Mendelian randomisation study exploring the associations of serum folate with pan and site-specific cancers

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Abstract

Background: Epidemiological studies report evidence for an association between folate, an essential B vitamin, and the risk of several common cancers. However, both protective and harmful effects have been reported, and effects may differ by cancer site. These associations suggest that modulating dietary folate, or its synthetic form folic acid, could be used to modify population-wide cancer risk. However, observational studies are liable to biases, including residual confounding and reverse causation, thus limiting causal inference. Using Mendelian randomisation (MR), we investigated the causal relationships of genetically determined serum folate with pan-cancer risk (all cancers excluding non-melanoma skin cancers); breast, prostate, ovarian, lung, and colorectal cancers; and malignant melanoma.

Methods: Using publicly available genome-wide association study (GWAS) summary data, we identified genetic instruments to proxy serum folate levels and analysed these using GWAS summary statistics of risk of pan-cancer and six site-specific cancers available from large consortia and the population-based cohort study UK Biobank (UKBB) within a two-sample Mendelian randomisation framework. We conducted MR using the inverse variance weighted (IVW) method and the likelihood-based approach. We performed sensitivity analyses to assess potential violations of MR assumptions.

Results: We identified three SNPs (rs1801133, rs1999594, rs7545014) robustly associated with serum folate in a healthy, young adult Irish population using publicly available GWAS summary data. There was little evidence that genetically increased serum folate was associated with risk of pan-cancer or six site-specific cancers. Meta-analysis showed odds ratios (OR) per standard deviation (SD) increase in $\log_{10}$ serum folate of 0.92 (95% confidence interval 0.78-1.07) for breast cancer, 0.87 (95% confidence interval 0.71-1.06) for prostate cancer, 0.87 (95% confidence interval 0.61-1.25) for ovarian cancer, 0.87 (95% confidence interval 0.57-1.34) for lung cancer, and 1.26 (95% confidence interval 0.84-1.88) for colorectal cancer. ORs for pan-cancers and malignant melanoma in UKBB were 0.86 (95% confidence interval 0.71-1.03) and 0.57 (95% confidence interval 0.30-1.10) respectively. The results were powered to detect modest effect sizes (>80% power [α=0.05] to detect ORs 1.1 (or its inverse 0.9) for the cancer GWAS consortia) and were consistent between the two statistical approaches used (IVW and likelihood-based).

Conclusions: There is little evidence that genetically increased serum folate may affect the risk of pan-cancer and six site-specific cancers. However, we may still be underpowered to detect clinically relevant but smaller magnitude effects. Our results provide some evidence that increasing levels of circulating folate through widespread supplementation or deregulation of fortification of foods with folic acid is unlikely to lead to moderate unintended population-wide increase in cancer risk.

Keywords

Cancer, diet, nutrition, folate, Mendelian randomisation

Introduction

Folate is an essential B vitamin found in foods such as dark leafy green vegetables, liver and legumes. Serum folate reflects recent folate intake and is the earliest biomarker to detect folate status[1]. Folic acid, the synthetic form of folate, is available as a dietary supplement and is used to fortify food such as bread flour in over 80 countries worldwide[2]. At the time of writing, many European countries, including the UK have yet to decide or have rejected a mandate of folic acid fortification, with some countries opting for a voluntary scheme or no population-wide intervention at all[2]. Recently, the UK government has released a report conducted by the Scientific Advisory Committee on Nutrition (SACN)[3] and proposed a public consultation on the mandatory fortification of flour with folic acid. Decisions by governments regarding this public health intervention are made based on all evidence of adverse effects including any potential cancer risk.

Folate has an essential role in the synthesis and methylation of DNA and is a crucial co-factor in one-carbon metabolism together with other B vitamins such as vitamins B2, B6, and B12[4]. In developing foetuses, insufficient folate increases the risk of neural tube defects, including spina bifida and anencephaly[5,6]. In adults, insufficient folate can lead to anaemia[7]. There is also a suggestion that low folate may contribute to carcinogenesis through aberrations in DNA methylation and uracil misincorporation, leading to DNA instability[8]. Folic acid supplementation was shown to have tumour-promoting effects in mouse models[9].

Epidemiological studies exploring associations of folate with the risk of developing site-specific cancers have been inconsistent. For instance, total folate, dietary folate and serum folate levels have been reported to have no associations with breast cancer[10,11], whilst in contrast, a meta-analysis of 26 case-control studies reports protective effects of higher dietary folate intake[12]. Likewise, some meta-analyses suggest positive associations between serum folate and prostate cancer[13], while others suggest no evidence of associations with folate intake[14,15]. These inconsistencies are also present for studies examining folate and colorectal cancer[16,17]. Much of the observational studies to date are limited due to small study sample sizes, measurement error, heterogeneity of the exposure measurement (dietary intake vs. supplement intake vs. circulating levels), timing of folate measurement (leading to possible reverse causation), and the use of data from both pre- and post- folic acid fortification study populations[18].

Several randomised control trials (RCTs) have been conducted exploring the effects of folic acid supplementation on a range of primary outcomes while having also recorded incident cancers. A 2013 pooled analysis of such RCTs compared folic acid supplementation and placebo for the incidence of cancers during treatment periods. The primary outcome of interest was overall cancer, with additional analyses within site-specific cancers. In total, 3,713 cancers were reported in around 50,000 participants with a weighted average treatment period of 5.2 years (range 1.8 to 7.4 years). The meta-analysis reported little evidence that folic acid treatment increased (or decreased) risk of overall cancer compared to placebo. Furthermore, there was no evidence of association in any of the site-specific cancers including colorectal, lung, ovarian, breast, malignant melanoma, and prostate cancer[19]. However, these trials are limited by the small number of incident cancer cases and the short duration of treatment time during the trials. The meta-analysis could not address whether there were any beneficial or adverse effects to folic acid supplementation within more prolonged periods after the trials had ended.

Mendelian randomisation (MR) is an instrumental variable analytical approach which utilises common genetic variants as instruments to proxy potentially modifiable risk factors. The aim of MR is to elucidate the causal effects of these risk factors on disease outcomes of interest[20,21]. Germline genetic variants are randomised and fixed at conception, enabling MR analysis to mitigate the major biases of observational studies such as residual confounding, measurement error and reverse causation. The aim of the current study was to apply MR within a two-sample framework to elucidate the causal associations of serum folate with pan-cancer risk; cancers of the breast, prostate, ovaries, lung, and colorectum; and malignant melanoma.

Materials and Methods

Genetic instrument selection for serum folate

We conducted a search of published genome-wide association studies (GWAS) using MR-Base[22] and PubMed (https://www.ncbi.nlm.nih.gov/pubmed/). Studies with single nucleotide polymorphisms (SNPs) that were robustly associated at P-value $<5x10^{-8}$ with serum folate levels and involving participants of European ancestry were prioritised. We identified a moderately sized GWAS of serum folate in a healthy, young adult Irish population consisting of 2,232 individuals with full summary statistics available[23]. Additionally, GWAS within an Italian population and a large GWAS in an Icelandic population were identified[24,25]. Food manufacturers in the Republic of Ireland (at the time of writing) voluntarily fortify foods with folic acid, in line with the UK[26]. Given the similar ancestry, dietary intakes and current policy in fortification we choose to utilise the genetic association results for the Irish study to match these demographics to these of the participants from UKBB, which was used as the outcome sample in the 2-sample MR framework, and which consists of UK participants. For the large GWAS consortia datasets; participants were recruited to studies from Europe (including the UK) and the USA.

Five SNPs (rs1801133, rs1999594, rs12085006, rs7545014, and rs7554327) from Shane *et al*.[23] located within a 100kb region around the methylenetetrahydrofolate reductase (*MTHFR*) gene and identified among individuals of European ancestry were identified as potential instruments. We excluded rs12085006 and rs7554327 as they are in near-perfect linkage disequilibrium (LD) with rs1999594 (R^2 1.00) and rs7545014 (R^2 0.99) respectively. Detailed information on the selected genetic instruments is provided in Table 1.

Data on the genetic epidemiology of cancers

We retrieved summary statistics of the genetic effects for the selected instruments on the risk of site-specific cancers from large, recently published GWAS. Four large consortia had publicly available summary statistics for breast cancer (BCAC - Breast Cancer Association Consortium), prostate cancer (PRACTICAL - Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome), ovarian cancer (OCAC - Ovarian Cancer Association Consortium), and lung cancer (ILCCO - International Lung Cancer Consortium). Summary statistics were made available for colorectal cancer from the Genetic and Epidemiology of Colorectal Cancer Consortium (GECCO), the Colorectal Cancer Transdisciplinary Study (CORECT), and the Colon Cancer Family Registry (CCFR) consortia (GECCO-CORECT-CCFR). In Supplementary methods we further describe each of these datasets. Information on quality control, imputation and statistical analysis for each GWAS has been previously reported[27–31].

The UK Biobank is a population-based health research resource consisting of approximately 500,000 people, aged between 38 years and 73 years, who were recruited between the years 2006 and 2010 from across the UK[32]. We performed GWAS for cancers of the breast, prostate, ovaries, lung, colorectal and malignant melanoma in the population-based UK Biobank (UKBB) cohort[33]. Cancers were identified by linkage of each participant in the cohort to the UK Cancer Registry. Cases were defined as having a diagnosed cancer throughout the life-course of the UKBB study participants. That is, the cancer diagnosis occurred either before or after enrolment to the UKBB study. A list of the ICD09 and ICD10 codes used to define each site-specific cancer are included in Supplementary Table S1. We also performed GWAS for pan-cancer in UKBB using data for all cancer sites (ICD9:140.0-208.9 and ICD10:C00-C97 specific codes with the exclusion of ICD10:C44.0-C44.9; ICD9:173.0-173.9 non-melanoma skin cancers). Further details on the definition of cases and controls, quality control, imputation, GWAS and statistical analysis can be found in the Supplementary Methods and

Supplementary Table S1. All three instruments for serum folate were available in each of the GWAS consortia and in UKBB.

Mendelian randomisation analysis

We conducted two-sample MR analyses to appraise the potential causality of associations between serum folate and the risk of pan-cancer and six site-specific cancers (breast, prostate, ovarian, colorectal, lung and malignant melanoma)[34].

The beta-coefficients for the associations of each SNP with serum folate levels were reported on the Log_{10} scale. These were converted to the standard deviation (SD) scale to represent an SD change in log_{10} transformed serum folate with each additional effect allele (see supplementary material). We harmonised the SNPs so that the effect alleles were the serum folate increasing alleles.

The three SNPs are located within a 100kb region around the *MTHFR* gene on chromosome 1 and are in weak LD with each other (all R^2 <0.45) (see Supplementary Table S2). The use of multiple correlated SNPs (such as these) introduces bias in the precision of the overall causal effect estimates. To mitigate this bias of over precision, we used extensions of the fixed-effect inverse variance weighted (IVW) method and the likelihood-based approach to account for the correlation structure between the SNPs using a matrix of SNP correlations[35,36]. A matrix of correlations was constructed using the TwoSampleMR R package, which uses reference data on participants of European ancestry within the 1000 Genomes project (Phase 3)[37].

Fixed-effects IVW meta-analysis was performed to pool the MR estimates from the GWAS consortia studies and UKBB for the following cancers: breast, prostate, ovarian, colorectal and lung. Cochran's Q statistic was used to assess heterogeneity between studies.

Sensitivity analyses

The validity of the effect estimates and interpretation in MR analyses are reliant on the following assumptions[38]: i) the selected genetic instruments are robustly associated with serum folate; ii) the genetic instruments affect cancer only through their effect on serum folate; and iii) the instruments are independent of any confounders of the association between serum folate and cancer.

To evaluate the first MR assumption, we estimated the variance in serum folate explained (R^2) by each SNP as well as the strength of the instruments represented by the F-statistic. The R^2 and the F-statistic can be used to evaluate the strength of our instruments and to indicate weak instrument bias[39]. Derivation of the R^2 and the F-statistic is given in the Supplementary methods. To evaluate potential violation of the second and third assumption, we performed look-ups for each of our instruments using the MR-Base PheWAS (http://phewas.mrbase.org/) tool to determine the presence of associations with secondary phenotypes that could be potential confounders of the association. Due to the limited number of folate SNPs, and their correlation, we were unable to assess potential violations of the second assumption of MR (no horizontal pleiotropy) using statistical methods (MR-Egger, weighted median and mode estimators).

Cochran's Q statistic was calculated to assess heterogeneity across SNPs in the causal estimate, with the null hypothesis being that such differences between individual-SNP effect sizes are due to chance[40]. Where there was evidence of heterogeneity (P-value <0.05), a (multiplicative) random-effects IVW and maximum likelihood MR analysis[41] was performed, accounting for the correlation between SNPs.

To further elucidate the potential impact of using correlated SNPs as an instrument we derived the magnitude to which increasing folate might affect the risk of cancer by calculating the ratio of coefficients (Wald ratios)[42] for each SNP individually. The corresponding SEs were derived using the delta method[43]. In addition, we explored systematically whether an individual SNP was driving the main MR association results

by performing a leave-one-out analysis, whereby IVW estimates are derived iteratively by excluding each SNP in turn.

Statistical power

Power calculations were performed using the online tool mRnd (http://cnsgenomics.com/shiny/mRnd/) as described previously[44]. The statistical power to detect a range of odds ratios (ORs) (1.05, 1.1, 1.2, 1.3, 1.4, and 1.5 or their respective inverse) per SD change in $\log_{10}$ serum folate levels given the sample size in each cancer GWAS, a type 1 error of 5%, and a total variance explained by the instruments of 5% (see below), are given in Supplementary Table S3.

The number of cancer cases ranged from 1,277 for ovarian cancer in UKBB to 122,977 for breast cancer in BCAC. We had >80% power to detect modest effect sizes (OR 1.1 or its inverse 0.9) in our MR analysis for consortia studies of cancer of the breast (BCAC), prostate (PRACTICAL), and colorectum (GECCO-CORECT-CCFR) cancers as well as pan-cancers from UKBB. We had a lower power to detect an OR of 1.1 for the consortia studies of ovarian (OCAC, 77%) and lung (ILCCO, 42%) cancers. For UKBB, where cases were not enriched within the dataset, power to detect an OR of 1.1 ranged from 13% to 100%. Supplementary Table S3 describes the sample sizes for each cancer study along with power to detect a range of OR including retrospective power to detect the reported MR ORs.

Analyses were conducted in R software version 3.5.1 using TwoSampleMR and MRInstruments[22], MendelianRandomization[45], meta, and matafor R packages. All reported P-values are two-tailed.

Results

Table 1 describes the associations for each SNP comprising our instrument with serum folate, after rescaling to the SD scale. In total, the genetic instruments explained 4.9% of the variance in serum folate levels. The corresponding F-statistic (113.8) suggests that weak instrument bias was unlikely[39].

Mendelian randomisation estimates for the association between serum folate and cancer

Our Mendelian randomisation effect estimates showed consistent inverse relationships for all cancer outcomes (except colorectal cancer) using both the IVW method and the likelihood-based approach. As effect estimates were very similar between the IVW method and the likelihood-based approach, all subsequent analyses utilise the IVW estimates. Together, the MR effect estimates confer a possible protective effect of increasing serum folate on the risk of cancers. In contrast, the colorectal cancer causal estimates suggest increasing risk with increasing serum folate. These results were concordant between those of the consortia studies and UKBB. However, our estimates are imprecise with 95% confidence intervals crossing the null suggesting that there was little evidence of causal associations (Table 2).

Overall, there was little evidence of heterogeneity using the Cochran's Q statistic between effect estimates for each of the three serum folate SNPs (P-value for heterogeneity >0.1) in our MR analyses. This is with the exception of breast cancer in UKBB (P-value for heterogeneity 0.03) and colorectal cancer in GECCO-CORECT-CCFR (P-value for heterogeneity 0.002) (Table 2). Random effects IVW and likelihood approach is reported for these two cancers. Supplementary Figure S1 show scatter plots of associations between serum folate SNPs and the risk of each of the cancer studies analysed. Results for individual single SNP MR analysis (using Wald ratios) are provided in Supplementary Table S4. There no strong evidence for causal associations between any of the individual SNPs and cancer. Overall, effect estimates were concordant with that of the IVW MR analysis.

Figure 1 shows a forest plot depicting our MR causal estimates for each cancer study as well as the pooled effects using fixed-effects IVW meta-analysis for breast, prostate, ovarian, lung and colorectal cancer. Pooled estimates were concordant in magnitude and direction of effect to those of the individual studies, conferring a protective effect on the risk of cancer with increasing serum folate. Again, there was little evidence of causal associations with confidence intervals crossing the null. In addition, there was little evidence of heterogeneity between the studies in each meta-analysis (P-value for heterogeneity >0.6). Supplementary Table S5 gives meta-analysis causal estimates and between-study heterogeneity results.

Sensitivity analyses and evaluation of Mendelian randomisation assumptions

After look-up within the MR-Base PheWAS database, we found some evidence from GWAS that the three SNPs were associated with additional phenotypes at genome-wide significance (P-value $<1x10^{-5}$) (Supplementary Table S6). All three SNPs were associated with blood cell traits; rs1801133 is associated with mean corpuscular haemoglobin, mean corpuscular volume, plateletcrit (a measure of total platelet mass), and red cell distribution width[46]. Whilst rs7545014 and rs1999594 are associated with plateletcrit and platelet count[46]. In addition, rs1801133 is associated with several vascular phenotypes including diastolic blood pressure and hypertension in UKBB as well as birthweight of first child and hip circumference. Rs1999594 is additionally associated with diastolic blood pressure and rs7545014 is associated with the operative procedure to excise umbilicus; both within UKBB.

Leave-one-out analysis suggests that it is unlikely that any individual SNP was driving the IVW MR results we report as shown by the forest plot illustrated in Supplementary Figure S2. Except for colorectal cancer, all leave-one-out analyses were concordant in direction and strength of effect.

Discussion

We found no strong evidence that genetically increasing serum folate was causally associated with pan-cancer and six site-specific cancers. Although we found little evidence of causal associations, the MR effect estimates tended towards those of being protective for cancer risk with increasing serum folate levels.

Breast cancer

In line with our findings for breast cancer, the latest WCRF-CUP reported little evidence of associations between folate intake and breast cancer risk[47]. The WCRF-CUP aimed to systematically review and meta-analyse observational studies and RCTs associating nutritional risk factors with site-specific cancers. In the meta-analysis of 19,251 breast cancer cases, dietary folate intake was not reported to be associated with cancer risk (risk ratio (RR) per 50 µg/day, 0.99; 95% CI 0.98-1.01). Likewise, results from the meta-analysis of 6,094 cases associating total folate intake with breast cancer also reported no evidence of association (RR 1.01; 95% CI 0.96-1.06)[47]. Subsequent meta-analyses have also reported concordant results to those reported in our MR study. The European Prospective Investigation into Cancer and Nutrition (EPIC) cohort recently reported protective effects of plasma folate on the risk of breast cancer albeit with little statistical evidence (OR 0.93; 95% CI 0.83-1.05)[48].

A recent systematic review and meta-analysis have demonstrated a U-shaped dose-effect relationship between dietary folate intake and breast cancer risk in prospective studies. Daily intake of folate between 153 and 400 µg showed a reduced breast cancer risk compared to those with low folate intake (<153 µg), but not for those >400 µg[49]. We were unable to explore potential non-linear relationships within our MR study owing to lack of individual-level data.

More recently, authors included within our study published findings for an MR of circulating concentrations of micro-nutrients and risk of breast cancer using data from BCAC[50]. Serum folate was included in an MR of breast cancer risk using instruments identified from Grarup *et al.*[25]. In common with our study, SNP rs1801133 was included within the serum folate instrument. Per 1 SD increase in serum folate (nmol/L), the authors reported an OR 1.06 (95% CI 0.94-1.20). The effect estimates are at odds with those we have presented (OR 0.94; 95% CI 0.78-1.12). However, both are imprecise and have confidence intervals that overlap. When comparing the causal effect estimates of rs1801133; the SNP in common with both studies; we see a more comparative causal estimate (OR 1.03; 95% CI 0.89-1.18 Papadimitriou *et al*.[50] vs. OR 1.04; 95% CI 0.82-1.26). It is important to note that our MR study reports causal effect estimates per 1 SD increase in $\log_{10}$ serum folate (nmol/L) while the estimates for Papadimitriou *et al.* are reported per 1 SD increase in serum folate on the natural scale (nmol/L).

Prostate cancer

Several studies have focused on the associations between serum folate and prostate cancer and have been meta-analysed by the WCRF-CUP, which reported no evidence of a dose-response relationship in 7 prospective and nested case-control studies (5,938 cases) (RR 1.01 per 5 nmol/L; 95% CI 1.00-1.02)[47]. These adverse effect estimates contrast with the protective ORs reported in our MR study, although the observational effect estimate overlaps the MR confidence interval and both studies show little evidence of association.

More recent studies are inconsistent. In a pooled analysis of 23 case-control studies, the *MTHFR* C677T variant rs1801133 was found to be protective against prostate cancer (OR per each additional T allele, 0.83; 95% CI 0.70-1.02) albeit with a wide confidence interval that included a possibly harmful effect [51]. The suggested protective effect of the folate reducing 677TT allele runs counter to our MR study whereby each additional C allele (the folate increasing allele) confers a protective effect of similar magnitude (OR 0.87, see Figure 1). In a pooled nested case-control study (6,875 cases, average follow-up of 8.9 years) higher serum folate was associated with increased risk of prostate cancer (OR 1.13; 95% CI 1.02-1.26)[13], while other meta-analyses of folate intake and prostate cancer risk find no associations but do report protective effects in line with our MR study (OR 0.97; 95%CI 0.89-1.06)[14]. However, the same publication also reports strong observational evidence of positive associations between serum folate and prostate cancer (OR 1.43; 95%CI 1.06-1.93), in contrast to our causal analyses[14].

Colorectal cancer

In our study, colorectal cancer was the only site-specific cancer to suggest increased risk with increasing serum folate, albeit with a wide confidence interval that included a possible protective effect. A 2018 systematic review[52] reported little evidence of an effect of folic acid supplementation on colorectal cancer risk in a meta-analysis of RCTs (OR 1.07; 95%CI 0.86-1.14) with effect estimates similar in magnitude to those reported in our MR analysis. For observational studies, the WCRF-CUP meta-analysed 10 studies (6,986 cases) which examined the association between dietary folate and colorectal cancer reporting a null relationship (RR 0.99 per 200 µg/day; 95% CI 0.96-1.02)[47]. A large, recent meta-analysis (24,816 cases) reported a reduced colorectal cancer risk when comparing highest (median, > 441 µg/day) with lowest (median, 212 µg/day) folate intake (RR 0.88; 95% CI 0.81-0.95)[17]. Using genetic studies, a meta-analysis of 67 studies reported strong evidence of association between the *MTHFR* 677TT genotype (which results in lower serum folate) and lower colorectal cancer risk under conditions of high folate intake[53].

Ovarian cancer

Studies exploring the relationships between folate and risk of ovarian cancer are few. The WCRF-CUP, which was last updated in 2013, reported no significant associations between dietary folate and ovarian cancer in a

dose-response meta-analysis (1,158 cases) (RR 0.96; 95% CI 0.88-1.05)[47]. These results are in line with the weakly protective MR results.

Lung cancer

In line with our MR results, the WCRF-CUP reported no significant associations between dietary folate intake and lung cancer risk in a dose-response meta-analysis of 9 studies (4,900 cases) (RR 0.99; 95% CI 0.95-1.02)[47]. The report also described possible U-shaped relationships between dietary folate and lung cancer (P-value < 0.01)[47]. A 2014 meta-analysis of 5 RCTs also found no evidence of associations between folic acid supplementation and lung cancer incidence (RR 1.00; 95% CI 0.84-1.21)[54].

Malignant melanoma

Epidemiological studies relating folate and malignant melanoma risk are limited. An inverse relationship was reported for a meta-analysis of three RCTs evaluating treatment with combined supplements, including folic acid and risk for malignant melanomas (RR 0.47; 95% CI 0.23-0.94) with similar magnitudes of effect to our current MR study. However, the sample size for this analysis was very small (38 malignant melanoma cases)[55]. A meta-analysis of 13 RCTs found no association between folic acid supplementation and malignant melanoma, though results were limited by low number of cases (N = 126) and a relatively short follow up period (average 5.2 years)[19]. More recently, a large meta-analysis of prospective cohorts reported a modest increased risk of malignant melanoma (1,328 cases over a 26-year follow-up) for folate intake from food only (HR 1.36; 95% CI 1.13-1.64), though this did not replicate when looking at total folate intake[56].

Pan-cancer

Most studies published to date have focused on site-specific cancers. In this study, we have performed a genome-wide association analysis for pan-cancers. This allowed us to appraise the impact of folate on cancer risk across all sites in the general population. Proposed mechanisms for the formation of cancer via folate stems from the effects of perturbation of the one-carbon metabolism pathway effects of methylation and DNA repair and synthesis mechanisms which are common to the pathogenesis of many cancers[57]. However, we found little evidence of causal associations with pan-cancer, although the effect estimates were protective and of similar magnitude to those of the site-specific cancers. Likewise, a recent pooled analysis of RCTs showed that folic acid supplements had little effect on the risk of total cancer incidence (RR 1.06; 95% CI 0.99-1.13)[19]. The number of cancer cases was modest (3,713 cases) and the mean follow-up time for included studies was five years, limiting conclusions of long-term impacts of folic acid supplementation.

In cancer treatment, antifolates are key compounds which inhibit enzymes in the folate metabolic pathway disrupting tumour growth and progression[58]. Due to the high proliferation of cells and demand for DNA, increasing levels of folate may promote the growth of precursor or established tumours in animal models[18,59]. Conversely, in normal tissues, insufficient folate levels may impair DNA replication and repair, providing possible mechanisms for the initiation of cancer through gene mutation and chromosomal aberrations. Indeed, administration of folate has shown to reverse these effects[60]. This may support the general findings of protective effect estimates as reported for the multiple cancers within this MR study and in previously published studies. The role of folate in cancer treatment strategies, as well as the proposed mechanisms for carcinogenesis and progression, suggests that potential associations between folate and cancer may not be in terms of risk per se, but rather in progression and survival.

Folate intake has been reported to have a protective effect in cancers at sites not included in this current study, including oral cavity and pharyngeal cancer, bladder, oesophageal, and pancreatic cancer[18]. Further work is needed to establish extensive well-powered publicly available GWAS data in order to elucidate the causal effects on these cancers, which is outside the scope of this current study.

Strengths and limitations

This study's major strength is the use of two-sample MR, which is less prone to biases from confounding, reverse causation and measurement error that is seen in observational studies using directly measured phenotypes. Observational studies have tended to focus on dietary and/or supplement intake of folic acid using methods such as food frequency questionnaire and diet diaries. These methods have inherent limitations such as recall bias and insufficient food composition tables leading to measurement error. Although a genetic proxy of an exposure provides a more objective means with which to asses a causal relationship; measurement error such as that described above can bias the casual estimates. In our study, we used genetic proxies for serum folate level. Circulating biomarkers provide a more proximal measure of nutrition status and is more objectively measured[61].

Several important factors should ideally be met for instruments to be considered robust. One factor is that variants have a biologically plausible relationship to the exposure (though this is not mandatory), i.e. located at or near genes with established pathways relevant to the exposure. The lead GWAS SNP rs1801133 (C667T;A222V) resides within the Methylenetetrahydrofolate reductase (*MTHFR*) gene, reduces *MTHFR* activity and increases it thermolability, in turn lowering serum folate levels, particularly in individuals with low dietary folate intake[62]. This satisfies the criteria of biological plausibility and thus strengthens the robustness of our serum folate instrument. A second factor to consider is that multiple independent variants from different loci that affect the exposure independently should be used to allow for formal tests of violations of instrumental variable assumptions. With so few SNPs identified as instruments for serum folate, we were unable to satisfy this criterion.

Further strengths were the ability to additionally perform GWAS in the UKBB enabling a comparison of population cohort effect estimates with those of case ascertained consortia studies and ultimately allowing meta-analysis, further increasing statistical power to detect modest effect estimates.

We also have several limitations that impact our interpretation of findings. We were unable to extend our analysis to allow for stratified analyses by factors of interest such as alcohol intake, BMI, sex, age, menopausal status and smoking. Our causal estimators assumed a linear relationship, and we were also unable to test for deviations from this. Several methods have recently been developed to explore non-linear relationships within an MR framework; however, these approaches are underpowered and require access to individual-level data[63].

We had greater than 90% power to detect our reported MR ORs for breast, prostate, ovarian, lung and colorectal cancer in the consortia GWAS meta-analysis datasets. However, we had lower power for the cancers appraised using UKBB. Where possible, we performed meta-analysis within each site-specific cancer; which increases statistical power; but we may still be underpowered to detect clinically relevant but smaller magnitude effects. Furthermore, statistical power in MR is dependant on the amount of variance in the exposure variable explained by the genetic variants. The three SNPs within our MR were in weak LD with each other; therefore, it is likely that the variance explained is lower than that of the sum of the three SNPs (R^2 5%). Further work to identify additional SNPs robustly associated with serum folate in larger GWAS and meta-analysis will go some way to improving statistical power.

The SNPs included in our instrument were found to be associated with vascular traits, and cell and platelet measures in MR-Base PheWAS. Grarup *et al*.[25] conducted GWAS of serum folate in an Icelandic population and evaluated possible pleiotropic effects by screening their database of common disease and risk factors. rs1801133 was found to be associated with thoracic aortic aneurysm, though this is unlikely to affect the risk of cancer. Previous studies have reported associations between folate and vascular traits[64] as well as between vascular traits and cancer risk[65,66]. The serum folate instruments used in our MR are located within

or near the *MTHFR* gene, which is directly involved in the one-carbon metabolism pathway. This suggests that the associations with vascular traits could reflect the downstream effects of folate on cancer rather than pleiotropic effects. Though there is no compelling evidence, we cannot rule out the possibility of associations with potential confounders or the presence of pleiotropy.

This study was conducted in European populations and so may not be generalisable to other populations. Both the Republic of Ireland and the UK voluntarily fortify cereals, which may help reduce the prevalence of folate-deficient individuals. Further work may be needed to explore causal effects in populations in which both samples (sample 1 and sample 2) included in the MR analyses derive from countries with no such voluntary or mandatory fortification of food items.

Conclusions

We found little evidence of causal associations between genetically increasing serum folate levels and the risk of pan-cancer, and cancer of the breast, prostate, ovaries, lung, colorectum; and malignant melanoma. Further work is needed to replicate our findings, strengthen the folate instrumental variable, and explore causal associations in the risk of cancer subtypes. The focus of this study was on the risk in developing cancer; however, the use of antifolates in cancer treatments and reported associations of folate supplementation and tumour-progression lends itself to further exploration of causal effects in cancer prognosis and mortality. In combination with existing literature, our results provide evidence that widespread supplementation or deregulation of fortification of foods with folic acid will not lead to an unintended population-wide increase in cancer risk.

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Contributors

SJL, RMM and KB conceived and designed the study. KB conducted the analyses. KB wrote the manuscript with input from all authors. Correspondence and material requests should be addressed to KB

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Declaration of interests

None

Tables

Table 1 Genetic variants included in the instrumental variable and their associations with serum folate

Variant	chr:pos	Locus	Alleles		EAF	Per-allele estimate			R^2
			Effect	Other		Beta[ab]	SE[b]	P-value	
rs1801133	1:11778965	*MTHFR*	C	T	0.66	0.062	0.009	2.82E-11	0.020
rs7545014	1:11857240	*LOC390997*	C	T	0.56	0.050	0.009	1.01E-08	0.015
rs1999594	1:11881803	*RNU5E*	T	C	0.45	0.050	0.009	1.43E-08	0.014

chr, chromosome; pos, position (Build 38); Locus, nearest gene reported [Shane et al. 2017]; EAF, effect allele frequency where the effect allele is the folate increasing allele; SE, standard error; MTHFR, Methylenetetrahydrofolate Reductase; LOC390997, SET Binding Factor 1 Pseudogene; RNU5E, RNA-U5E Small Nuclear 1; R^2, proportion of variance explained; [a] Linear regression adjusted for age and sex; [b] Regression coefficients were re-scaled to represent SD change per each additional effect allele.

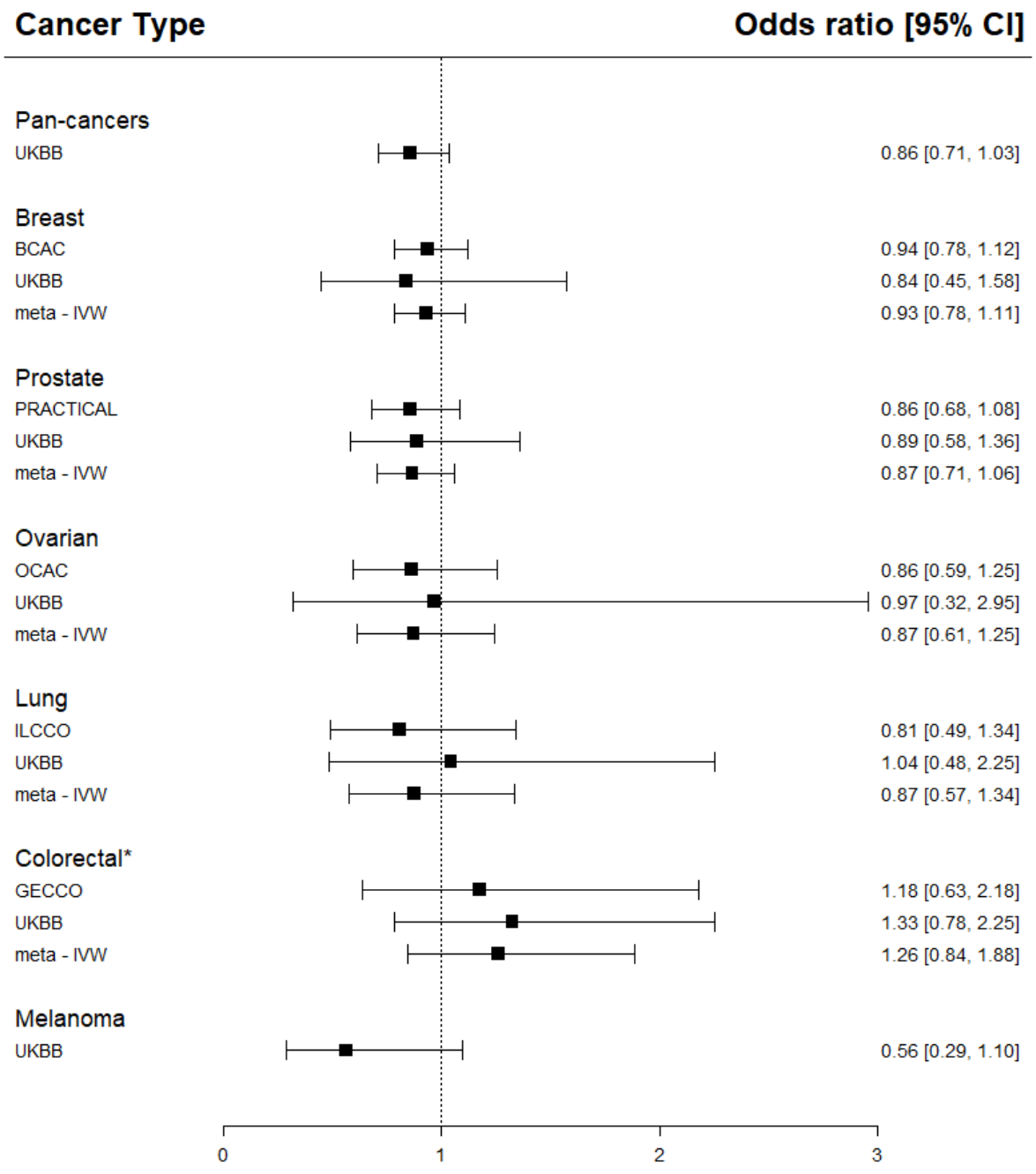
Table 2 Mendelian randomisation estimates between genetically increasing serum folate and risk of cancer

Cancer type	Study	Inverse variance weighted estimate				Maximum likelihood estimate				Q	P_{het}
		OR	LCI	UCI	P-value	OR	LCI	UCI	P-value		
Pan-cancers	UKBB	0.858	0.713	1.033	0.106	0.856	0.707	1.036	0.110	1.297	0.523
Breast	BCAC	0.938	0.784	1.123	0.489	0.936	0.778	1.125	0.480	2.871	0.238
	UKBB*	0.837	0.445	1.575	0.582	0.820	0.421	1.600	0.561	6.839	0.033
Prostate	PRACTICAL	0.859	0.681	1.085	0.202	0.850	0.666	1.085	0.193	4.206	0.122
	UKBB	0.889	0.581	1.361	0.589	0.886	0.575	1.366	0.584	1.829	0.401
Ovarian	OCAC	0.862	0.593	1.255	0.439	0.860	0.587	1.258	0.437	1.351	0.509
	UKBB	0.969	0.318	2.954	0.956	0.968	0.310	3.020	0.955	2.586	0.275
Lung	ILCCO	0.810	0.488	1.344	0.415	0.809	0.486	1.348	0.416	0.376	0.829
	UKBB	1.045	0.484	2.253	0.911	1.046	0.479	2.288	0.910	2.227	0.329
Colorectal	GECCO*	1.177	0.635	2.181	0.605	1.223	0.614	2.435	0.567	12.568	0.002
	UKBB	1.326	0.781	2.252	0.296	1.330	0.778	2.273	0.298	0.577	0.749
Melanoma	UKBB	0.557	0.295	1.053	0.072	0.555	0.288	1.069	0.078	0.404	0.817

*OR, odds ratio; LCI, lower 95% confidence interval; UCI, upper 95% confidence interval; Cochran's Q and P_{het} is the P-value for heterogeneity between instrumented SNP causal estimates in the IVW analysis; OR and 95% CI reflect the casual risk estimate of cancer per genetically determined standard deviation increase in serum folate; * causal estimators are derived using random effects for both IVW and maximum likelihood models.*

Figures

Figure 1 Forest Plot of Mendelian randomisation causal association estimates between serum folate and cancers



*The odds ratios (OR) were derived using the inverse variance weighted method and correspond to a 1 SD increase in log_{10} serum folate levels. Meta – IVW correspond to the fixed effects IVW meta-analysis results; * there will be over precision in the IVW meta-analysis estimate as a sub-sample of UKBB (7% of the UKBB MR sample) was included in the GECCO-CORECT-CCFR colorectal GWAS meta-analysis.*

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