sample size of GWAS in lycopene, zinc, and vitamin B6 may contribute to imprecision in the selection of SNPs. Therefore, there is a need for larger GWAS to identify more genetic variants for nutrients. Another limitation in MR analysis is horizontal pleiotropy, but there was no indication of pleiotropic effects in MR-Egger analyses. A further limitation is that the relationship among nutrients is complex, and it may be misleading to examine nutrients individually without considering others. Although some endogenous nutrients are related to exogenous dietary intake in dose-dependently manners, changes in circulating levels of nutrients do not necessarily and completely reflect the variations in dietary intake of nutrients. Besides, genetic associations with lycopene, vitamin A, D and E were estimated

while adjusted for body mass index according to the original GWAS, which might increase the risk for collider bias in the current MR investigation. Unfortunately, the GWASs for these nutrients without adjustment for BMI were not available, thus these observed associations should be cautiously interpreted.

Conclusion

In conclusion, this MR study observed that genetically predicted lycopene, vitamin D, and vitamin K1 levels were inversely associated with CD risk, whereas the genetically predicted magnesium levels were positively associated with CD risk. We also found evidence that genetically predicted lycopene, phosphorus, selenium, zinc, folate, and vitamin E levels have an inverse effect on the risk of UC, and genetic prediction of high calcium and magnesium levels were associated with increased risk of UC.

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Figure legends

Figure 1. A flowchart of study design. CD, Crohn's disease; UC, ulcerative colitis; IIBDGC,

The International Inflammatory Bowel Disease Genetics Consortium; IVW, inverse-variance

weighted; MR-PRESSO, MR Pleiotropy RESidual Sum and Outlier.

Figure 2. Associations of antioxidants, minerals, and vitamins with risk of Crohn's disease. CI,

confidence interval; OR, odds ratio.

Figure 3. Associations of antioxidants, minerals, and vitamins with risk of ulcerative colitis. CI,

confidence interval; OR, odds ratio.