Mendelian Randomization of Circulating Polyunsaturated Fatty Acids and Colorectal Cancer Risk

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Background: Results from epidemiologic studies examining circulating 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 PUFA levels could help elucidate an association between PUFA levels 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 (ORLA = 0.96; 95% confidence interval (CI) = 0.93–0.98; P = 5.2 × 10^{-4}) and α-linolenic acid (ORALA = 0.95; 95% CI = 0.92–0.97; P = 5.4 × 10^{-5}), whereas modest increased risks were observed for arachidonic acid (ORAA = 1.06; 95% CI = 1.03–1.08; P = 3.3 × 10^{-3}), eicosapentaenoic acid (OEPXA = 1.04; 95% CI = 1.01–1.07; P = 2.5 × 10^{-3}), and docosapentaenoic acid (ORDPA = 1.03; 95% CI = 1.01–1.06; P = 1.2 × 10^{-5}). 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) are 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.

References

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15Cancer Epidemiology, Biomarkers, & Prevention | Research Article

ABSTRACT

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Conclusions: Our study suggests that higher circulating shorter-chain PUFAs (i.e., LA and ALA) are 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.

References

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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 x 10–6); or (iii) low minor allele frequencies (MAF; ref. 16). All imputed SNPs had an R2 > 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
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, rs3796713, rs174538, rs780094, rs3734398, and rs2236212. The SNPs used in the six different genetic instruments were calculated as follows: 

\[
\sum_{i} \frac{(2\beta_i^2 \text{MAF}(1 - \text{MAF})}{\text{variance(PUFA)}} \times 100, \text{where } n \text{ is the number of independent SNPs,} \\
\beta_i \text{ is effect estimate from GWAS, and variance is PUFAs specific (22).}
\]

Each PUFAs-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). SNPs used in each instrument are independent with linkage disequilibrium (LD; as measured using the correlation coefficient, \( r^2 \)) less than 0.1.

| 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                   | rs3796713, 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: \( \sum_{i} (2\beta_i^2 \text{MAF}(1 - \text{MAF})/\text{variance(PUFA)}) \times 100 \), where \( n \) is the number of independent SNPs, \( \beta_i \) is effect estimate from GWAS, and variance is PUFAs specific (22).

\(^b\)Each PUFAs-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). SNPs used in each instrument are independent with linkage disequilibrium (LD; as measured using the correlation coefficient, \( r^2 \)) less than 0.1.

\(^c\)SNPs used in each instrument are independent with linkage disequilibrium (LD; as measured using the correlation coefficient, \( r^2 \)) less than 0.1.

**Table 1.** SNPs identified from published GWAS used to construct genetic instruments for PUFAs.
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 PUFA 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 PUFA 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 PUFA, 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 rs16696692) 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 (ORLA = 0.96, 95% CI = 0.93–0.98, P = 5.2 × 10^{-4}; ORALA = 0.95, 95% CI = 0.92–0.97, P = 5.4 × 10^{-5}). An increased colorectal cancer risk was observed per SD increase in circulating longer-chain omega-3 fatty acids, EPA (OREPA = 1.04, 95% CI = 1.01–1.07, P = 2.5 × 10^{-4}) and DPA (ODPA = 1.03, 95% CI = 1.01–1.06, P = 1.2 × 10^{-3}). 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 (ORAA = 1.06, 95% CI = 1.03–1.08, P = 3.3 × 10^{-3}).

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; Pinteraction for LA = 1.5 × 10^{-2} and Pinteraction for ALA = 0.04) and regular aspirin/NSAID use (Pinteraction for AA = 0.05, Pinteraction for ALA = 0.04, and Pinteraction for EPA = 1.4 × 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 (ORALA, ≥65 years = 0.93, 95% CI = 0.89–0.96, P = 5.4 × 10^{-3}; ORALA, ≥65 years = 0.92, 95% CI = 0.89–0.96, P = 2.7 × 10^{-3}). Whereas among individuals <65 years, no statistically significant associations were observed. For longer-chain omega-3 PUFA (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 (ORAA, ≥65 years = 1.08, 95% CI = 1.04–1.12, P = 2.7 × 10^{-3}) and no association was observed among those <65 years (ORAA, <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 (ORAA, aspirin/NSAID nonuser = 1.08, 95% CI = 1.04–1.11, P = 8.3 × 10^{-5}), whereas no association was observed among aspirin/NSAID users (ORAA, 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 (ORALA, aspirin/NSAID nonuser = 0.93, 95% CI = 0.90–0.96, P = 9.7 × 10^{-5}). Similar increased colorectal cancer risks were observed for higher levels of circulating longer-chain omega-3 EPA (OREPA, aspirin/NSAID nonuser = 1.07, 95% CI = 1.03–1.10, P = 1.7 × 10^{-3}) and DPA (ODPA, aspirin/NSAID nonuser = 1.05, 95% CI = 1.02–1.09, P = 2.4 × 10^{-5}) 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 PUFA 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 (ORshort LA, aspirin/NSAID nonuser = 0.89, 95% CI = 0.84–0.95, P = 7.8 × 10^{-4}; ORshort ALA, aspirin/NSAID nonuser = 0.87, 95% CI = 0.81–0.93, P = 4.1 × 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 (ORlong AA, aspirin/NSAID nonuser = 1.12, 95% CI = 1.05–1.20, P = 7.6 × 10^{-3}) and DPA (ODPA, aspirin/NSAID nonuser = 1.07, 95% CI = 1.00–1.15, P = 3.9 × 10^{-2}) among aspirin/NSAID nonusers.

Among those with lower levels of genetically predicted circulating PUFAs, use of aspirin/NSAID was associated with reduced colorectal cancer risk, with colorectal cancer risk reductions ranging from 24% (ORraw AA, aspirin/NSAID user = 0.76, 95% CI = 0.70–0.82, P = 8.4 × 10^{-5}) to 29% (ORraw LA, aspirin/NSAID user = 0.71, 95% CI = 0.65–0.77, P = 3.3 × 10^{-5}). Generally, among aspirin/NSAID users, higher levels of genetically predicted PUFA (named LA and ALA) did not further reduce colorectal cancer risk compared with lower levels of PUFA (ORhigh LA, aspirin/NSAID user = 0.68, 95% CI = 0.63–0.73, P = 2.0 × 10^{-5}; ORhigh ALA, aspirin/NSAID user = 0.65, 95% CI = 0.65, 95% CI = 0.60–0.71, P = 3.2 × 10^{-5}). For longer-chain PUFAs (i.e., omega-6: AA, and omega-3: 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.

| Subgroup               | Cases/controls | Omega-6 PUFAs |          |          |       |       |
|------------------------|----------------|---------------|----------|----------|-------|-------|
|                        |                | LA (ORa 95% CI P) | AA (ORa 95% CI P) |             |       |       |
| Overall                | 11,016/13,732  | 0.96 (0.93-0.98) 5.2 x 10^{-4} | 1.06 (1.03-1.08) 3.3 x 10^{-5} | 0.95 (0.92-0.97) 5.4 x 10^{-5} |           |       |
| Sex                    |                |               |          |          |       |       |
| Female                 | 5,810/7,327    | 0.94 (0.91-0.97) 4.2 x 10^{-4} | 1.07 (1.03-1.10) 3.2 x 10^{-4} | 0.94 (0.90-0.97) 2.3 x 10^{-4} |           |       |
| Male                   | 5,206/6,405    | 0.96 (0.94-1.02) 0.26 | 1.04 (1.00-1.08) 0.04 | 0.97 (0.95-1.00) 0.07 |           |       |
| Age                    |                |               |          |          |       |       |
| <65 years              | 5,770/7,096    | 0.98 (0.95-0.102) 0.36 | 1.03 (0.99-1.07) 0.08 | 0.97 (0.94-1.01) 0.11 |           |       |
| ≥65 years              | 5,246/6,636    | 0.93 (0.89-0.96) 5.4 x 10^{-5} | 1.18 (1.14-2.12) 2.7 x 10^{-5} | 0.92 (0.89-0.96) 2.7 x 10^{-5} |           |       |
| Smoking                |                |               |          |          |       |       |
| Ever                   | 6,090/7,526    | 0.97 (0.93-1.00) 0.06 | 1.05 (1.01-1.09) 5.7 x 10^{-3} | 0.95 (0.92-0.99) 8.7 x 10^{-3} |           |       |
| Never                  | 4,745/6,121    | 0.94 (0.91-0.98) 3.4 x 10^{-3} | 1.06 (1.02-1.10) 3.4 x 10^{-3} | 0.94 (0.91-0.98) 3.3 x 10^{-3} |           |       |
| 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 (0.94-1.05) 0.40 |           |       |
| No                     | 6,919/7,672    | 0.94 (0.91-0.97) 2.3 x 10^{-4} | 1.04 (1.01-1.11) 8.3 x 10^{-6} | 0.93 (0.90-0.96) 9.7 x 10^{-6} |           |       |
| Cancer site            |                |               |          |          |       |       |
| Rectal                 | 2,849/3,695    | 0.96 (0.92-1.00) 0.06 | 1.06 (1.01-1.11) 9.7 x 10^{-3} | 0.95 (0.91-0.99) 0.01 |           |       |
| Colon                  | 7,907/11,575   | 0.96 (0.93-0.98) 1.9 x 10^{-3} | 1.05 (1.02-1.08) 6.0 x 10^{-4} | 0.95 (0.93-0.99) 7.8 x 10^{-4} |           |       |
| Proximal colon         | 4,319/6,352    | 0.95 (0.92-0.99) 6.2 x 10^{-3} | 1.05 (1.01-1.09) 5.3 x 10^{-3} | 0.95 (0.92-0.99) 5.6 x 10^{-3} |           |       |
| Distal colon           | 3,439/4,372    | 0.95 (0.92-0.99) 1.5 x 10^{-2} | 1.06 (1.02-1.10) 3.3 x 10^{-3} | 0.95 (0.91-0.99) 4.5 x 10^{-3} |           |       |
| Body mass index         |                |               |          |          |       |       |
| ≤81.5 kg/m²            | 96/121         | 0.89 (0.65-1.20) 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 |
| 81.5-24.9              | 3,426/4,842    | 0.95 (0.90-0.99) 1.4 x 10^{-2} | 1.06 (1.01-1.10) 1.4 x 10^{-2} | 0.94 (0.90-0.99) 0.91 | 1.05 (1.00-1.11) 3.6 x 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 (0.93-1.00) 0.09 | 1.05 (1.01-1.09) 0.04 | 0.94 (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) 0.31 | 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 |

Subgroup-specific interaction terms were calculated using nested models for the multiplicative interaction term via a likelihood ratio test with a χ² distribution with 1 degree of freedom.

Notes: All models adjusted for age, sex, study, and top principal components for European ancestry. ORa represents 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.
Table 3. Additive interaction between genetically predicted PUFA intake and regular aspirin/NSAID use in the GECCO.

| Polyunsaturated fatty acid levels | Aspirin/NSAID use | Cases/controls | OR\(^b\) (95% CI) | P      | RERI\(^c\) (95% CI)\(^d\) |
|----------------------------------|-------------------|---------------|-------------------|--------|---------------------------|
| LA                              | Low               | 3.590/3.722   | 1.00              |        |                           |
| High                            | No                | 3.329/3.950   | 0.89 (0.84–0.95)  | \(7.8 \times 10^{-4}\) |                           |
| Low                             | Yes               | 1.545/2.505   | 0.71 (0.65–0.77)  | \(3.3 \times 10^{-7}\) |                           |
| High                            | Yes               | 1.515/2.559   | 0.68 (0.63–0.74)  | \(2.0 \times 10^{-10}\) | 0.085 (–0.004 to 0.170)   |
| AA                              | Low               | 3.321/4.002   | 1.00              |        |                           |
| High                            | No                | 3.598/3.670   | 1.15 (1.07–1.23)  | \(4.4 \times 10^{-5}\) |                           |
| Low                             | Yes               | 1.505/2.619   | 0.76 (0.70–0.82)  | \(8.4 \times 10^{-12}\) |                           |
| High                            | Yes               | 1.553/2.442   | 0.82 (0.76–0.89)  | \(1.9 \times 10^{-6}\) | –0.082 (–0.185 to 0.021) |
| ALA                             | Low               | 3.603/3.667   | 1.00              |        |                           |
| High                            | No                | 3.316/4.005   | 0.87 (0.81–0.93)  | \(4.1 \times 10^{-5}\) |                           |
| Low                             | Yes               | 1.566/2.457   | 0.72 (0.67–0.78)  | \(2.4 \times 10^{-16}\) |                           |
| High                            | Yes               | 1.492/2.624   | 0.65 (0.60–0.71)  | \(3.2 \times 10^{-25}\) | 0.059 (–0.028 to 0.146)   |
| EPA                             | Low               | 4.046/4.476   | 1.00              |        |                           |
| High                            | No                | 2.873/3.196   | 1.12 (1.05–1.20)  | \(7.6 \times 10^{-6}\) |                           |
| Low                             | Yes               | 1.807/3.111   | 0.76 (0.70–0.82)  | \(1.9 \times 10^{-21}\) |                           |
| High                            | Yes               | 1.252/1.950   | 0.80 (0.74–0.87)  | \(4.4 \times 10^{-8}\) | –0.081 (–0.182 to 0.021) |
| DPA                             | Low               | 3.848/4.105   | 1.00              |        |                           |
| High                            | No                | 3.071/3.567   | 1.07 (1.00–1.15)  | \(3.9 \times 10^{-2}\) |                           |
| Low                             | Yes               | 1.665/2.706   | 0.76 (0.70–0.82)  | \(8.2 \times 10^{-18}\) |                           |
| High                            | Yes               | 1.393/2.355   | 0.77 (0.71–0.83)  | \(8.1 \times 10^{-11}\) | –0.063 (–0.161 to 0.035) |
| DHA                             | Low               | 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)  | \(5.1 \times 10^{-16}\) |                           |
| High                            | Yes               | 1.252/1.921   | 0.80 (0.73–0.87)  | \(2.5 \times 10^{-7}\) | 0.024 (–0.075 to 0.124)   |

*Genetically predicted PUFA intake dichotomized at the median (i.e., wGS < median = “Low” and wGS ≥ median = “High”).

aAdditive interaction assessed using the RERI = OR\(_{\text{high}}\) - OR\(_{\text{low}}\) - OR\(_{\text{null}}\) + 1 (e.g., Linoleic acid RERI = 0.68 – 0.71 - 0.89 + 1 = 0.08).

bAll models adjusted for age, sex, study, and top principal components for European ancestry.

cAdditive interaction estimated using method of Hosmer and Lemeshow (26).

d95% 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\(_{\text{highLA, aspirin/NSAID user}}\) = 0.82, 95% CI = 0.76–0.89, \(P = 1.9 \times 10^{-6}\); OR\(_{\text{highEPA, aspirin/NSAID user}}\) = 0.80, 95% CI = 0.74–0.87, \(P = 4.4 \times 10^{-6}\); OR\(_{\text{highDPA, aspirin/NSAID user}}\) = 0.77, 95% CI = 0.71–0.83, \(P = 8.1 \times 10^{-11}\); OR\(_{\text{highDHA, aspirin/NSAID user}}\) = 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 (Pheterogeneity = 3.6 \times 10^{-1}), 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\(_{\text{DPA}}\) = 0.93, 95% CI = 0.88–0.97, \(P = 2.1 \times 10^{-7}\)).

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% (\(P_{\text{interaction}} = 0.05\)), and reduced risk by 7% for ALA (\(P_{\text{interaction}} = 0.04\)). 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 (34). Furthermore, results for AA from May-Wilson and colleagues (34) 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, OR₉₅–₉₀ = 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 (15). 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-adjusted 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 α = 0.05 was assessed and 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 of increasing long-chain omega-3 PUFAs may have 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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**Acknowledgments**

N.K. Khankari is supported by NIH NCICR09 CA215386. M.C. Borges is supported by a Skills Development Fellowship from the UK Medical Research Council (Grant number MR/P010541/1). P.C. Haycock is supported by CRUK Population Research Postdoctoral Fellowship CS2274/20138. M. Song is supported by the American Cancer Society (grant number MRSG-17-220-01 – NEC) and the US NIH grants (K99 CA215314, R00 CA215314). The following acknowledgements are for GECCO.

**ASTERISK (French Association Study Evaluating Risk for Sporadic Colorectal Cancer):** The authors thank all participants and cooperating clinicians, and Ute Handte-Daub, Ute Bensch, Muhabbet Celik, and Ursula Elber for excellent technical assistance.

**Harvard cohorts:** The study protocol was approved by the institutional review boards of the Brigham and Women’s Hospital and Harvard T.H. Chan School of Public Health, and those of participating registries as required. The authors thank the participants and staff of the HPFS, NHS, and PHS for their valuable contributions as well as the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WV.

**DACHS (Darmkrebs: Chancen der Verhütung durch Screening):** The authors thank all participants and cooperating clinicians, and Ute Handte-Daub, Ute Bensch, Muhabbet Celik, and Ursula Elber for excellent technical assistance.

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The authors assume full responsibility for analyses and interpretation of these data.

**PLCO (Prostate, Lung, Colorectal Cancer, and Ovarian Cancer Screening Trial):** The authors thank the PLCO Cancer Screening Trial screening center investigators and the staff from Information Management Services Inc. and Westat Inc. Most importantly, they thank the study participants for their contributions that made this study possible.

**PMH (Postmenopausal Hormone study):** The authors thank the study participants and staff of the Hormones and Colon Cancer study.
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WHI (Women’s Health Initiative): The authors thank the WHI investigators and staff for their dedication, and the study participants for making the program possible. A full listing of WHI investigators can be found at http://www.whi.org/researchers/Documents%20Written%20Paper/WHI%20investigator%20list.pdf.

The following acknowledgments are for CORECT: CoCare: Biospecimens were provided by the CoCare Consortium, funded by the Fred Hutchinson Cancer Research Center. Other investigators may have received specimens from the same subjects.

CPS-II (American Cancer Society Cancer Prevention Study II): The authors thank the CPS-II PI CA143247, and R01 CA142327, to G. Casey). The Colon CFR/CORECT Illumina GWAS was supported by funding from the NCI, NIH (grant number U01 CA097497, to B. Gruber). The Colon CFR participant recruitment and collection of data and biospecimens used in this study were supported by the NCI, NIH (grant number U10 CA078551) and through cooperative agreements with the following Colon CFR center cores: Australian Colorectal Cancer Family Registry (NCI/NIH grant numbers U01 CA074778 and U01 U24 CA097735), USC Consortium Colorectal Cancer Family Registry (NCI/NIH grant numbers U01 U24 CA074799), Mayo Clinic Cooperative Family Registry for Colon Cancer Studies (NCI/NIH grant number U01 U24 CA074780), Ontario Familial Colorectal Cancer Family Registry (NCI/NIH grant number U01 U24 CA074794), and University of Hawaii Colorectal Cancer Family Registry (NCI/NIH grant number U01 U24 CA074806). Additional support for case ascertainment was provided by the Surveillance, Epidemiology and End Results (SEER) Program of the NCI to Fred Hutchinson Cancer Research Center (control nos. N01-CN-67009 and N01-PC-35142 and contract no. HHSN26120000012I), the Hawaii Department of Health (control nos. N01-PC-67001 and N01-PC-35137 and contract no. HHSN261201000037C), and the California Department of Public Health (contracts HHSN261201000015C awarded to the University of Southern California, and the following state cancer registries: AZ, CO, MN, NC, NH, and by the Victoria Cancer Registry and Ontario Cancer Registry).

DACHS: This work was supported by the German Research Council (BR 1704/6-1, BR 1704/6-2, BR 1704/6-3, CH 1711-1, HO 5172-2, I. HE 5982-1, K. 2354-3, 1, RO 22708/1, and BR 1704/17-1), the Interdisciplinary Research Program of the National Center for Tumor Diseases (NCT), Germany, and the German Federal Ministry of Education and Research (01KHO404, 01ER0814, 01ER0815, 01ER1505A, and 01ER1508B).

DALS (Diet, Activity, and Lifestyle Study): NIH (R01 CA48998, to M.L. Slattery), Harvard cohorts (HPFS, NHS, PHS): HPFS is supported by the NIH (P01 CA50597, UM1 CA61752, U01 CA61755, R01 CA137178, R01 CA151993, R35 CA197735, K07 CA096073, and P05 CA127003), NHS by the NIH (R01 CA137718, P01 CA087969, UM1 CA186107, R01 CA151993, R35 CA197735, K07 CA096073, and P05 CA127003), and PHS by the NIH (R01 CA074212).

GECCO: NCI, NIH, U.S. Department of Health and Human Services (U01 CA64930, U01 CA137088, R01 CA509045). This research was funded in part through the NCI/NCCancer Center Support Grant P30 CA015704.

MECC (Multiethnic Cohort): NIH (R37 CA54281, P01 CA33619, R01 CA63464, U01 CA164937). Also part of CORECT funding acknowledgments.

OFCCR (Ontario Familial Colorectal Cancer Registry): NIH, through funding allocated to the Ontario Registry for Studies of Familial Colorectal Cancer (U01 CA074783); see CORECT section above. Additional funding toward genetic analyses of OFCCR acknowledgments are Research Fund, the Canadian Institutes of Health Research, and the Ontario Institute for Cancer Research, through generous support from the Ontario Ministry of Research and Innovation.

PLOC: Intramural Research Program of the Division of Cancer Epidemiology and Genetics and supported by contracts from the Division of Cancer Prevention, NCI, and National Cancer Institute, NIH. DHHS Funding was provided by NIH, Genes, Environment and Health Initiative (GEIH) Z01 CP 012029, NIHU01 HG004446, and NHGRI U01 HG004438.

PMH: NIH (R01 CA76366, to P.A. Newcomb).

VITAL (Vitamins and Lifestyle): NIH (K05 CA154377).

The WHI program is funded by the National Heart, Lung, and Blood Institute, NIH, U.S. Department of Health and Human Services through contracts HHSN262201000046C, HHSN262201000010C, HHSN262201000022C, HHSN262201000034C, NIHHSN262201000034C, and NIHHSN27212010004C.

The following funding information is for studies included in CORECT:

ATBC (Alpha-Tocopherol, Beta Carotene Cancer Prevention Study): The ATBC Study was supported by the U.S. Public Health Service contracts (N01-CN-54165, N01-RC-45055, N01-RC-37004, and HHSN262120100006C) from the National Cancer Institute.

CoCare: This work was supported by the NIH [grant numbers R01 CA189194 (to L.M. Ulrich), U01 CA206110 (C.M. Ulrich/Li/Sorged/Figueiredo/Coldrzt, 2P30CA015704-40 (Gilliland), R01 CA207371 (C.M. Ulrich/Li)], the Matthais Lacks-Foundation, the German Consortium for Translational Cancer Research, and the EU TRANSCAN initiative.

CORECT Study: The CORECT Study was supported by the NCI/NIH, U.S. Department of Health and Human Services (grant numbers U19 CA148107, R01 CA81488, P30 CA014089, R01 CA0197350, P01 CA916569, R01 CA201407) and National Institutes of Environmental Health Sciences, NIH (grant number T32 ES013678).

The American Cancer Society funds the creation, maintenance, and updating of the Cancer Prevention Study-II (CPS-II) cohort. This study was conducted with Institutional Review Board approval.

ESTHER/VERDI (Epidemiologische Studie zu Chancen der Verhütung, Früherkennung und optimierten Therapie chronischer Erkrankungen in der älteren Bevölkerung; Verlauf der diagnostischen Abklärung bei Krebspatienten): This work was supported by grants from the Baden-Württemberg Ministry of Science, Research and Arts and the German Cancer Aid.

Kentucky: This work was supported by the following: (i) Clinical Investigator Award from Damon Runyon Cancer Research Foundation (CI-8) and (ii) NICHIC136726, the authors acknowledge the staff at the Kentucky Cancer Registry.

MCCS: Cohort recruitment was funded by VicHealth and Cancer Council Victoria. The MCCS was further supported by Australian NHMRC grants 309348, 200657, 251553, and 504711 and by infrastructure provided by Cancer Council Victoria. Cases and their vital status were ascertained through the Victorian Cancer Registry (VCR) and the Australian Institute of Health and Welfare (AIHW), including the National Death Index and the Australian Cancer database.

MECC (Molecular Epidemiology of Colorectal Cancer Study): This work was supported by the NIH, U.S. Department of Health and Human Services (R01 CA81488, to B.B. Gruber and G. Rennert).

MSKCC: The work at Sloan Kettering in New York was supported by the Robert and Kate Nielsens Foundation for Inherited Cancer Genomics and the Romeo Milo Foundation. Moffitt: This work was supported by funding from the NIH (grant numbers R01 CA189184, P30 CA076292), Florida Department of Health Bankhead-Coley Grant 09BN-13, and the University of South Florida Oehler Foundation. Moffitt contributions were supported in part by the Total Cancer Care Initiative, Collaborative Data Services Core, and Tissue Core at the H. Lee Moffitt Cancer Center & Research Institute, a National Cancer Institute-designated Comprehensive Cancer Center (grant number P30 CA076292).

NFCCR (Newfoundland Case-Control Study): This work was supported by an Interdisciplinary Health Research Team grant from the Canadian Institutes of Health Research (CIHR) (RFT 43821); the NIH, U.S. Department of Health and Human Services (U01 CA74783); and PHS National Cancer Institute of Canada grants (18223 and 18226). The authors acknowledge the contribution of Alexandre Belisle and the genotyping team of the McGill University and Genome Quebec Innovation Centre, Montréal, Canada, for genotyping the Sequenom panel in the NFCCR samples.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received July 26, 2019; revised October 3, 2019; accepted January 23, 2020; published first February 12, 2020.

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