

Genome-wide interaction study of dietary intake of fibre, fruits, and vegetables with risk of colorectal cancer



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Summary

Background Consumption of fibre, fruits and vegetables have been linked with lower colorectal cancer (CRC) risk. A genome-wide gene-environment (G × E) analysis was performed to test whether genetic variants modify these associations.

Methods A pooled sample of 45 studies including up to 69,734 participants (cases: 29,896; controls: 39,838) of European ancestry were included. To identify G × E interactions, we used the traditional 1-degree-of-freedom (DF) G × E test and to improve power a 2-step procedure and a 3DF joint test that investigates the association between a genetic variant and dietary exposure, CRC risk and G × E interaction simultaneously.

Findings The 3-DF joint test revealed two significant loci with p-value < 5 × 10⁻⁸. Rs4730274 close to the *SLC26A3* gene showed an association with fibre (p-value: 2.4 × 10⁻³) and G × fibre interaction with CRC (OR per quartile of fibre increase = 0.87, 0.80, and 0.75 for CC, TC, and TT genotype, respectively; G × E p-value: 1.8 × 10⁻⁷). Rs1620977 in the *NEGR1* gene showed an association with fruit intake (p-value: 1.0 × 10⁻³) and G × fruit interaction with CRC (OR per quartile of fruit increase = 0.75, 0.65, and 0.56 for AA, AG, and GG genotype, respectively; G × E p-value: 0.029).

Interpretation We identified 2 loci associated with fibre and fruit intake that also modify the association of these dietary factors with CRC risk. Potential mechanisms include chronic inflammatory intestinal disorders, and gut function. However, further studies are needed for mechanistic validation and replication of findings.

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Keywords: Diet; Fibre; Gene-environment interaction; Colorectal cancer; GWAS

Research in context

Evidence before this study

It is unknown whether genetic polymorphisms modify the associations of fruits, vegetables and dietary fibre with colorectal cancer risk as current evidence is limited. Previous genome-wide diet-gene interaction studies were underpowered due to insufficient sample size. Additionally, new statistical techniques have recently been developed that aim to further increase the statistical power of the interaction analyses.

Added value of this study

In this large-scale genome-wide interaction analysis, we found two $G \times E$ interactions for fibre, fruits, and colorectal cancer risk. The most significant finding was rs4730274 close to the

SLC26A3 gene, which provides supportive evidence for an interaction between fibre consumption, chronic inflammatory intestinal disorders, overall gut function, and colorectal cancer risk development. A second signal involved rs1620977 in the *NEGR1* gene and this has been linked with obesity and food preference.

Implications of all the available evidence

Our study identified a genetic polymorphism that could modify the protective effect of dietary fibre on colorectal cancer risk through various mechanisms. Additional studies are needed to understand functional implications and to replicate these findings both in European ancestry and other racial/ethnic populations.

Introduction

Colorectal cancer (CRC) is one of the most common cancer types at a global level, responsible for almost 2 million new cancer cases and over 900,000 related deaths in 2020.¹ Current evidence suggests that high consumption of fruits, vegetables, wholegrains and foods containing dietary fibre are associated with a lower CRC risk.² The World Cancer Research Fund classified the evidence for relationship between wholegrains and foods containing dietary fibre with CRC as strong while the evidence for fruits and vegetables and CRC associations was classified as limited.²

Recent genome-wide association studies (GWAS) have identified over 200 independent loci associated with CRC risk explaining up to 35% of total heritability according to twin studies.^{3–8} It has been suggested that gene-environment interactions ($G \times E$) might be able to explain some of this missing heritability.⁹ However, previous genome-wide diet-gene interaction analyses have reported only one significant interaction between rs4143094 (10p14/near *GATA3*) and intake of processed meat.^{10,11} Other, earlier studies focusing solely on CRC susceptibility loci did not find evidence of strong gene-diet interactions, except for an interaction for vegetable consumption and rs16892766, on chromosome 8q23.3, near the genes *EIF3H* and *UTP23*.^{12,13} A recent umbrella review of gene environment evidence and CRC assigned this finding a weak plausibility score due to the strong genetic effect and the weak environmental effects of vegetable intake on CRC risk.¹⁴ Currently, we are still at the early stages of exploring gene-diet interactions and prior studies were limited by the relatively small sample sizes.

These prior studies which conducted traditional interaction analyses were probably underpowered due to

insufficient sample size (16,739 to 18,509 participants). Additional new statistical techniques have recently been developed that aim to increase the statistical power of the interaction analyses including joint tests, which consider both main and interaction effects, and two-step methods, which apply a filtering step before the interaction testing thus prioritizing single nucleotide polymorphisms (SNPs) and decreasing the burden of multiple testing.^{15–17}

Accordingly, the aim of our study was to identify new gene-environment interactions for the consumption of fruits, vegetables, and fibre with risk of CRC cancer by applying both traditional and modern techniques to a large-scale dataset of up to 69,734 participants from three genetic consortia with information on the consumption of fruits, vegetables, and dietary fibre.

Methods

Study population

Up to 45 studies of individuals of European ancestry from three CRC genetic consortia: the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO), the Colorectal Cancer Transdisciplinary Study (CORECT) and the Colon Cancer Family Registry (CCFR) were included in this analysis (Supplemental Table S1).³ For cohort studies, nested case-control sets were assembled via risk-set sampling, whereas cancer-free controls were used for case-control studies. Controls were mostly matched on age at enrolment or diagnosis, sex, race, and enrolment date/trial group, when applicable. Cases were defined as colorectal adenocarcinoma and were confirmed by medical records, pathology reports, or death certificate information. In total, 69,599 (cases: 29,820; controls: 39,779), 69,734 (cases: 29,896;

controls: 39,838), and 44,890 (cases: 20,749; controls: 24,141) participants were included in the analyses of fruits, vegetables, and fibre respectively. Analyses were limited to individuals of European ancestry, based on self-reported race and clustering of principal components with the 1000 Genomes EUR population.

Exposure definition

Information on dietary intake of fruits, vegetables, and total fibre was ascertained through food frequency questionnaires and diet histories while data on demographics and additional factors were collected using in-person interviews and/or structured questionnaires within a period ranging from three months to two years prior to diagnosis for case-control studies and at enrolment for cohort studies. For both fruits and vegetables consumption, within each study multiple questions were used to derive the total consumption of each dietary variable. Most studies reported fruits and vegetables consumption as servings per day. For total fibre, grams/day was the most common measure across contributing studies and it was derived by multiplying intakes of all fibre containing foods in the dietary questionnaires by the fibre content data in nutrient databases. All data were centrally harmonised at Fred Hutchinson Cancer Center.¹² In summary, a multi-step approach was implemented for the data harmonization using a priori defined common data elements (CDEs) to which all questionnaires and data dictionaries were mapped to. Definitions, permissible values, and standardized coding were implemented into a single database via SAS and T-SQL and the overall data were checked for potential outlying values and other errors. The harmonized consumption data were expressed either as servings per day (fruits, vegetables) or grams per day (fibre) and entered into the analysis as sex- and study-specific quartiles, where the quartile groups were coded with the median value of the quartile within each study and sex. In the case-control studies, the study and sex-specific quartiles were based on the control distribution as this best represents the underlying study populations.

Genotyping and imputation

Details on quality control and genotyping were previously published and the genotyping arrays used are summarized in [Supplemental Table S1](#).^{3,18} In brief, SNPs were excluded for: missing call rate more than 2–5%, departure from Hardy–Weinberg equilibrium (HWE) ($p < 1 \times 10^{-4}$), differences between self-reported and genotypic sex, and discordant genotype calls within duplicate samples. All individual studies genotyped using the same build (GRCh37). Autosomal SNPs were imputed to the Haplotype Reference Consortium (HRC) r1.1 panel through the University of Michigan Imputation Server and converted into a binary format for data

management and analyses using the BinaryDosage R package (<https://cran.r-project.org/web/packages/BinaryDosage>).^{19,20} The imputed SNPs were filtered based on a pooled minor allele frequency (MAF) of less than 1% and imputation accuracy R^2 over 0.8. Overall, 7,250,911 SNPs that passed the quality control and were present in all participating studies were included in our analysis. Principal component analysis (PCA) for population stratification assessment was performed using PLINK1.9 on 30,000 randomly sampled imputed SNPs with MAF >5% and $R^2 > 0.99$.

Statistics

Main effects

Logistic regression models adjusted for age (continuous), sex, total energy consumption (kcal/day, continuous divided by 1000), and three population-stratification principal components were used to estimate the association between fibre, fruits, and vegetables intakes and CRC cancer in each of the participating studies. The study-specific results were then meta-analyzed using random-effects models (Hartung-Knapp) to calculate the overall summary odds ratios (ORs) and 95% confidence intervals (CIs).²¹ Presence of heterogeneity was examined and quantified using the Cochran's Q and I^2 statistic respectively.²² Additional stratified analyses were conducted to assess the relationship between the three exposures with CRC risk by sex, tumour site (proximal colon, distal colon, or rectum), and study design (case-control or cohort study). All meta-analyses were performed using the R package Meta.

Interaction effects

Genome-wide interaction scans were performed to identify interaction effects using the R package $G \times E$ ScanR (<https://cran.r-project.org/web/packages/GxEScanR>), which implements a series of different tests such as the traditional logistic regression models, case-only analyses and joint tests of G-E association. Imputed SNP dosages were modelled as continuous variables and a p-value threshold of 5×10^{-8} was chosen to denote the significant associations.²³ In the context of the current study the following notation was used to describe the methods used. *E* corresponds to the exposures of interest (fibre, fruits, or vegetables), *G* corresponds to SNPs, *D* corresponds to CRC status, and *C* corresponds to the adjustment covariates in the models. Logistic regression models were implemented to test for multiplicative scale interaction: $\text{logit}(\text{Pr}(D = 1|G)) = \beta_0 + \beta_G G + \beta_E E + \beta_{G \times E} G \times E + \beta_C C$. The adjustment covariates included age at the reference time, sex, total energy intake (kcal/day), three population stratification principal components, and study as a fixed effect which adjusts for the potential for the individual studies to act as potential confounders. In this case we tested the hypothesis $H_0 : \beta_{G \times E} = 0$, which corresponds to the multiplicative interaction. Apart from this traditional

approach we also applied two more advanced $G \times E$ techniques as our primary tests. The 3-degrees of freedom (DF) test further expands the main hypothesis and considers both the primary D|G the G|E associations in the combined case/control population: $H_0 : \beta_G = \beta_{G \times E} = \delta_G = 0$, where δ_G represents the association between G and E in a combined case-control sample, in an effort to increase the overall statistical power.²⁴ This test examines a fundamentally different hypothesis than a $G \times E$ analysis since significant results can occur due to strong D|G effect, $G \times E$ effect, and/or G|E correlation. However, as it has been shown that an underlying $G \times E$ interaction can induce both D|G and G|E associations in a case-control sample, the 3-DF test increases the chance that important interacting loci will be identified and can be further investigated in additional studies.²⁴ We also implemented a two-step approach that gives priority to potential interactions after weighting $G \times E$ interaction tests (step 2) based on ranks of an independent filtering statistic (step 1).^{25,26} Given the independence between steps 1 and 2, this approach can reduce the burden of multiple comparisons and increase the statistical power to detect significant interactions. In the weighted testing framework proposed by Ioanita-Laza et al.,²⁶ SNPs are partitioned based on their Step-1 p-value into exponentially larger bins, each assigned with an increasingly more stringent threshold for interaction test significance. Bins of size $k_1 = 5, k_2 = 2k_1, k_3 = 2k_2$ etc, were recommended with each bin having step 2 significance threshold $\frac{\alpha}{(2)(5)}, \frac{\alpha}{(4)(10)}, \frac{\alpha}{(8)(20)}$, etc., where $\alpha = 0.05$. Therefore, SNPs with higher step 1 ranks were prioritized and interaction testing of these SNPs conducted at more liberal thresholds. The step 1 filtering statistic that was applied was a combination of the marginal D|G associations and the G|E association in a combined case-control population (EDGE).²⁵ Since many SNPs are in linkage disequilibrium, it is likely that the top bins are populated by correlated markers from the same locus. Therefore, we implemented a modified two-step approach accommodating for the correlated SNPs while properly controlling for type I error.²⁷ Specifically, SNPs were partitioned into bins based on the step 1 Pvalue thresholds, which were calculated based on the original predetermined bin sizes, assuming uniform distribution of 1 million independent tests. For step 2 GxE testing, we accounted for the influx of correlated markers into each bin by correcting for the effective number of tests, estimated using principal component analysis performed on genotype correlated matrices for each bin.²⁸ For any significant findings, the models were further adjusted for body mass index (BMI), diabetes, alcohol, red, and processed meat consumption to examine the robustness of the results.

We examined the extent of genomic inflation using quantile-quantile (Q-Q) plots and by calculating the genomic inflation factor (λ). Because λ

scales with sample size, we also calculated λ_{1000} , which corresponds to the genomic inflation factor for a study of 1000 cases and 1000 controls.^{29,30}

Regional plots were generated for significant hits which present the strength of association, the extent of association signal and linkage disequilibrium (LD) with other SNPs, as well as the position of loci in relation to genes in the region. The software LocusZoom v1.3 was used to generate these plots.³¹ Measures of LD were estimated using European populations of the 1000 Genomes Project study population. Potential expression quantitative trait loci (eQTL) relationships were explored using the Genotype-Tissue Expression (GTEx V8) and the University of Barcelona and University of Virginia genotyping and RNA sequencing project (BarcUVa-Seq)^{32,33} using the available online tool at <https://barcuvaseq.org/cotrex/> (accessed on January 2023). Based on the results from eQTL-gene associations, we then tested the predicted expression of the eQTL-associated gene of interest for an interaction with fibre, fruits, and vegetables consumption in data from the three consortia involved in this study.

Functional annotation plots were also created to visualize chromatin accessibility across the functional datasets and to plot $-\log_{10}$ (p-value) signal tracks. We used ATAC-seq, DNASE-seq, H3K27ac histone ChIP-seq, and H3K4me1 histone ChIP-seq datasets (Supplemental Table S2) of primary tissue from healthy colon and tumour primary tissue samples from Scacheri et al.,³⁴ as well as from three colorectal cancer cell lines (SW480, HCT116, COLO205). These datasets were processed through ENCODE ATAC-seq/DNASE-seq³⁵ and histone ChIP-seq pipelines³⁶ to perform alignment and peak calling. $-\log_{10}$ (p-value) tracks were extracted from the MACS2 step of the pipeline for visualization in genome browsers. Irreproducible Discovery Rate (IDR)³⁷ peak calls for ATAC-seq and DNASE-seq datasets, as well as naive overlap peak calls for histone ChIP-seq datasets, were determined from the ENCODE pipelines. The pyGenomeTracks³⁸ software package was used to visualize chromatin accessibility across the functional datasets and to plot $-\log_{10}$ (p-value) signal tracks. Peaks across samples from the same assay were concatenated across datasets, cropped to within 200 bp centered on the peak summit, and merged using bedtools³⁹ merge.

Interaction analyses for rare variants

As a separate secondary analysis, we further conducted $G \times E$ testing for rare variants. Interaction tests of the three exposures of interest and aggregated rare variant sets at the gene and enhancer level were performed using the Mixed effects Score Tests for interactions (MiSTi) method.⁴⁰ This regression framework includes the interaction between E and the burden component of rare variants as the fixed effect and heterogeneous $G \times E$ effects as random effects. A Fisher's combination

approach under MiSTi (fMiSTi) was applied to combine both the fixed and random effects for discovering $G \times E$ interactions (42), after adjusting for age at reference time, sex, study, and three population-stratification principal components. Since 25,000 genes were tested, a p-value threshold of 2×10^{-6} ($\alpha = 0.05/25,000$) was considered as statistically significant. The analysis was conducted using the MiSTi R package (42).

Ethics

All participants gave written informed consent and studies were approved by their respective Institutional Review Boards.

Role of funders

The funders had no role in the design of the study; the collection, analysis, and interpretation of the data; the writing of the manuscript; or the decision to submit the manuscript for publication.

Results

Consumption of fibre, fruits, and vegetables and CRC risk

Table 1 shows the baseline characteristics of the participants included in the three analyses. Cancer cases were older, had higher BMI and total energy intake, had a greater prevalence of family history of CRC, higher prevalence of type 2 diabetes, and were more likely to have ever smoked cigarettes. Additionally, CRC cases consumed less total dietary fibre (grams/day: 1.49 ± 1.14 versus 1.56 ± 1.14), fruits (servings/day: 1.34 ± 1.04 versus 1.45 ± 1.07), and vegetables (servings/day: 1.40 ± 1.03 versus 1.46 ± 1.08) than controls (p-value < 0.001 for all three exposures).

In the meta-analyses, inverse associations were observed between fibre (OR per quartile increase = 0.79; 95% CI 0.74, 0.85), fruits (OR per quartile increase = 0.79; 95% CI 0.72, 0.86), and vegetables (OR per quartile increase = 0.82; 95% CI 0.73, 0.93) and CRC risk (Fig. 1). The inverse associations were similar by sex (minimum P-het: 0.26 for fibre intake) and cancer subsite (minimum P-het: 0.17 for fibre intake) (Fig. 1). There was evidence of heterogeneity in all three analyses which was driven by the case-control studies (minimum $I^2 = 55\%$; P-het < 0.001) (Supplemental Figure S1). In general, the inverse associations were stronger for case-control studies (Fruits-OR = 0.69; 95% CI = 0.57, 0.83; Vegetables-OR = 0.66; 95% CI = 0.48, 0.91; Fibre-OR = 0.71; 95% CI = 0.64, 0.79) than cohort studies (Fruits-OR = 0.88; 95% CI = 0.83, 0.92; Vegetables-OR = 0.94; 95% CI = 0.89, 0.99; Fibre-OR = 0.86; 95% CI = 0.81, 0.92) (Supplemental Figure S1).

Interaction analysis results

Genomic control inflation and quantile-quantile (QQ) plots for the SNP-diet interactions for risk of CRC did

not show evidence for residual population stratification (Supplemental Figure S2).

Fibre

The 3-DF joint test revealed a significant hit for rs4730274, which maps upstream of the *SLC26A3* gene (Supplemental Figure S3a; 3-DF p-value: 3.8×10^{-8}). This variant was not directly associated with CRC (G|D p-value: 0.33), but there was a moderate association with fibre intake (G|E p-value: 2.4×10^{-3}), and an interaction with fibre intake on CRC risk ($G \times E$ p-value: 1.8×10^{-7}) (Table 2). Stratifying by genotype of rs4730274 showed that the strength of the inverse association between fibre and CRC increased with every copy of the T allele: OR per quartile of fibre increase = 0.87 for those with CC genotype; OR = 0.80 for those with TC genotype; OR = 0.75 for those with TT genotype (Table 3). The functional annotation analysis showed that rs4730274 and some SNPs in LD with it are in open chromatin suggesting enhancer activity in normal colon, cancer tissues, and CRC cell lines (Supplemental Figure S4a). Furthermore, several SNPs in LD with rs4730274 were eQTLs for *DLD* gene in the BarcUVa-Seq and GTEx transverse colon tissue data but not in GTEx sigmoid colon tissue data (Supplemental Tables S3–S5). We also detected statistically significant interactions between fibre and the expression levels of the *SLC26A3* and *DLD* genes in relation to CRC. For *SLC26A3* a negative interaction (p-value = 0.0052) was observed with the inverse association between fibre intake and CRC risk becoming stronger as the expression levels of *SLC26A3* increased, in contrast a positive interaction was observed for *DLD* (p-value = 0.0021) (Supplemental Table S6).

Fruits

Based on the 3-DF joint test, rs1620977 in *NEGR1* showed a highly significant combined effect (Supplemental Figure S3b; 3-DF p-value: 3.4×10^{-8}) with no direct CRC associations (D|G p-value: 0.77), but with a highly significant association with fruit intake (G|E p-value: 1.0×10^{-8}), and a modest interaction with fruits on risk of CRC ($G \times E$ p-value: 0.03) (Table 2). For this SNP the associations between fruit intake and CRC risk became stronger with every copy of the G allele: OR per quartile of fruit increase = 0.75 for those with AA genotype; OR = 0.65 for those with AG genotype; OR = 0.56 for those with GG genotype (Table 3). There was no evidence for enhancer activity for rs1620977 but a correlated SNP aligned with open chromatin regions (Supplemental Figure S4b). No eQTLs were observed for rs1620977 or other SNPs in LD with it (Supplemental Tables S3–S5).

Vegetables

The analysis for vegetables did not identify any interaction effects for either the 3-DF or the 2-step methods. Several hits were identified in the 3-DF analysis, but

| | Fruits | | Vegetables | | Fibre | |
|--|----------------|--------------|--------------|--------------|--------------|--------------|
| | Controls | Cases | Controls | Cases | Controls | Cases |
| Age | | | | | | |
| Mean (SD) | 63.5 (9.4) | 64.3 (10.8) | 63.5 (9.4) | 64.3 (10.8) | 64.5 (9.7) | 64.8 (10.5) |
| Sex | | | | | | |
| Men | 20,208 | 15,357 | 20,238 | 15,396 | 11,032 | 9877 |
| Women | 19,571 | 14,463 | 19,600 | 14,500 | 13,109 | 10,872 |
| BMI | | | | | | |
| Mean (SD) | 27.0 (4.6) | 27.4 (4.9) | 27.0 (4.6) | 27.4 (4.9) | 26.8 (4.7) | 27.4 (5.0) |
| Missing | 1460 (3.7%) | 1501 (5.0%) | 1462 (3.7%) | 1508 (5.0%) | 473 (2.0%) | 582 (2.8%) |
| Total energy intake Mean (SD) | 1900 (719) | 1960 (768) | 1900 (719) | 1970 (769) | 1900 (716) | 1960 (767) |
| Family history of CRC | | | | | | |
| No | 25,180 (63%) | 20,612 (69%) | 25,219 (63%) | 20,676 (69%) | 18,361 (76%) | 14,613 (70%) |
| Yes | 4002 (10%) | 4043 (14%) | 4019 (10%) | 4062 (14%) | 2356 (10%) | 2822 (14%) |
| Missing | 10,597 (27%) | 5165 (17%) | 10,600 (27%) | 5158 (17%) | 3424 (14.2%) | 3314 (16.0%) |
| Education level (highest completed) | | | | | | |
| Less than High School | 8084 (20%) | 7427 (25%) | 8096 (20%) | 7455 (25%) | 6013 (25%) | 6188 (30%) |
| High School/GED | 6139 (15%) | 6025 (20%) | 6156 (15%) | 6054 (20%) | 4047 (17%) | 3581 (17%) |
| Some College | 9627 (24%) | 6542 (22%) | 9653 (24%) | 6562 (22%) | 6122 (25%) | 4942 (24%) |
| College/Graduate School | 12,088 (30%) | 7968 (27%) | 12,118 (30%) | 7979 (27%) | 7734 (32%) | 5701 (27%) |
| Missing | 3841 (11%) | 1858 (6%) | 3815 (11%) | 1846 (6%) | 225 (1%) | 337 (2%) |
| Smoking, never/ever | | | | | | |
| No | 19,298 (49%) | 13,525 (45%) | 19,326 (49%) | 13,530 (45%) | 11,460 (47%) | 9385 (45%) |
| Yes | 19,869 (50%) | 15,656 (53%) | 19,896 (50%) | 15,727 (53%) | 12,224 (51%) | 10,953 (53%) |
| Missing | 612 (1%) | 639 (2%) | 616 (1%) | 639 (2%) | 457 (2%) | 411 (2%) |
| T2D (ever diagnosed) | | | | | | |
| No | 34,970 (88%) | 24,811 (83%) | 35,024 (88%) | 24,873 (83%) | 20,516 (85%) | 16,982 (82%) |
| Yes | 3484 (9%) | 3638 (12%) | 3489 (9%) | 3650 (12%) | 2335 (10%) | 2490 (12%) |
| Missing | 1325 (3%) | 1371 (5%) | 1325 (3%) | 1373 (5%) | 1290 (5%) | 1277 (6%) |
| Red meat (servings/day) | | | | | | |
| Mean (SD) | 0.55 (0.54) | 0.63 (0.58) | 0.55 (0.54) | 0.63 (0.58) | 0.66 (0.63) | 0.66 (0.63) |
| Missing | 189 (0.5%) | 193 (0.6%) | 191 (0.5%) | 193 (0.6%) | 102 (0.4%) | 103 (0.5%) |
| Processed meat (servings/day) | | | | | | |
| Mean (SD) | 0.31 (0.35) | 0.39 (0.44) | 0.31 (0.35) | 0.39 (0.44) | 0.29 (0.36) | 0.35 (0.42) |
| Missing | 2952 (7%) | 3211 (11%) | 2977 (8%) | 3238 (11%) | 1047 (5%) | 561 (3%) |
| Alcohol Consumption | | | | | | |
| No | 14,317 (36%) | 12,826 (43%) | 14,328 (36%) | 12,856 (43%) | 11,184 (46%) | 10,271 (50%) |
| Low (1–28 g/day) | 20,293 (51%) | 12,751 (43%) | 20,324 (51%) | 12,781 (43%) | 10,794 (45%) | 8164 (39%) |
| Moderate (>28 g/day) | 4766 (12%) | 3867 (13%) | 4779 (12%) | 3880 (13%) | 1906 (8%) | 2084 (10%) |
| Missing | 403 (1%) | 376 (1%) | 407 (1%) | 379 (1%) | 257 (1%) | 230 (1%) |
| Total fruit intake | | | | | | |
| Mean (SD) | 1.45 (1.07) | 1.34 (1.04) | 1.45 (1.07) | 1.34 (1.04) | 1.51 (1.10) | 1.39 (1.11) |
| Missing | | | 196 (0.5%) | 158 (0.5%) | 264 (1%) | 230 (1%) |
| Total vegetables intake | | | | | | |
| Mean (SD) | 1.46 (1.07) | 1.40 (1.03) | 1.46 (1.08) | 1.40 (1.03) | 1.53 (1.11) | 1.47 (1.11) |
| Missing | 137 (0.3%) | 82 (0.3%) | | | 214 (0.9%) | 175 (0.8%) |
| Total fibre intake | | | | | | |
| Mean (SD) | 1.56 (1.11) | 1.47 (1.12) | 1.56 (1.11) | 1.47 (1.12) | 1.56 (1.14) | 1.49 (1.14) |
| Missing | 15,902 (40.0%) | 9301 (31%) | 15,911 (40%) | 9322 (31%) | | |

g, grams; SD, standard deviation.

Table 1: Baseline characteristics of the participants by control-case status in the three analyses.

they were all driven by strong D|G or G|E effects with none showing a significant interaction effect (all G × E p-value >0.05, data not shown).

Secondary and sensitivity analyses

The interaction analysis for rare variants did not identify any significant interactions for any of the three

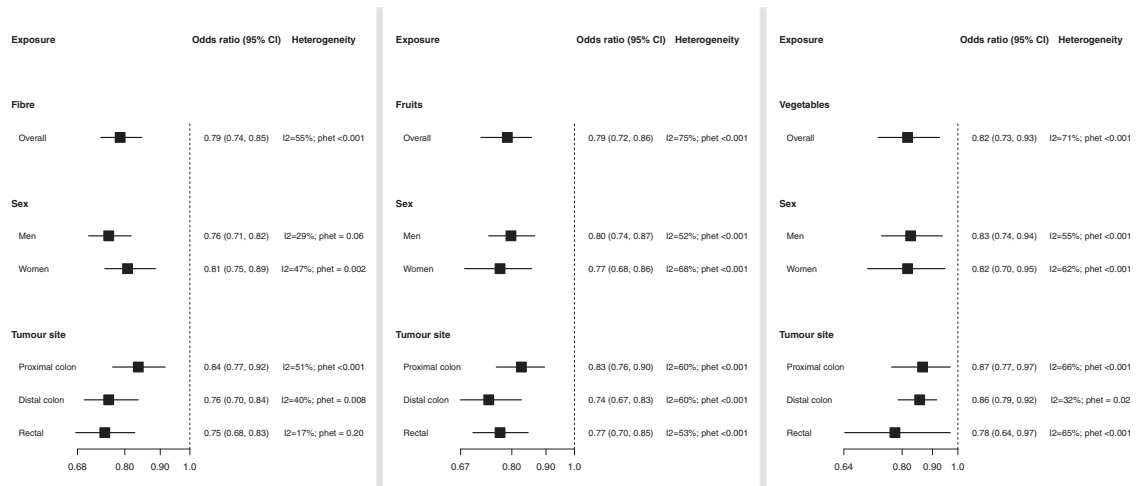


Fig. 1: Results from meta-analysis of association between (a) fibre, fruits, vegetables, and colorectal cancer, overall and stratified by sex and tumour site. Models adjusted for age, sex, and total energy intake. Intake of fibre (g/day), fruits, and vegetables (servings per day) were coded as median of intake sex/study specific quartiles, modelled as continuous variables.

exposures (min p-value = 4.3×10^{-6} in the fibre analysis). Further adjustments for seven additional confounders (i.e., smoking, diabetes, family history of CRC, BMI, alcohol, and red and processed meat) did not change the strength of the interactions (Supplemental Table S7).

Discussion

In this large-scale genome-wide interaction analysis of almost 70,000 participants, we found evidence of interactions between fibre intake and rs4730274 close to *SLC26A3* gene, in relation to CRC risk and an additional hit was observed for fruits intake and rs1620977 in the *NEGR1* gene.

The SNP rs4730274, which maps upstream of the *SLC26A3*, showed the most significant interaction effect in our analysis. This SNP has been linked with ulcerative colitis (UC) and inflammatory bowel disease (IBD) in a previous GWAS study of almost 60,000 participants, with the C allele being associated with a lower risk.⁴¹ Current evidence suggests that dietary fibre can help to maintain remission in patients with IBD and reduce lesions of the intestinal mucosa.⁴² It has also been reported that a high-fibre diet in patients with UC in remission decreased markers of inflammation and reduced intestinal dysbiosis in faecal samples.⁴³ On the

other hand, it is well documented that patients with IBD are at higher risk of CRC.⁴⁴ Therefore, it seems feasible that the protective effect of fibre is stronger in people with genetic liability to these chronic inflammatory intestinal disorders.

The *SLC26A3* gene is located on chromosome 7 and encodes a 764-amino acid protein that is primarily expressed in the digestive tract and more specifically mainly on the cell membrane inside the lumen.⁴⁵ *SLC26A3* is a member of the *SLC26A* transporter family, which includes multifunctional anion exchangers, and is involved in the regulation of Cl⁻ absorption and HCO₃⁻ secretion.⁴⁵ Mutations of this gene lead to congenital chloride diarrhoea (CLD), an autosomal recessive disorder that enhances colonic proliferation and up-regulation of ion transporters in the colon.⁴⁵ Down-regulation of *SLC26A3* has been observed in colon adenomas and adenocarcinomas, colon cancer cell lines, and has been linked with higher CRC incidence.^{46–48} Studies in mice provided further support for the tumour-suppressive role with *SLC26A3* knockout mice showing symptoms of CLD like humans and increased proliferation of colonic crypt epithelium denoting an important role of *SLC26A3* in colon tumourigenesis.⁴⁹ Therefore, *SLC26A3* probably functions as a tumour suppressor; however, the exact mechanisms are not well defined although it has been suggested that its ability to export

| Exposure | SNP | Chr | Position | Gene | OA | EA | EAF | Method | p-value D G | p-value G E | p-value GxE | p-value 3DF |
|----------|-----------|-----|-------------|-------------------------|----|----|------|------------------|-------------|----------------------|----------------------|----------------------|
| Fibre | rs4730274 | 7 | 107,479,719 | upstream <i>SLC26A3</i> | C | T | 0.52 | Joint test (3DF) | 0.33 | 2.4×10^{-3} | 1.8×10^{-7} | 3.8×10^{-8} |
| Fruits | rs1620977 | 1 | 72,729,142 | <i>NEGR1</i> | A | G | 0.71 | Joint test (3DF) | 0.77 | 1.0×10^{-8} | 2.9×10^{-2} | 3.4×10^{-8} |

DF, degrees of freedom; SNP, single nucleotide polymorphism; Chr, chromosome; Position, base pair position based on NCBI Build37. ^aNo interaction effects were observed for vegetables.

Table 2: Main results from genome-wide interaction scans of fibre, fruits, and vegetables.^a

| Fibre | rs4730274 (7:107,479,719) | | |
|--------|---------------------------|-------------------|-------------------|
| | CC (n = 10,335) | TC (n = 22,310) | TT (n = 12,245) |
| | 0.87 (0.81, 0.94) | 0.80 (0.76, 0.84) | 0.75 (0.70, 0.80) |
| Fruits | rs1620977 (1:72,729,142) | | |
| | AA (n = 5715) | AG (n = 28,368) | GG (n = 35,516) |
| | 0.75 (0.59, 0.97) | 0.65 (0.59, 0.73) | 0.56 (0.50, 0.62) |

CRC, colorectal cancer; SNP, single nucleotide polymorphism. ^aNo interaction effects were observed for vegetables.

Table 3: Associations between consumption of fibre, fruits, and vegetables^a and CRC risk, stratified by genotypes of SNPs identified in the interaction analysis.

HCO₃⁻ from the cells might play a role as this reduces intracellular alkalinization, which facilitates cell proliferation.⁵⁰ Finally, downregulation of *SLC26A3* may also be involved in the pathogenesis of UC.^{51,52} Given that chronic inflammation is a hallmark of cancer⁵³ and a prominent driver of CRC development⁵⁴ and the fact that patients with UC have a higher risk of CRC, this might be additional evidence of the tumour suppressive role of *SLC26A3*.

Current evidence suggests there could be a connection between consumption of dietary fibre and the expression levels of *SLC26A3* and CRC. More specifically, fibre promotes the growth of bacterial populations in the gut which in turn increases the expression of *SLC26A3*.^{55,56} Additionally, fibre is metabolized by gut microbiota to short chain fatty acids (SCFA) which have beneficial anti-inflammatory and anti-carcinogenic effects.⁵⁷ However, most SCFAs are ionized and transporters are needed for their absorption.⁵⁸ One of these transporters is *SLC26A3* which show affinities for all three major SCFA (acetate, propionate, and butyrate).⁵⁸ Therefore, there may be synergistic effects between fibre, expression levels of *SLC26A3*, and CRC which are also consistent with our finding that the inverse association between fibre intake and CRC risk is stronger with increased *SLC26A3* expression.

In our analysis we also found some strong normal colon eQTLs for SNPs in LD with rs4730274 and the *DLD* gene, which also showed an interaction effect with fibre on CRC risk. This gene is the third catalytic enzyme of three mitochondrial enzyme complexes: branched-chain alpha-ketoacid dehydrogenase (BCKDH); α -ketoglutarate dehydrogenase (α KGDH); and pyruvate dehydrogenase (PDH).⁵⁹ The main role of PDH is to convert pyruvate to acetyl-CoA as a part of the carbohydrate oxidation pathway.⁶⁰ There is a lack of evidence of changes in the expression of the genes encoding PDH due to cancer but PDH kinase 1 (PDK1), which is often overexpressed in cancer cells and has been strongly implicated in tumourigenesis, phosphorylates and inactivates PDH.⁶⁰ However, it is currently unclear how the *DLD* gene could interact with fibre

consumption and CRC, suggesting that *SLC26A3* is the more likely candidate gene.

We found that the association of fruits with CRC was modified by rs1620977 in the *NEGR1* gene. The SNP rs1620977 has been linked with BMI in previous GWAS with the G allele corresponding to lower values,^{61–63} however our sensitivity analyses after adjusting for additional confounders including BMI did not show any change in the original results. A recent GWAS reported that rs1620977 was associated with fruit consumption at a genome-wide significance level (p-value = 1.2×10^{-18}) with A allele been linked with higher intake.⁶⁴ The *NEGR1* gene is highly expressed in the brain and particularly in the hypothalamus and plays an important role in neuronal outgrowth during neurogenesis.⁶⁵ It has been implicated in regulation of body weight with several SNPs in this gene identified in GWAS of BMI.^{61,66} Furthermore, previous GWAS and individual studies have found associations between SNPs in the same gene in very low to moderate LD ($0.06 < R^2 < 0.59$) with rs1620977 and dietary intake of fruits, meat (beef, pork, processed meat), fat, carbohydrates, and fibre.^{64,67,68} *NEGR1* is an extracellular adhesion protein that binds to cell membrane rafts and promotes cell-to-cell attachment and aggregation, such properties are important in tumour cell migration and invasion during metastasis.⁶⁹ Therefore, *NEGR1* might be involved in malignant transformation through the regulation of intercellular and cell-to-matrix interactions.⁷⁰ *NEGR1* has been shown to be downregulated in various cancer types, including CRC, suggesting a tumour suppression role.⁷¹ Overall, there is some evidence connecting *NEGR1* with diet and CRC since it might also play a role in the choice, preference, and intake of specific type of foods and macronutrients, however, current evidence is limited and further research is needed.⁶⁷

The current study has several strengths. The large sample size gave us increased statistical power to identify G \times E interactions by applying an agnostic genome-wide scan approach. Additionally, we applied a series of new statistical approaches such as the 3-DF joint test, and two-step approach to further increase our power to identify new signals. Finally, the data harmonization and unified quality control across all pooled studies allowed us to evaluate various putative confounders.

A limitation of our study is the fact that information on consumption of fruits, vegetables, and fibre was based on a single measurement through questionnaires which are prone to measurement error and cannot capture long-term consumption efficiently. We also were not able to conduct analyses by fibre type, so it is unknown if the interaction effects we observed differ for soluble and insoluble fibre. Additionally, several of the included studies had a case-control design which are further prone to additional biases like recall and participation bias, and which can lead to biased and inflated results, however the inverse associations were observed for case-control and cohort studies when analyzed separately. Furthermore,

the initial models were adjusted for a small number of confounders which could potentially affect the marginal associations of the three exposures of interest with CRC. Regarding the interaction analyses however, for interaction findings to be confounded the confounder not only has to confound the main effect but also the interactions. Given gene-environment independence, and no interaction of the confounder with the genetic factor it is unlikely that these variables act as confounders.⁷² However, further adjustments for potential confounders yielded similar results with our initial analyses, which gives us confidence for the validity of our results. The two new statistical approaches used in the current study are not without some limitations as well. The two-step approach, even though more statistically efficient, still requires large sample sizes to detect modest-sized interactions. In addition, a significant 3-DF test can occur because of a strong DG effect, $G \times E$ effect, and/or GE correlation and it might not necessarily provide better insights of the mechanisms through which a SNP might affect a trait.^{24,25} Finally, the analysis was conducted in participants of European ancestry and therefore the results may not be generalisable to other populations. However, it is important to follow up in additional population groups where $G \times E$ efforts are limited or underpowered. An important step is the recent colorectal cancer GWAS including individuals of east Asian ancestry.⁸ Additional harmonization of epidemiological data is required however before expanding the $G \times E$ testing.

In summary, we conducted the largest $G \times E$ study to date and we found two $G \times E$ interactions for fibre, fruits, and CRC risk. Our most significant finding was rs4730274 close to the *SLC26A3* gene, which provides supportive evidence for an interaction between fibre consumption, chronic inflammatory intestinal disorders, overall gut function, and CRC development. Additional studies are needed to understand functional implications and to replicate these findings both in European ancestry and other racial/ethnic populations.

Contributors

Conceptualization: Gauderman, Peters, Kim, Kawaguchi, Morrison, Thomas, Lewinger, Data curation: Kim, Kawaguchi, Qu, Diez-Obrero, Morrison, Peters, Harrison, Huyghe, Data verification: Kim, Kawaguchi, Qu, Diez-Obrero, Morrison, Peters, Harrison, Huyghe.

Formal analysis: Kim, Kawaguchi, Diez-Obrero, Morrison.

Methodology: Gauderman, Peters, Kim, Kawaguchi, Morrison, Thomas, Lewinger.

Writing—original draft: Papadimitriou.

Writing—Review and editing: All authors.

Supervision: Peters, Gauderman, Murphy.

All authors read and approved the final version of the manuscript.

Data sharing statement

The dataset used in the current study is available from the corresponding authors on reasonable request.

Declaration of interests

ESK is a co-investigator in a grant from National Institutes of Health (R01CA196569). JW is Stock shareholder of Gilead Sciences Inc. AK has received consulting fees for Illumina Inc., has participated on data safety

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Appendix A. Supplementary data

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