Mendelian Randomization of Circulating Polyunsaturated Fatty Acids and Colorectal Cancer Risk

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ABSTRACT

Background: Results from epidemiologic studies examining polyunsaturated fatty acids (PUFA) and colorectal cancer risk are inconsistent. Mendelian randomization may strengthen causal inference from observational studies. Given their shared metabolic pathway, examining the combined effects of aspirin/NSAID use with PUFAs could help elucidate an association between PUFAs and colorectal cancer risk.

Methods: Information was leveraged from genome-wide association studies (GWAS) regarding PUFA-associated SNPs to create weighted genetic scores (wGS) representing genetically predicted circulating blood PUFAs for 11,016 non-Hispanic white colorectal cancer cases and 13,732 controls in the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO). Associations per SD increase in the wGS were estimated using unconditional logistic regression. Interactions between PUFA wGSs and aspirin/NSAID use on colorectal cancer risk were also examined.

Results: Modest colorectal cancer risk reductions were observed per SD increase in circulating linoleic acid [$OR_{LA} = 0.96$; 95% confidence interval (CI) = 0.93–0.98; $P = 5.2 \times 10^{-4}$] and α-linolenic acid ($OR_{ALA} = 0.95$; 95% CI = 0.92–0.97; $P = 5.4 \times 10^{-5}$), whereas modest increased risks were observed for arachidonic ($OR_{AA} = 1.06$; 95% CI = 1.03–1.08; $P = 3.3 \times 10^{-5}$), eicosapentaenoic ($OR_{EPA} = 1.04$; 95% CI = 1.01–1.07; $P = 2.5 \times 10^{-3}$), and docosapentaenoic acids ($OR_{DPA} = 1.03$; 95% CI = 1.01–1.06; $P = 1.2 \times 10^{-2}$). Each of these effects was stronger among aspirin/NSAID nonusers in the stratified analyses.

Conclusions: Our study suggests that higher circulating shorter-chain PUFAs (i.e., LA and ALA) were associated with reduced colorectal cancer risk, whereas longer-chain PUFAs (i.e., AA, EPA, and DPA) were associated with an increased colorectal cancer risk.

Impact: The interaction of PUFAs with aspirin/NSAID use indicates a shared colorectal cancer inflammatory pathway. Future research should continue to improve PUFA genetic instruments to elucidate the independent effects of PUFAs on colorectal cancer.

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Introduction

Colorectal cancer is the third most commonly diagnosed cancer worldwide with an estimated 746,000 males and 614,000 females diagnosed in 2012 (1). Diet has been shown to play an important role in colorectal cancer development (2, 3). One nutrition-related inflammatory metabolite, prostaglandin E2 (PGE-2), is known to influence colorectal carcinogenesis (4) via promotion of tumor cell proliferation (5, 6) and silencing of tumor suppressor and DNA repair genes (7). PGE-2 is generated via metabolism of omega-6 polyunsaturated fatty acid (PUFA) arachidonic acid (AA) via the COX-2 enzyme (4) and is often overexpressed in colorectal cancer(8, 9). While omega-3 PUFAs are also metabolized by COX-2, they produce a different array of noninflammatory eicosanoids that have not been implicated in carcinogenesis. Thus, PGE-2 levels may be competitively reduced by increasing levels of omega-3 PUFAs in the diet, which could be a potential strategy for colorectal cancer prevention.

Dietary intake of PUFAs has been studied in relation to colorectal cancer incidence; however, results from epidemiologic investigations have been inconsistent (10–12). One possible reason for these discrepancies in the epidemiologic literature may be related to error in accurately assessing dietary PUFA intake. For example, differential recall of dietary intake in case–control studies of colorectal cancer could lead to biased effect estimates. In cohort studies, repeated measurements would be ideal but are not feasible, and a prediagnostic measurement of PUFAs using an objective dietary biomarker may not accurately reflect dietary intake since the etiologically relevant period for colorectal cancer development is unclear. The observed inconsistencies could also be due to biases related to inappropriate confounding control, selection bias, or reverse causation. In addition to these methodologic considerations, it is important to consider aspirin and nonsteroidal anti-inflammatory drug (NSAID) use in tandem with PUFAs given their shared metabolic pathway via COX-2 and resulting PGE-2 production. A limited number of studies have examined the interaction between PUFAs and aspirin/NSAID use on colorectal cancer risk with inconsistent results (13, 14).

The goal of our study was to estimate potentially unbiased associations between genetically predicted circulating PUFAs with colorectal cancer using the Mendelian randomization approach. The Mendelian randomization approach uses genetic variants as instrumental variables for an exposure and given alleles are randomly assorted during conception (akin to a randomized trial); results from such analyses are less susceptible to confounding and other biases (15). Our study was conducted among non-Hispanic whites using data from two large colorectal cancer consortia. Given the shared metabolism via COX-2, we further assessed the combined effects of genetically predicted circulating PUFAs and aspirin/NSAID use on colorectal cancer risk.

Methods

Study population

This study leverages the Genetics and Epidemiology of Colorectal Cancer Consortium (GECCO) consortium and the Colon Cancer Family Registry (CCFR), a pooled dataset of 14 studies of colorectal cancer with a total of 11,018 cases and 13,735 controls, all European ancestry. Details regarding the characteristics of individual studies included in the consortium have been published (16–18). Briefly, medical records, pathologic reports, or death certificates were used to confirm colorectal cancer cases. Genotyped SNPs that did not meet the following criteria were excluded: (i) call rate <98%; (ii) lack of Hardy–Weinberg equilibrium in the controls ($P < 1 \times 10^{-4}$); or (iii) low minor allele frequencies (MAF; ref. 16). All imputed SNPs had an $R^2 > 0.3$. Additional details regarding genotyping are published elsewhere (19). Our study used individual-level and summary statistics data from GECCO to conduct primary and sensitivity analyses. In addition, summary statistics were available from the ColoRectalTransdisciplinary Study (CORECT) consortium, a pooled dataset comprised of 17 studies with a total of 18,682 cases and 11,225 controls. Study-specific sample sizes and genotyping platforms are provided in Supplementary Table S1. All study participants provided written informed consent and all studies included in the consortia were approved by their respective institutional review boards.

Instrumental variable selection

SNPs identified from published omega-6 and omega-3 PUFA genome-wide association studies (GWAS) conducted among individuals of European ancestry (20, 21) were used as the genetic instruments for this Mendelian randomization analysis. The previous

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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Table 1. SNPs identified from published GWAS used to construct genetic instruments for PUFAs.

PUFAs (chain length)	Number of SNPs used in instrument	% Variation explained[a]	1 SD increase in wGS[b] (%)	Independent SNPs included in instrument[c]
Omega-6				
LA (18:2)	4	8.8–23.6	1.18	rs10740118, rs174547, rs2727270, rs16966952
AA (20:2)	2	33.1	1.11	rs174547, rs16966952
Omega-3				
ALA (18:3)	1	1.0	0.01	rs174547
EPA (20:5)	2	2.1	0.06	rs3798713, rs174538
DPA (22:5)	3	11.6	0.06	rs780094, rs3734398, rs174547
DHA (22:6)	1	0.7	0.08	rs2236212

[a]Percent variation explained per instrument calculated as follows: $\sum_{i}^{n}[2\beta_i^2(\text{MAF})(1-\text{MAF})/\text{variance(PUFA)}] \times 100$, where n is the number of independent SNPs, β is effect estimate from GWAS, and variance is PUFA specific (22).
[b]Each PUFA-specific wGS represents a genetically predicted level of PUFAs, which represent an increase in total percent of plasma fatty acids. Weights used to create the wGS were obtained from previous GWAS (20, 21).
[c]SNPs used in each instrument are independent with linkage disequilibrium (LD; as measured using the correlation coefficient, r^2) less than 0.1.

GWAS were conducted among the same individuals as part of the Cohorts for Heart and Aging Research in Genomic Epidemiology (i.e., CHARGE) Consortium. They reported associations between SNPs and plasma levels of omega-6 and omega-3 PUFAs (i.e., as a percentage of total fatty acids). The following nine SNPs were selected as they were all genome-wide significant (i.e., $P < 5 \times 10^{-8}$) and independent at $r^2 < 0.1$: rs10740118, rs174547, rs2727270, rs16966952, rs3798713, rs174538, rs780094, rs3734398, and rs2236212. The SNPs used in the six different genetic instruments (one instrument per PUFA) are summarized in **Table 1** and further details are provided in Supplementary Table S2. Using the β estimates and effect allele frequencies specific to each SNP *i*, and the variance in PUFA levels from published GWAS (20, 21), the percent variation explained by the *n* SNPs included in the six different genetic instruments were calculated as follows: $\sum_{i}^{n}[2\beta_i^2(\text{MAF})(1-\text{MAF})/\text{variance}\,(\text{PUFA})] \times 100$ (22). In GECCO, the average imputation quality for imputed SNPs was $r^2 = 0.98$ (range: 0.97–0.99). In CORECT, the average imputation quality was $r^2 = 0.99$ (range: 0.98–0.99).

Construction of weighted genetic scores

Weighted genetic scores (wGS) were created using individual-level genotyped data in GECCO. For each PUFA, a wGS was constructed per individual as follows: $wGS = \sum_{i}^{n} \beta_i * dosage_i$; where n is the number of independent SNPs used for each PUFA instrument, β_i is the effect estimate (i.e., increase in percent of total plasma fatty acids) for SNP *i* (obtained from two GWAS examining omega-3 and omega-6 PUFAs within the same population; refs. 20, 21), and dosage$_i$ (range from 0–2) is the number of the effect alleles (i.e., alleles representing increased fatty acids levels) an individual possesses for SNP *i*. All GECCO participants had six different PUFA wGSs representing genetically predicted circulating PUFA levels measured as a percentage of total plasma fatty acids. Excluding docosahexaenoic acid (DHA)'s correlation with linoleic acid (LA), (AA), and (ALA), the PUFA wGSs were highly correlated (Supplementary Table S3). No wGSs were simultaneously included in a single model.

Statistical analysis

Unconditional logistic regression adjusted for age, sex, study, and top three principal components for European ancestry was conducted to estimate associations between 1 SD increase in genetically predicted circulating PUFAs and colorectal cancer risk in GECCO. Matching factors including age, sex, and study were included in the models to avoid any bias due to control selection (23). We also adjusted for principal components of European ancestry to account for bias due to population stratification (24, 25). We also explored the association between each PUFA wGS with potential confounders including education (highest level completed), family history (first-degree relative), regular aspirin/NSAID use (at any point during a participant's lifetime), body mass index (BMI; kg/m^2), ever smoking (yes/no), alcohol use (g/day; compared with nondrinkers), folate intake (μg/day from diet), red meat consumption (serving/day), fruit and vegetable intake (servings/day), and sedentary behavior (hours/week; Supplementary Table S4). Only education, family history, aspirin/NSAID use, BMI, and fruit intake were found to be significantly associated ($P < 0.05$) with the six different PUFA wGSs. Results from the fully adjusted model were identical to those from the minimally adjusted models.

Analyses were stratified by potential effect measure modifiers including sex, age [i.e., <65 years (median age), ≥65 years], smoking use, regular aspirin/NSAID use, and BMI (i.e., ≤18.5 kg/m^2, 18.5–24.9, 25–30, and >30). Statistically significant differences (P <0.05) in strata were assessed via the likelihood ratio test using nested models for the multiplicative interaction term. Polytomous regression was conducted to estimate stratum-specific estimates by cancer site (i.e., rectal vs. colon, and separately for proximal and distal colon cancer).

Additive interactions were also conducted to assess the combined effects of genetically predicted circulating PUFA levels and aspirin/NSAID use on colorectal cancer risk. All six PUFA-specific wGSs were dichotomized at the median representing "low" and "high" circulating levels. Using a common referent category, additive interactions were assessed statistically via calculation of the relative excess risk due to interaction (RERI) and its corresponding 95% confidence intervals (95% CI; ref. 26). All analyses were conducted using SAS Enterprise 7.13 and "TwoSampleMR" package curated by MR-Base (27) in R 3.5.1 (R Foundation for Statistical Computing; https://www.r-project.org/).

Sensitivity analyses

Several sensitivity analyses were conducted in GECCO and CORECT. A fixed-effects inverse-variance weighted Mendelian randomization analysis (28) was conducted using summary statistics from PUFA GWAS and from the two consortia, GECCO and COREECT. The remaining analyses assessed the validity of the genetic instruments utilized in this study. Egger regression estimated a bias-reduced

Mendelian randomization association in the presence of directional pleiotropy (i.e., when the average pleiotropic effects of all SNPs used in the instrument are either positive or negative), provided the effects of the instrument on the exposure is not correlated with any pleiotropic effects. Statistically significant intercepts from Egger regression indicate directional pleiotropy and was applied when three or more independent SNPs were included in the instrument [LA and docosapentaenoic acid (DPA); ref. 29]. The weighted-median approach estimated the Mendelian randomization effect assuming at least 50% of SNPs used in the genetic instrument are invalid (30). Corresponding 95% CIs for the weighted-median estimate were calculated using bootstrapped SEs. The weighted-median estimate was only conducted for the PUFAs with more than two SNPs in the instrument, and was not conducted for AA, ALA, DPA, or DHA. The multivariable Mendelian randomization was adjusted for the potential pleiotropic effects of the SNPs included in one PUFA instrument on circulating levels of other PUFAs and utilized all nine GWAS-identified SNPs and their PUFA-specific beta estimates (31, 32). Finally, for instruments with more than two SNPs, a "leave-one-out" analysis was conducted where the inverse-variance Mendelian randomization association was reestimated after excluding the most influential SNP (determined via largest estimated change in MR after exclusion; ref. 27). All sensitivity analyses using summary statistics were scaled to represent 1 SD increase in genetically predicted circulating PUFA levels.

Results

The variants used in the six different PUFA genetic instruments are listed in **Table 1**. The instruments for ALA and DHA included one SNP each explaining 1.0% (i.e., rs174547) and 0.7% (i.e., rs2236212) percent of variation in PUFA levels, respectively. The instruments for eicosapentaenoic acid (EPA) and DPA explained a higher proportion of variance in fatty acid levels with 2.1% and 11.6%, respectively. Comparatively, the SNPs associated with omega-6 PUFAs, LA and AA, explained a higher percent variation in fatty acid levels. Four SNPs were significantly associated with and explained anywhere between 8.8% and 23.6% of the variation in circulating LA levels (reported range from studies included in the omega-6 GWAS; ref. 20). For AA, two SNPs (i.e., rs174547 and rs16966952) together explained more than 33% of variation in AA fatty acid levels, with rs174547 accounting for most of the variation explained.

Main effects and stratified analyses

In **Table 2**, a 1 SD increase in wGSs for shorter-chain omega-6 and omega-3 fatty acids (i.e., LA and ALA) was associated with 4% to 5% reduced colorectal cancer risk ($OR_{LA} = 0.96$, 95% CI = 0.93–0.98, $P = 5.2 \times 10^{-4}$; $OR_{ALA} = 0.95$, 95% CI = 0.92–0.97, $P = 5.4 \times 10^{-5}$). An increased colorectal cancer risk was observed per SD increase in circulating longer-chain omega-3 fatty acids, EPA ($OR_{EPA} = 1.04$, 95% CI = 1.01–1.07, $P = 2.5 \times 10^{-3}$) and DPA ($OR_{DPA} = 1.03$, 95% CI = 1.01–1.06, $P = 1.2 \times 10^{-2}$). No association was observed for DHA. The largest observed increased risk was for AA, the longer-chain omega-6 PUFA, where a 6% increased colorectal cancer risk was observed ($OR_{AA} = 1.06$, 95% CI = 1.03–1.08, $P = 3.3 \times 10^{-5}$).

Stratified analyses are also presented in **Table 2**. Overall, most associations showed little evidence for varying by strata of different effect measure modifiers. Potential exceptions included a statistically significant multiplicative interaction for age (<65 years vs. ≥65 years; $P_{\text{interaction for LA}} = 1.5 \times 10^{-2}$ and $P_{\text{interaction for ALA}} = 0.04$) and regular aspirin/NSAID use ($P_{\text{interaction for AA}} = 0.05$, $P_{\text{interaction for ALA}} = 0.04$, and $P_{\text{interaction for EPA}} = 1.4 \times 10^{-2}$). Among those ≥65 years, 1 SD increase in genetically predicted circulating ALA and LA reduced colorectal cancer risk by 7% and 8%, respectively ($OR_{LA,\ \geq 65\ years} = 0.93$, 95% CI = 0.89–0.96, $P = 5.4 \times 10^{-5}$; $OR_{ALA,\ \geq 65\ years} = 0.92$, 95% CI = 0.89–0.96, $P = 2.7 \times 10^{-5}$). Whereas among individuals <65 years, no statistically significant associations were observed. For longer-chain omega-3 PUFAs (i.e., EPA, DPA, and DHA), no differences across the age-stratified results were observed. For the longer-chain omega-6, 1 SD increase in circulating AA levels was associated with an 8% increased colorectal cancer risk among those ≥65 years ($OR_{AA,\ \geq 65\ years} = 1.08$, 95% CI = 1.04–1.12, $P = 2.7 \times 10^{-5}$) and no association was observed among those <65 years ($OR_{AA,\ <65\ years} = 1.03$, 95% CI = 0.99–1.07, $P = 0.08$). Among aspirin/NSAID nonusers, a similar 8% increased risk was observed per SD increase in circulating AA ($OR_{AA,\ aspirin/NSAID\ nonuser} = 1.08$, 95% CI = 1.04–1.11, $P = 8.3 \times 10^{-6}$), whereas no association was observed ($OR_{AA,\ aspirin/NSAID\ user} = 1.02$, 95% CI = 0.98–1.07, $P = 0.34$) among users. For the short-chain omega-3 PUFA ALA, those individuals who were aspirin/NSAID nonusers were observed to have a 7% reduced colorectal cancer risk per 1 SD increase in circulating ALA levels ($OR_{ALA,\ aspirin/NSAID\ nonuser} = 0.93$, 95% CI = 0.90–0.96, $P = 9.7 \times 10^{-6}$). Similar to longer-chain omega-6 AA, increased colorectal cancer risks were observed for higher levels of circulating longer-chain omega-3s EPA ($OR_{EPA,\ aspirin/NSAID\ nonuser} = 1.07$, 95% CI = 1.03–1.10, $P = 1.7 \times 10^{-4}$) and DPA ($OR_{DPA,\ aspirin/NSAID\ nonuser} = 1.05$, 95% CI = 1.02–1.09, $P = 2.4 \times 10^{-3}$) among aspirin/NSAID nonusers; however, this multiplicative interaction was only statistically significant for EPA. Whereas among regular aspirin/NSAID users, null associations were observed for PUFAs in the stratified analysis.

Additive interaction with aspirin/NSAID use

In **Table 3**, additive interaction between PUFA-specific wGSs and regular use of aspirin/NSAID via a common referent category (i.e., "low" circulating PUFA levels and aspirin/NSAID nonusers) are presented. Among those who were not regular aspirin/NSAID users (i.e., aspirin/NSAID nonusers), high levels of circulating shorter-chain PUFAs (i.e., omega-6 LA and omega-3 ALA) was associated with an 11%–13% reduction in colorectal cancer risk ($OR_{high\ LA,\ aspirin/NSAID\ nonuser} = 0.89$, 95% CI = 0.84–0.95, $P = 7.8 \times 10^{-4}$; $OR_{high\ ALA,\ aspirin/NSAID\ nonuser} = 0.87$, 95% CI = 0.81–0.93, $P = 4.1 \times 10^{-5}$). A 15% increased colorectal cancer risk was observed for higher levels of genetically predicted circulating longer-chain omega-6 AA among aspirin/NSAID nonusers ($OR_{AA,\ aspirin/NSAID\ nonuser} = 1.15$, 95% CI = 1.07–1.23, $P = 4.4 \times 10^{-5}$). Similar increased colorectal cancer risks were observed for higher circulating levels of longer-chain omega-3 PUFAs EPA ($OR_{EPA,\ aspirin/NSAID\ nonuser} = 1.12$, 95% CI = 1.05–1.20, $P = 7.6 \times 10^{-4}$) and DPA ($OR_{DPA,\ aspirin/NSAID\ nonuser} = 1.07$, 95% CI = 1.00–1.15, $P = 3.9 \times 10^{-2}$), among aspirin/NSAID nonusers.

Among those with lower levels of genetically predicted circulating PUFAs, use of aspirin/NSAIDs was associated with reduced colorectal cancer risk, with colorectal cancer risk reductions ranging from 24% ($OR_{low\ AA,\ aspirin/NSAID\ user} = 0.76$, 95% CI = 0.70–0.82, $P = 8.4 \times 10^{-12}$) to 29% ($OR_{lowLA,\ aspirin/NSAID\ user} = 0.71$, 95% CI = 0.65–0.77, $P = 3.3 \times 10^{-17}$). Generally, among aspirin/NSAID users, higher levels of genetically predicted PUFAs (namely LA and ALA) did not further reduce colorectal cancer risk compared with lower levels of PUFAs ($OR_{high\ LA,\ aspirin/NSAID\ user} = 0.68$, 95% CI = 0.63–0.73, $P = 2.0 \times 10^{-20}$; $OR_{highALA,\ aspirin/NSAID\ user} = 0.65$, 95% CI = 0.65, 95% CI = 0.60–0.71, $P = 3.2 \times 10^{-25}$). For longer-chain PUFAs (i.e., omega-6: AA, and omega-3s: EPA, DPA, and DHA), among aspirin/NSAID users, the effect of higher circulating levels of these PUFAs modestly attenuated the colorectal cancer risk reductions observed

Table 2. Overall and stratified associations for genetically predicted PUFAs and colorectal cancer risk using individual-level data in the GECCO.

		Omega-6 PUFAs				Omega-3 PUFAs							
		LA		AA		ALA		EPA		DPA		DHA	
Subgroup	Cases/controls	OR[a] (95% CI)	*P*	OR[a] (95% CI)	*P*	OR[a] (95% CI)	*P*	OR[a] (95% CI)	*P*	OR[a] (95% CI)	*P*	OR[a] (95% CI)	*P*
Overall	11,016/13,732	0.96 (0.93–0.98)	5.2×10^{-4}	1.06 (1.03–1.08)	3.3×10^{-5}	0.95 (0.92–0.97)	5.4×10^{-5}	1.04 (1.01–1.07)	2.5×10^{-3}	1.03 (1.01–1.06)	1.2×10^{-2}	1.01 (0.98–1.03)	0.53
Sex													
Female	5,810/7,327	0.94 (0.91–0.97)	4.2×10^{-4}	1.07 (1.03–1.10)	3.2×10^{-4}	0.94 (0.90–0.97)	2.3×10^{-4}	1.06 (1.02–1.10)	1.1×10^{-3}	1.05 (1.01–1.08)	1.1×10^{-2}	1.01 (0.98–1.05)	0.52
Male	5,206/6,405	0.98 (0.94–1.02)	0.26	1.04 (1.00–1.08)	0.04	0.97 (0.93–1.00)	0.07	1.02 (0.98–1.06)	0.39	1.02 (0.98–1.06)	0.39	1.00 (0.97–1.04)	0.87
$P_{interaction}$[b]		0.15		0.40		0.29		0.14		0.34		0.73	
Age													
<65 years	5,770/7,096	0.98 (0.95–1.02)	0.36	1.03 (0.99–1.07)	0.08	0.97 (0.94–1.01)	0.11	1.03 (0.99–1.07)	0.09	1.02 (0.98–1.05)	0.37	0.99 (0.95–1.02)	0.47
≥65 years	5,246/6,636	0.93 (0.89–0.96)	5.4×10^{-5}	1.08 (1.04–1.12)	2.7×10^{-5}	0.92 (0.89–0.96)	2.7×10^{-5}	1.05 (1.01–1.09)	1.0×10^{-2}	1.05 (1.01–1.09)	5.7×10^{-3}	1.03 (0.99–1.07)	0.12
$P_{interaction}$[b]		1.5×10^{-2}		0.06		0.04		0.47		0.14		0.10	
Smoking													
Ever	6,090/7,526	0.97 (0.93–1.00)	0.06	1.05 (1.01–1.09)	5.7×10^{-3}	0.95 (0.92–0.99)	8.7×10^{-3}	1.04 (1.00–1.08)	3.2×10^{-2}	1.03 (0.99–1.06)	0.14	1.01 (0.97–1.05)	0.57
Never	4,745/6,121	0.94 (0.91–0.98)	3.4×10^{-3}	1.06 (1.02–1.10)	3.4×10^{-3}	0.94 (0.91–0.98)	3.3×10^{-3}	1.04 (1.00–1.08)	3.6×10^{-2}	1.04 (1.00–1.08)	3.2×10^{-2}	1.00 (0.96–1.04)	0.88
$P_{interaction}$[b]		0.33		0.71		0.63		0.83		0.67		0.55	
Aspirin/NSAID use													
Yes	3,058/5,061	0.98 (0.94–1.03)	0.40	1.02 (0.98–1.07)	0.34	0.98 (0.94–1.03)	0.40	1.00 (0.95–1.04)	0.88	1.00 (0.96–1.05)	0.92	1.03 (0.98–1.08)	0.22
No	6,919/7,672	0.94 (0.91–0.97)	2.3×10^{-4}	1.08 (1.04–1.11)	8.3×10^{-6}	0.93 (0.90–0.96)	9.7×10^{-6}	1.07 (1.03–1.10)	1.7×10^{-4}	1.05 (1.02–1.09)	2.4×10^{-3}	1.01 (0.97–1.04)	0.72
$P_{interaction}$[b]		0.11		0.05		0.04		1.4×10^{-2}		0.06		0.45	
Cancer site													
Rectal	2,849/13,732	0.96 (0.92–1.00)	0.06	1.06 (1.01–1.10)	9.7×10^{-3}	0.95 (0.91–0.99)	0.01	1.06 (1.02–1.11)	4.4×10^{-3}	1.04 (1.00–1.09)	4.7×10^{-2}	0.99 (0.95–1.04)	0.72
Colon	7,907/13,732	0.96 (0.93–0.98)	1.9×10^{-3}	1.05 (1.02–1.08)	6.0×10^{-4}	0.95 (0.93–0.98)	7.8×10^{-4}	1.03 (1.00–1.06)	4.0×10^{-2}	1.03 (0.99–1.06)	0.07	1.01 (0.98–1.04)	0.39
Proximal colon	4,319/13,732	0.95 (0.92–0.99)	6.2×10^{-3}	1.05 (1.01–1.09)	5.3×10^{-3}	0.95 (0.92–0.99)	5.6×10^{-3}	1.03 (0.99–1.07)	0.06	1.03 (0.99–1.07)	0.07	1.00 (0.97–1.04)	0.80
Distal colon	3,439/13,732	0.95 (0.92–0.99)	1.5×10^{-2}	1.06 (1.02–1.10)	3.3×10^{-3}	0.95 (0.91–0.98)	4.5×10^{-3}	1.04 (0.99–1.08)	0.7	1.03 (0.98–1.07)	0.16	1.02 (0.98–1.06)	0.35
$P_{homogeneity}$		0.95		0.96		0.99		0.75		0.99		0.79	
Body mass index													
≤18.5 kg/m^2	96/121	0.89 (0.65–1.21)	0.45	1.10 (0.81–1.50)	0.54	0.88 (0.65–1.21)	0.46	1.20 (0.85–1.69)	0.30	1.10 (0.82–1.49)	0.52	0.96 (0.72–1.30)	0.81
18.5–24.9	3,426/4,842	0.95 (0.90–0.99)	1.4×10^{-2}	1.06 (1.01–1.10)	1.4×10^{-2}	0.94 (0.90–0.99)	0.01	1.05 (1.00–1.11)	3.6×10^{-2}	1.02 (0.98–1.07)	0.28	1.03 (0.98–1.07)	0.25
25.0–30.0	4,114/5,211	0.96 (0.93–1.01)	0.09	1.05 (1.01–1.10)	1.4×10^{-2}	0.95 (0.91–0.99)	0.03	1.04 (0.99–1.09)	0.09	1.03 (0.99–1.07)	0.18	1.01 (0.97–1.05)	0.67
>30.0	2,243/2,443	0.95 (0.90–1.01)	0.11	1.06 (1.00–1.13)	0.04	0.94 (0.88–0.99)	0.04	1.04 (0.98–1.11)	0.22	1.05 (0.98–1.11)	0.14	1.01 (0.96–1.07)	0.66
$P_{interaction}$[b]		0.88		0.98		0.94		0.87		0.85		0.88	

[a]All models adjusted for age, sex, study, and top principal components for European ancestry. ORs represent associations per 1 SD increase in PUFA-specific wGS, which corresponds to the following increase in percent of total plasma fatty acids: 1.18% increase in LA; 1.11% increase in AA; 0.01% increase in ALA; 0.06% increase in EPA; 0.06% increase in DPA; and 0.08% increase in DHA.
[b]$P_{interaction}$ calculated using nested models for the multiplicative interaction term via a likelihood ratio test with a χ^2 distribution with 1 degree of freedom.

Table 3. Additive interaction between genetically predicted PUFA intake and regular aspirin/NSAID use in the GECCO.

Polyunsaturated fatty acid levels[a]	Aspirin/NSAID use	Cases/controls	OR[b] (95% CI)	*P*	RERI[c] (95% CI)[d]
LA					
Low	No	3,590/3,722	1.00		
High	No	3,329/3,950	0.89 (0.84–0.95)	7.8×10^{-4}	
Low	Yes	1,545/2,505	0.71 (0.65–0.77)	3.3×10^{-17}	
High	Yes	1,513/2,559	0.68 (0.63–0.74)	2.0×10^{-20}	0.083 (−0.004 to 0.170)
AA					
Low	No	3,321/4,002	1.00		
High	No	3,598/3,670	1.15 (1.07–1.23)	4.4×10^{-5}	
Low	Yes	1,505/2,619	0.76 (0.70–0.82)	8.4×10^{-12}	
High	Yes	1,553/2,442	0.82 (0.76–0.89)	1.9×10^{-6}	−0.082 (−0.185 to 0.021)
ALA					
Low	No	3,603/3,667	1.00		
High	No	3,316/4,005	0.87 (0.81–0.93)	4.1×10^{-5}	
Low	Yes	1,566/2,437	0.72 (0.67–0.78)	2.4×10^{-15}	
High	Yes	1,492/2,624	0.65 (0.60–0.71)	3.2×10^{-25}	0.059 (−0.028 to 0.146)
EPA					
Low	No	4,046/4,476	1.00		
High	No	2,873/3,196	1.12 (1.05–1.20)	7.6×10^{-4}	
Low	Yes	1,807/3,111	0.76 (0.70–0.82)	1.9×10^{-11}	
High	Yes	1,251/1,950	0.80 (0.74–0.87)	4.4×10^{-8}	−0.081 (−0.182 to 0.021)
DPA					
Low	No	3,848/4,105	1.00		
High	No	3,071/3,567	1.07 (1.00–1.15)	3.9×10^{-2}	
Low	Yes	1,665/2,706	0.76 (0.70–0.82)	8.2×10^{-12}	
High	Yes	1,393/2,355	0.77 (0.71–0.83)	8.1×10^{-11}	−0.063 (−0.161 to 0.035)
DHA					
Low	No	4,052/4,627	1.00		
High	No	2,867/3,045	1.05 (0.98–1.13)	0.13	
Low	Yes	1,806/3,140	0.72 (0.67–0.78)	5.1×10^{-18}	
High	Yes	1,252/1,921	0.80 (0.73–0.87)	2.5×10^{-7}	0.024 (−0.075 to 0.124)

[a]Genetically predicted PUFA intake dichotomized at the median (i.e., wGS < median = "Low" and wGS ≥ median = "High").
[b]All models adjusted for age, sex, study, and top principal components for European ancestry.
[c]Additive interaction assessed using the RERI = OR_{11} − OR_{10} − OR_{01} + 1 (e.g., Linoleic acid RERI = 0.68 − 0.71 − 0.89 + 1 = 0.08).
[d]95% CI for RERI estimated using method of Hosmer and Lemeshow (26).

compared with lower levels of AA, EPA, DPA, and DHA. However, the additive interactions presented did not significantly deviate from an additive model as measured via the RERI and corresponding 95% CIs. Overall, colorectal cancer risk reductions (likely driven by aspirin/NSAID use) were still observed in this subgroup ($OR_{highAA,\ aspirin/NSAID\ user} = 0.82$, 95% CI = 0.76–0.89, $P = 1.9 \times 10^{-6}$; $OR_{highEPA,\ aspirin/NSAID\ user} = 0.80$, 95% CI = 0.74–0.87, $P = 4.4 \times 10^{-8}$; $OR_{highDPA,\ aspirin/NSAID\ user} = 0.77$, 95% CI = 0.71–0.83, $P = 8.1 \times 10^{-11}$; $OR_{highDHA,\ aspirin/NSAID\ user} = 0.80$, 95% CI = 0.73–0.87, $P = 2.5 \times 10^{-7}$).

Summary statistics and sensitivity analyses results

The inverse-variance weighted fixed-effects Mendelian randomization results (Supplementary Table S5) using summary statistics were identical to those from the individual-level wGS results. For PUFAs with more than one SNP included in the instrument, statistically significant heterogeneity was observed for the inverse-variance weighted fixed-effects MR estimates for DPA ($P_{heterogeneity} = 3.6 \times 10^{-4}$), indicating possibility for directional pleiotropy (i.e., when the effect on the outcome for each SNP included in the instrument is in the same direction; ref. 15). The results in CORECT were identical to GECCO. Results from the weighted-median analyses were identical to the inverse-variance weighted fixed-effects MR, indicating that our estimates are robust when assuming half the variants included in the instrument are invalid (30). No estimates from the multivariable MR approaches were statistically significant, which evaluated potential pleiotropy of SNPs included in one instrument on other PUFAs (31, 32). Results from the "leave-one-out" analysis (only possible for LA and DPA) indicated that rs174547 was the most influential SNP in these two instruments, and removal of rs174547 from the PUFA instruments did not affect the overall results. The one exception being for DPA in the CORECT consortium where removal of rs174547 resulted in a 7% reduced colorectal cancer risk ($OR_{DPA} = 0.93$, 95% CI = 0.88–0.97, $P = 2.1 \times 10^{-3}$).

Discussion

In our study conducted among over 24,000 non-Hispanic white individuals from the GECCO consortium, we observed a 6% increased colorectal cancer risk among those with higher genetically predicted circulating levels of omega-6 PUFA AA. Modest increased risks were observed for EPA and DPA. Modest risk reductions were observed for longer-chain omega-6 PUFA LA, and longer-chain omega-3 PUFAs ALA. These associations remained statistically significant among those ≥65 years and among aspirin/NSAID nonusers. When stratified by aspirin/NSAID use, 1 SD increase in circulating AA increased risk of colorectal cancer by 8% ($P_{interaction} = 0.05$), and reduced risk by 7% for ALA ($P_{interaction} = 0.04$). Regular users of aspirin/NSAIDs were

observed to have 18%–35% reduced risk of colorectal cancer regardless of their genetically predicted levels of PUFAs. Our main effects results were confirmed using the summary statistics Mendelian randomization approach.

Not all the associations observed were consistent with our biologic hypothesis regarding omega-6 and omega-3 PUFAs. For example, a modest 4% reduction in colorectal cancer risk was observed for increases in genetically predicted short-chain omega-6 LA levels, which is a precursor to AA levels and subsequently PGE-2. One potential explanation for the risk reduction observed for the LA may be related to two variants included in the instrument that are part of the *FADS1* and *FADS2* genes (i.e., rs174547 and rs2727270, respectively) and are responsible for the conversion of LA to AA. When incorporating these SNPs in the instrument, increased genetically predicted levels of LA will result in lower downstream levels of AA and PGE-2, which could potentially reduce colorectal cancer risk. We also observed modest increased risks for higher genetically predicted levels of potentially anti-inflammatory omega-3 PUFAs EPA and DPA. However, the risk reduction is consistent with a previous meta-analysis of LA intake on colorectal cancer risk (33), and with a previous Mendelian randomization study (also included data from the CCFR) conducted by May-Wilson and colleagues among seven European cohorts ($OR_{LA} = 0.95$, 95% CI = 0.93–0.98) (34). Furthermore, results for AA from May-Wilson and colleagues ($OR_{AA} = 1.05$, 95% CI = 1.02–1.07) are nearly identical to those presented in our study. Results for EPA, DPA, and DHA were in the same direction (except for EPA); however, the effect sizes reported in May-Wilson and colleagues have larger magnitudes but are less precise. We also observed slightly stronger associations among older (i.e., ≥65 years) compared with younger individuals for many of the PUFAs, which could be an indication of the cumulative effects of being genetically predisposed to higher PUFA levels on colorectal cancer risk.

The benefits of taking aspirin/NSAID on colorectal cancer risk have been studied extensively (35, 36). GECCO has also reported risk reductions with aspirin/NSAID use (OR = 0.71, 95% CI = 0.66–0.77; ref. 37), and the magnitude of the risk reduction was similar to the associations reported among the subgroup of aspirin/NSAID users when considering the interactions with circulating PUFAs. Notably, in the Nurses' Health Study, long-term aspirin use (i.e., >10 years) and NSAID use reduced colorectal cancer risk by 32%, and risk was reduced by over 50% (OR = 0.47, 95% CI = 0.31–0.71) among women taking more than 14 (325mg) tablets per week (35). The benefits of long-term aspirin use were corroborated in randomized and observational studies (36). The recommendation to the United States Preventive Task Force for long-term aspirin use as a preventive strategy for colorectal cancer was indicated for 10 years postinitiation (38). In our study, aspirin/NSAID use was defined as regular use over an individual's lifetime and this definition varied according to study. Thus, it is possible that heterogeneity in assessment of aspirin intake may affect the association between long-term aspirin use and colorectal cancer risk in our study; however, the associations observed are consistent with previous investigations.

Hall and colleagues examined the interaction between PUFA levels and aspirin use on colorectal cancer risk among men in the Physicians' Health Study (PHS; ref. 14). They reported reduced colorectal cancer risk with higher intake of long-chain omega-3 PUFAs (i.e., Quartile 4 vs. Quartile 1, $OR_{Q4vs.Q1} = 0.34$, 95% CI = 0.15–0.82) among nonaspirin users. Similar to our results, the potential added benefit of increasing long-chain omega-3 intake among aspirin users was minimal when compared with non–aspirin users with low omega-3 intake (14). Among the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS) participants, the potential modification of marine omega-3 dietary intake by aspirin/NSAID use on colorectal cancer risk was evaluated but no significant heterogeneity was reported (13). Another study examined prediagnostic levels of the urinary PGE-2 metabolite (PGE-M) on colorectal adenoma risk stratified by aspirin use (>2 tablets per week) in the NHS (39). Aspirin use was only beneficial among individuals with high levels of PGE-M. AA uptake by COX-2 is reduced in the presence of NSAIDs in colon cancer cells (40). Similarly, reduced binding of DHA to COX-2 was observed when combined with a selective COX-2 inhibitor celecoxib (41). Inhibition of PUFA metabolism via the COX-2 enzyme in the presence of aspirin may help to explain the potential antagonism observed for the interaction between PUFAs and aspirin on colorectal cancer risk.

Our study has several strengths. First, we utilized data from two large consortia of approximately 25,000 and 30,000 subjects (for GECCO and CORECT, respectively) to estimate potentially unbiased association between PUFAs and colorectal cancer risk using the Mendelian randomization approach. The availability of individual-level GECCO data and several covariates was helpful for assessing the association between the PUFA-specific wGSs with colorectal cancer risk factors. This is one way to assess the validity of the genetic instrument in a Mendelian randomization analysis (i.e., the instrument should not be associated with confounders of the exposure–disease association; ref. 15). We adjusted for additional covariates that were found to be associated with the six different PUFA wGS; however, the results from the adjusted models were identical to the minimal-lyadjusted models. We also conducted stratified analyses to estimate the association between genetically predicted PUFAs among different subgroups. Several Mendelian randomization sensitivity analyses were conducted to assess the robustness of the results in the presence of pleiotropy, but these analyses are likely underpowered due to the limited number of independent SNPs included. Finally, we are one of the few studies to assess the additive interaction between genetically predicted circulating PUFAs along with aspirin/NSAID use on colorectal cancer risk.

While our study has many strengths, there are several opportunities for improvement in future investigations. There was indication of directional pleiotropy in the Mendelian randomization sensitivity analyses (for DPA), and for some of the PUFAs, we were unable to estimate an effect for sensitivity analyses using summary statistics (i.e., Egger regression, weighted-median approach, leave-one-out analysis) due to the limited number of SNPs used in the genetic instrument. Several of the wGSs were highly correlated with one another in the individual-level analysis, which would affect the estimation of independent PUFA effects. However, incorporating additional SNPs as part of the genetic instrument in the future will increase the percent variation explained and subsequently increase the strength of the genetic instrument. Stronger genetic instruments will ultimately help to further elucidate independent PUFA effects and provide a better opportunity to assess influence of pleiotropy on the Mendelian randomization estimates. Furthermore, using new weights from future GWAS that examine associations with longer-term PUFA biomarkers (e.g., adipose tissue and red blood cell) will help to clarify the potential causal role of PUFAs on colorectal cancer risk. The power to detect an OR at least 1.05 at an $\alpha = 0.05$ in our study ranged from approximately 5% (for DHA) to 62% (for AA), and is determined by the strength of the instrument (42). Furthermore, increasing the percent variation explained may allow for the detection of even smaller effects due to increased power. The associations derived from a Mendelian randomization analysis could help to identify the presence of a

potential causal association between exposure and outcome. Many comparisons were made in this analysis and thus the potential for false-positive associations exists. However, most associations in our analysis remain statistically significant even after Bonferroni correction for multiple comparisons. Furthermore, our genetic instruments utilized SNPs previously reported to be associated with circulating PUFAs that have previously shown to have influence on carcinogenesis in experimental studies, and thus the analyses undertaken in this article are based on an *a priori* biologic hypothesis. Finally, it would be worthwhile to conduct similar analyses in different populations to better understand the influence of PUFAs on colorectal cancer risk in populations where the ratio of omega-6 to omega-3 PUFAs may differ (e.g., Asians), and among populations where colorectal cancer risk is high (e.g., African Americans). Future investigations should consider identifying additional genetic variants associated with PUFA levels among different races that would facilitate conducting Mendelian randomization analyses in these populations.

Because of a substantial amount of missing data for continuous measures of aspirin/NSAID use, we were unable examine the interaction between long-term aspirin/NSAID use and circulating PUFAs on CRC risk. However, because selective COX-2 inhibitors may increase risk of cardiovascular disease with long-term use (43), examining the potential added benefit of omega-3 PUFA intake with long-term use of selective COX-2 inhibitors may be futile realistically (unless among high-risk population subgroups). Finally, it is possible that the results from the additive interaction are subject to residual confounding given aspirin/NSAID use was self-reported (44). Thus, future investigations with better long-term measures of aspirin/NSAID use should further examine the interaction with PUFAs, and also consider other potential biologic pathways.

In conclusion, we observed a 6% increased risk for colorectal cancer among those with higher genetically predicted circulating levels of omega-6 PUFA AA, and similarly modest increased risks for longer-chain omega-3 PUFAs EPA and DPA. Risk reductions were observed among those with higher genetically predicted circulating levels of short-chain omega-6 PUFA LA, and short-chain omega-3 PUFA ALA. Our study results indicate that among aspirin/NSAID users, the potential benefit of increasing long-chain omega-3 PUFAs may be minimal in terms of further reducing colorectal cancer risk. Results from the Mendelian randomization analysis using summary statistics corroborate our main effect findings. However, due to the limited number of variants used in some genetic instruments, an assessment of the influence of pleiotropy on our estimates could not be evaluated for all PUFAs. Given the small effects observed and the limited number of SNPs used in our genetic instruments, the clinical significance of our results is limited, and our results may only indicate a shared colorectal cancer inflammatory pathway for PUFAs and aspirin/NSAID use. Future Mendelian randomization studies should continue to improve the genetic instruments used that will help to further elucidate the effects of specific PUFAs on colorectal cancer risk.

Disclosure of Potential Conflicts of Interest

S.B. Gruber is founder of Brogent International LLC, reports receiving a commercial research grant from Myriad Genetics, and has ownership interest (including patents) in Brogent International LLC. No potential conflicts of interest were disclosed by the other authors.

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References

1. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA Cancer J Clin 2015;65:87–108.
2. Lippi G, Mattiuzzi C, Cervellin G. Meat consumption and cancer risk: a critical review of published meta-analyses. Crit Rev Oncol Hematol 2016;97:1–14.
3. Theodoratou E, Timofeeva M, Li X, Meng X, Ioannidis JPA. Nature, nurture, and cancer risks: genetic and nutritional contributions to cancer. Annu Rev Nutr 2017;37:293–320.
4. Wang D, DuBois RN. An inflammatory mediator, prostaglandin E2, in colorectal cancer. Cancer J 2013;19:502–10.
5. Kawamori T. Enhancement of colon carcinogenesis by prostaglandin E2 administration. Carcinogenesis 2003;24:985–90.
6. Wang D, Wang H, Shi Q, Katkuri S, Walhi W, Desvergne B, et al. Prostaglandin E(2) promotes colorectal adenoma growth via transactivation of the nuclear peroxisome proliferator-activated receptor delta. Cancer Cell 2004;6:285–95.
7. Xia D, Wang D, Kim S-H, Katoh H, DuBois RN. Prostaglandin E2 promotes intestinal tumor growth via DNA methylation. Nat Med 2012;18:224–6.
8. Eberhart CE, Coffey RJ, Radhika A, Giardiello FM, Ferrenbach S, Dubois RN. Up-regulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas.Gastroenterology 1994;107:1183–8.
9. Zhang H, Sun X-F. Overexpression of cyclooxygenase-2 correlates with advanced stages of colorectal cancer. Am J Gastroenterol 2002;97:1037–41.
10. Gerber M. Omega-3 fatty acids and cancers: a systematic update review of epidemiological studies. Br J Nutr 2012;107:S228–39.
11. Shen X-J, Zhou J-D, Dong J-Y, Ding W-Q, Wu J-C. Dietary intake of n-3 fatty acids and colorectal cancer risk: a meta-analysis of data from 489 000 individuals. Br J Nutr 2012;108:1550–6.
12. Sakai M, Kakutani S, Horikawa C, Tokuda H, Kawashima H, Shibata H, et al. Arachidonic acid and cancer risk: a systematic review of observational studies. BMC Cancer 2012;12:606.
13. Song M, Chan AT, Fuchs CS, Ogino S, Hu FB, Mozaffarian D, et al. Dietary intake of fish, ω-3 and ω-6 fatty acids and risk of colorectal cancer: A prospective study in U.S. men and women: Fish, ω-3 and ω-6 fatty acids and risk of CRC. Int J Cancer 2014;135:2413–23.
14. Hall MN, Campos H, Li H, Sesso HD, Stampfer MJ, Willett WC, et al. Blood levels of long-chain polyunsaturated fatty acids, aspirin, and the risk of colorectal cancer. Cancer Epidemiol Biomarkers Prev 2007;16:314–21.
15. Haycock PC, Burgess S, Wade KH, Bowden J, Relton C, Davey Smith G. Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. Am J Clin Nutr 2016;103:965–78.
16. Peters U, Jiao S, Schumacher FR, Hutter CM, Aragaki AK, Baron JA, et al. Identification of genetic susceptibility loci for colorectal tumors in a genome-wide meta-analysis. Gastroenterology 2013;144:799–807.
17. Hutter CM, Chang-Claude J, Slattery ML, Pflugeisen BM, Lin Y, Duggan D, et al. Characterization of gene-environment interactions for colorectal cancer susceptibility loci. Cancer Res 2012;72:2036–44.
18. Newcomb PA, Baron J, Cotterchio M, Gallinger S, Grove J, Haile R, et al. Colon cancer family registry: an international resource for studies of the genetic epidemiology of colon cancer. Cancer Epidemiol Biomarkers Prev 2007;16:2331–43.
19. Zanke BW, Greenwood CM, Rangrej J, Kustra R, Tenesa A, Farrington SM, et al. Genome-wide association scan identifies a colorectal cancer susceptibility locus on chromosome 8q24. Nat Genet 2007;39:989–94.
20. Guan W, Steffen BT, Lemaitre RN, Wu JHY, Tanaka T, Manichaikul A, et al. Genome-wide association study of plasma N6 polyunsaturated fatty acids within the cohorts for heart and aging research in genomic epidemiology consortium. Circ Cardiovascr Genet 2014;7:321–31.
21. Lemaitre RN, Tanaka T, Tang W, Manichaikul A, Foy M, Kabagambe EK, et al. Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. PLos Genet 2011;7:e1002193.
22. Shim H, Chasman DI, Smith JD, Mora S, Ridker PM, Nickerson DA, et al. A Multivariate genome-wide association analysis of 10 LDL subfractions, and their response to statin treatment, in 1868 caucasians. PLoS One 2015;10:e0120758.
23. Rothman KJ, Greenland S, Lash TL. Modern epidemiology. 3, [thoroughly revised, an update]. Philadelphia, PA: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2008. Available from: http://www.loc.gov/catdir/enhancements/fy0828/2007036316-t.html.
24. Campbell CD, Ogburn EL, Lunetta KL, Lyon HN, Freedman ML, Groop LC, et al. Demonstrating stratification in a European American population. Nat Genet 2005;37:868–72.
25. Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D. Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 2006;38:904–9.
26. Hosmer DW, Lemeshow S. Confidence interval estimation of interaction. Epidemiology 1992;3:452–6.
27. Hemani G, Zheng J, Elsworth B, Wade KH, Haberland V, Baird D, et al. The MR-Base platform supports systematic causal inference across the human phenome. eLife 2018;7. pii: e34408.
28. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data: mendelian randomization using summarized data. Genet Epidemiol 2013;37:658–65.
29. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol 2015;44:512–25.
30. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. Genet Epidemiol 2016;40:304–14.
31. Burgess S, Thompson SG. Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. Am J Epidemiol 2015;181:251–60.
32. Burgess S, Dudbridge F, Thompson SG. Re: "Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects." Am J Epidemiol 2015;181:290–1.
33. Zock PL, Katan MB. Linoleic acid intake and cancer risk: a review and meta-analysis. Am J Clin Nutr 1998;68:142–53.
34. May-Wilson S, Sud A, Law PJ, Palin K, Tuupanen S, Gylfe A, et al. Pro-inflammatory fatty acid profile and colorectal cancer risk: A Mendelian randomisation analysis. Eur J Cancer 2017;84:228–38.
35. Chan AT. Long-term use of aspirin and nonsteroidal anti-inflammatory drugs and risk of colorectal cancer. JAMA 2005;294:914–23.
36. Flossmann E, Rothwell PM. British Doctors Aspirin Trial and the UK-TIA Aspirin Trial. Effect of aspirin on long-term risk of colorectal cancer: consistent evidence from randomised and observational studies. Lancet North Am Ed 2007;369:1603–13.

37. Nan H, Hutter CM, Lin Y, Jacobs EJ, Ulrich CM, White E, et al. Association of aspirin and NSAID use with risk of colorectal cancer according to genetic variants. JAMA 2015;313:1133.
38. Chubak J, Whitlock EP, Williams SB, Kamineni A, Burda BU, Buist DSM, et al. Aspirin for the prevention of cancer incidence and mortality: systematic evidence reviews for the U.S. Preventive Services Task Force. Ann Intern Med 2016;164:814.
39. Bezawada N, Song M, Wu K, Mehta RS, Milne GL, Ogino S, et al. Urinary PGE-M levels are associated with risk of colorectal adenomas and chemopreventive response to anti-inflammatory drugs. Cancer Prev Res 2014;7:758–65.
40. Orido T, Fujino H, Kawashima T, Murayama T. Decrease in uptake of arachidonic acid by indomethacin in LS174T human colon cancer cells; a novel cyclooxygenase-2-inhibition-independent effect. Arch Biochem Biophys 2010;494:78–85.
41. Swamy MV, Cooma I, Patlolla JMR, Simi A, Reddy BS, Rao CV. Modulation of cyclooxygenase-2 activities by the combined action of celecoxib and decosahexaenoic acid: novel strategies for colon cancer prevention and treatment. Mol Cancer Ther 2004;3:215–21.
42. Burgess S. Sample size and power calculations in Mendelian randomization with a single instrumental variable and a binary outcome. Int J Epidemiol 2014;43:922–9.
43. Gunter BR, Butler KA, Wallace RL, Smith SM, Harirforoosh S. Non-steroidal anti-inflammatory drug-induced cardiovascular adverse events: a meta-analysis. J Clin Pharm Ther 2017;42:27–38.
44. Knol MJ, VanderWeele TJ. Recommendations for presenting analyses of effect modification and interaction. Int J Epidemiol 2012;41:514–20.