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Investigation of the Association between High Arachidonic Acid Synthesis and Colorectal Polyp Incidence within a Generally Healthy UK Population: A Mendelian Randomization Approach

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Abstract
Background: Arachidonic acid (ARA) is associated with colorectal cancer (CRC), a major public health concern. However, it is uncertain if ARA contributes to the development of colorectal polyps which are pre-malignant precursors of CRC. ***Objective:*** The study aimed to investigate the association between lifelong exposure to elevated ARA and colorectal polyp incidence. ***Methods:*** Summary-level GWAS data from European, Singaporean, and Chinese cohorts (n = 10,171) identified 4 single-nucleotide polymorphisms (SNPs) associated with blood ARA levels ($p < 5 \times 10^{-8}$). After pruning, 1 SNP was retained (rs174547; $p = 3.0 \times 10^{-971}$) for 2-stage Mendelian randomization. ***Results:*** No association between ARA and colorectal polyp incidence was observed (OR = 1.00; 95% CI: 0.99, 1.00; p value = 0.50) within the UK Biobank (1,391 cases; 462,933 total). ***Conclusions:*** Blood levels of ARA do not associate with colorectal polyp incidence in a general healthy population. Although not providing direct evidence, this work supports the contention that downstream lipid mediators, such as PGE_2 rather than ARA itself, are key for polyp formation during early-stage colorectal carcinogenesis.

Introduction

Colorectal cancer (CRC) is a major global public health concern, accounting for approximately 10% of all global cancer cases. Colorectal carcinogenesis is associated with nonmodifiable (e.g., age, ethnicity) and modifiable risk factors such as excess body weight [1]. Overall, diet is a major contributor to CRC cases that, depending on the population and food consumed, is often estimated to account for 5–20% of CRC cases, with some nutrients demonstrating a stronger effect on CRC risk than others [1–3]. Recently, a Mendelian randomization (MR) study

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demonstrated that the long-chain omega-6 polyunsaturated fatty acid arachidonic acid (ARA) is causally associated with risk of CRC (odds ratio [OR] = 1.08; 95% CI 1.05, 1.11 per SD; $p = 6.3 \times 10^{-8}$) [4]; however, it is not clear if ARA contributes to early predictors of CRC.

The majority of CRCs are believed to occur via the benign precursor colorectal polyp (conventional adenoma or serrated polyp) in a process that can take approximately 10 years [5]. Therefore, one can hypothesize that ARA exposure is also involved in colorectal polyp development and may offer an early opportunity to mitigate early stages of colorectal carcinogenesis. To date, a single case-control study (n = 909 cases, n = 855 controls) has applied MR to examine this and found no association (OR 1.07; 95% CI: 0.97, 1.02; $p = 0.41$) between a genetic variant within *fatty acid desaturase 1* (*FADS1*; rs174537), which is involved in the ARA synthesis from precursor PUFAs (i.e., linoleic acid), and colorectal adenomas adenoma risk [6, 7]. However, the study reported a required OR of 1.6 to achieve sufficient confidence and minimize the risk of a type II error (i.e., false positive). Given the magnitude of association observed between ARA and CRC (OR = 1.08), it is possible that the study was underpowered and that a larger study is needed to test for a casual association of smaller magnitude. Therefore, to overcome any limitation of power, build on emerging evidence, explore alternative variants, and provide greater certainty regarding the causal role of ARA exposure on colorectal polyp risk, we applied an MR approach in a large prospective UK cohort ($n \approx 500{,}000$) to ascertain if prolonged exposure to elevated ARA synthesis is causally associated with colorectal polyp formation.

Materials and Methods

We performed 2-sample MR using MR-Base [8] with summary data from publicly available GWAS databases. All studies and consortia accessed in the present study on MR-Base were approved by their respective Ethics Committee, and the subjects from all the cohorts provided written informed consent.

Sample 1

Single-nucleotide polymorphisms (SNPs), associated with ARA at a significance level of $p < 5 \times 10^{-8}$, were identified within MR-Base. From the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium (CHARGE; n = 8,631 white Europeans; 55% women) and the Singapore Chinese Health Study (SCHS; n = 1,540), 6 potential SNPs were identified with plasma ARA concentrations. The association between ARA and polyps will be investigated in a primarily white European population, which positions SNPs from CHARGE as most suitable. However, to maximize the number of potential instrumental variables (IVs) we will consider all promising SNPs and perform stratified cohort analyses if a mixture of SNPs is retained. Of the 6 SNPs identified, two were removed – rs1741 (*PDXDC1*) and rs16829840 (*TMEM39A*) – because their mechanism of association with ARA could not be identified, which risks violating two (i.e., "independence," "exclusion restriction") of the 3 core assumptions of the "IVs" in MR [9, 10]. The 3 core assumptions are as follows: (i) *relevance*, the variant is associated with the risk factor of interest; (ii) *independence*, the variant shares no common causes (confounding) with the outcome; and (iii) *exclusion restriction*, the variant does not affect the outcome except through the risk factor. Therefore, only rs174547, rs102275, rs174577, and rs174528 were evaluated for inclusion, all of which are associated with ARA synthesis [11]. Variants rs174547, rs102275, rs174577 are within the fatty acid desaturase cluster which is involved in PUFA desaturation, while rs174528 resides within the myelin regulatory factor gene. The role of myelin regulatory factor on PUFA levels is uncertain, but it does not suggest an alternative (i.e., horizontal pleiotropic) pathway to influence colorectal polyps, and the variant was considered for further assessment. All are highly correlated ($R^2 > 0.8$; $D' > 0.9$) with previously identified genetic markers for PUFA levels [12, 13].

Sample 2

The UK Biobank, with self-reported longitudinal data (updated 2018) on colorectal polyps (ukb-b-14210: 1,391 cases of colorectal polyp; 462,933 total), contained all 4 SNPs within its database, so proxy SNPs in high linkage disequilibrium ($r^2 \geq 0.80$) were not required. Participants were asked to self-report rectal or colon adenoma/polyps via the touchscreen questionnaire. Specifically, they were asked "*Has a doctor ever told you that you have any other serious medical conditions*" and then to select the condition from a panel of options, which included "*rectal or colon adenoma/polyps.*" Using MR-Base and the *ld_matrix* function (TwoSampleMR) in R (v.3.5.1), $R^2 > 0.80$ was observed between the 4 SNPs within the 1,000 Genome Project. After pruning for independence ($r^2 < 0.001$), rs174547 was retained as the IV for ARA. The rs174547 variant is in very high linkage disequilibrium ($R^2 > 0.89$) with the rs174537 variant that was tested previously [6], is strongly associated with ARA ($\beta = -1.69$ [0.02] % total fatty acids, p value 3.0×10^{-971}), has been used in previous MR studies as an IV for ARA, and demonstrates no association with BMI, smoking, or alcohol intake [4, 7, 8].

Statistical Methods

The online tool mRnd [14] estimated study power to be over 90% and an F-statistic >11 to detect a significant difference ($p < 0.05$) in ≥1% change in odds of polyp formation, assuming a conservative mean r^2 of 0.20 between our IV and exposure [7, 8]. With a single IV, the Wald estimate was used to evaluate the association between ARA and polyp formation, with carriers of rs174547 predicted to have a lower proportion of ARA than noncarriers. Briefly, the Wald estimate assumes that the association between the exposure and the outcome (i.e., our association of investigation; β_{EO}) is the quotient of the association between IV and the outcome (β_{GO}) and the IV and the exposure (β_{GE}): $\beta_{EO} = \beta_{GO}/\beta_{GE}$. Estimates ($\beta_{EO}$) were exported from MR-Base as log odds and then exponentiated for easier interpretation as ORs, which can be interpreted as odds of reporting one or more colorectal polyps per unit (1%) decrease of ARA (up to most recent reporting period).

Results

We report a nonsignificant association (OR = 1.00; 95% CI: 0.99, 1.00; *p* value = 0.50) for each 1% reduction of ARA and colorectal polyp risk in the UK Biobank cohort. To contextualize the results, the analysis was scaled to reflect the estimated 1.7% reduction of ARA following 3-month supplementation with fish oil (2 g eicosapentaenoic acid and 1 g docosahexaenoic acid) [7]. However, the difference in effect sizes between a 1% and a 1.7% reduction of ARA was negligible (log $OR_{1\%}$ = 0.000048 vs. log $OR_{1.7\%}$ = 0.000082) with no change in risk observed (OR = 1.0; 95% CI: 0.99, 1.00).

Discussion

We provide greater certainty regarding the association between ARA and colorectal polyp risk in a large UK population. Our results suggest that blood ARA levels are not directly associated with colorectal polyp formation.

The results suggest that despite existing evidence of a causal association of ARA on CRC (OR = 1.08; 95%: CI 1.05–1.11) [2], ARA does not directly contribute to colorectal polyp formation, an early risk factor of CRC. However, it is plausible that downstream products of ARA (i.e., ARA-derived eicosanoids), rather than ARA itself, are mediators of colorectal polyp formation and CRC risk (i.e., vertical pleiotropy). This downstream route of investigation is strongly supported by evidence from human and preclinical models that report associations between levels of ARA-metabolizing enzymes (cyclooxygenase; lipoxygenase; and cytochrome P450), and their products, such as prostaglandin E_2, with colorectal polyp numbers and their transition, and has been recently reviewed [15]. Although lower ARA is associated with lower overall eicosanoid synthesis, it may be that moderators of ARA metabolite synthesis (e.g., age and aspirin use) rather than ARA level itself are the key promoters of polyp formation and their transition to malignancy. Future investigations of interactions between ARA-derived metabolites and their moderators in more established cohorts are required to shed light on this matter.

We acknowledge two major limitations. First, our use of self-reported polyp incidence is at risk of underreporting in our generally healthy low-risk population (57.4 ± 8.4 years). As the cohort ages and reaches the age of routine polyp screening (i.e., ≥60 years), the validly self-reported data will need to be retested and validated. Second, our analysis used summary-level data and, therefore, assumes a common effect between randomly assorted exposure groups (i.e., high vs. low synthesizers). This decision was made to evaluate the presence of a generalizable association between ARA and polyp incidence within a diverse cohort; however, future analyses with individual level data with known confounders of ARA metabolism, prostaglandin E_2 synthesis, polyp detection/incidence, and CRC (such as age, BMI, sex, and medication) [1, 7] are required to uncover differences in effect sizes or interactions between groups. In short, this study provides evidence that ARA is not directly associated with colorectal polyp risk and directs future investigations to focus on products of ARA to appreciate the overarching association between ARA and CRC.

Statement of Ethics

This study assessed publicly available data from CHARGE, SCHS, and UK Biobank that is freely available on MR-Base. All studies and consortia accessed in the present study on MR-Base were approved by their respective Ethics Committee, and the subjects from all the cohorts provided written informed consent [17–19].

Conflict of Interest Statement

This work was supported by the Wellcome Trust (M.A.Z.) and a studentship from the Nutrition Society (R.M.). The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results. J.B.M. and M.A.H. declare no conflicts of interest.

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Author Contributions

Rachel Moon: conceptualization, original draft preparation, formal analyses, writing – review and editing, funding acquisition, and approval of the final manuscript. Bernadette Moore and Mark Hull: writing – review and editing, and approval of the final manuscript. Michael Zulyniak: conceptualization, supervision, methodology, writing – review and editing, funding acquisition, and approval of the final manuscript.

Data Availability Statement

All data are publically available (MR-Base). Further inquiries can be directed to the corresponding author.

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