**Title:**

**Polyunsaturated fatty acids and prostate cancer risk: a Mendelian randomization analysis from the PRACTICAL consortium**

Running head (42/50 characters): Polyunsaturated fatty acids and prostate cancer

**Authors:**

Nikhil K. Khankari1, Harvey J. Murff1, Chenjie Zeng1, Wanqing Wen1, Rosalind A. Eeles2,3, Douglas F. Easton4, Zsofia Kote-Jarai2, Ali Amin Al Olama4,Sara Benlloch4, Kenneth Muir5, Graham G. Giles6,7, Fredrik Wiklund8, Henrik Gronberg8, Christopher A. Haiman9, Johanna Schleutker10,11, Børge G. Nordestgaard12, Ruth C. Travis13, Jenny L. Donovan14, Nora Pashayan4,15, Kay-Tee Khaw16, Janet L. Stanford17,18, William J. Blot19, Stephen N. Thibodeau20, Christiane Maier21,22, Adam S. Kibel23,24, Cezary Cybulski25, Lisa Cannon-Albright26, Hermann Brenner27,28, Jong Park29, Radka Kaneva30, Jyotsna Batra31, Manuel R. Teixeira32, Hardev Pandha33, Wei Zheng1,\*, and the PRACTICAL consortium‡

**Affiliations:**

1Division of Epidemiology, Department of Medicine, Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN, 37203, USA

2The Institute of Cancer Research, 15 Cotswold Rd, Sutton, London, SM2 5NG, UK

3Royal Marsden NHS Foundation Trust, Fulham Rd, London, SW3 6JJ, UK

4Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Strangeways Research Laboratory, 2 Worts’ Causeway, Cambridge, CB1 8RN, UK

5Institute of Population Health, University of Warwick, Coventry, CV4 7AL, UK

6 Cancer Epidemiology Centre, Cancer Council Victoria, 615 St Kilda Rd, Melbourne, Victoria, 3004, Australia

7Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Victoria, 3010, Australia

8Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, SE-171 77, Sweden

9Department of Preventive Medicine, Keck School of Medicine, University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, California, 90089, USA

10Department of Medical Biochemistry and Genetics, University of Turku, Turku, FI-20014 Finland

11Institute of Biomedical Technology/BioMediTech, University of Tampere and FimLab Laboratories, Kalevantie 4, Tampere, FI-33014, Finland

12Department of Clinical Biochemistry, Herlev Hospital, Copenhagen University Hospital, Herlev Ringvej 75, DK-2730 Herlev, Denmark

13Cancer Epidemiology, Nuffield Department of Population Health University of Oxford, Oxford, OX3 7LF, UK

14School of Social and Community Medicine, University of Bristol, Canynge Hall, 39 Whatley Road, Bristol, BS8 2PS, UK

15University College London, Department of Applied Health Research, 1-19 Torrington Place, London, WC1E 7HB, UK

16Cambridge Institute of Public Health, University of Cambridge, Forvie Site, Robinson Way, Cambridge CB2 0SR, UK

17 Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington, 98109, USA

18Department of Epidemiology, School of Public Health, University of Washington, Seattle, Washington, 98195, USA

19 International Epidemiology Institute, 1455 Research Blvd., Suite 550, Rockville, MD 20850, USA

20Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, 55905, USA

21Institute of Human Genetics, University Hospital Ulm, Albert-Einstein-Allee 11, Ulm, 89081, Germany

22Department of Urology, University Hospital Ulm, Albert-Einstein-Allee 11, Ulm, 89081, Germany

23Brigham and Women's Hospital/Dana-Farber Cancer Institute, USA, 45 Francis Street- ASB II-3, Boston, MA 02115, USA

24Washington University, 660 S. Euclid Ave, St Louis, Missouri, 63110, USA

25International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical University, Rybacka 1, Szczecin, Poland

26Division of Genetic Epidemiology, Department of Medicine, University of Utah School of Medicine, Salt Lake City, UT, 84108, USA

27Division of Clinical Epidemiology and Aging Research & Division of Preventive Oncology, German Cancer Research Center, Heidelberg, 69120, Germany

28German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, 69120, Germany

29Division of Cancer Prevention and Control, H. Lee Moffitt Cancer Center, 12902 Magnolia Dr, Tampa, Florida, 33612, USA

30 Molecular Medicine Center and Department of Medical Chemistry and Biochemistry, Medical University - Sofia, 2 Zdrave St, 1431, Sofia, Bulgaria

31Australian Prostate Cancer Research Centre-Queensland, Institute of Health and Biomedical Innovation and Schools of Life Science and Public Health, Queensland University of Technology, Brisbane, 4102, Australia

32Department of Genetics, Portuguese Oncology Institute, Porto, Portugal and Biomedical Sciences Institute (ICBAS), Porto University, 4200-072-Porto, Portugal

33The University of Surrey, Guildford, Surrey, GU2 7XH, UK

‡Additional members from the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium to be provided in the Supplement. Information of the consortium can be found at http://practical.ccge.medschl.cam.ac.uk/.

\*Corresponding author:

Wei Zheng, MD, PhD, MPH

Vanderbilt Epidemiology Center

Vanderbilt University Medical Center

2525 West End Ave, Suite 800

Nashville, TN 37203-1738

Phone: 615-936-0682

Email: wei.zheng@vanderbilt.edu

**Funding:**

Dr. Nikhil K. Khankari was supported by NIH grant R25CA160056.

**Abstract (word count = 200/200)**

**Background**: Prostate cancer is a common cancer worldwide with no established modifiable lifestyle factors to guide prevention. The associations between polyunsaturated fatty acids (PUFAs) and prostate cancer risk have been inconsistent. Using Mendelian randomization, we evaluated associations between PUFAs and prostate cancer risk.

**Methods**: We used individual-level data from a consortium of 22,721 cases and 23,034 controls of European ancestry. Externally-weighted PUFA-specific polygenic risk scores (wPRSs), with explanatory variation ranging from 0.65-33.07%, were constructed and used to evaluate associations with prostate cancer risk per one standard deviation (SD) increase genetically-predicted plasma PUFA levels using multivariable-adjusted unconditional logistic regression.

**Results**: No overall association was observed between the genetically-predicted PUFAs evaluated in this study and prostate cancer risk. However, risk reductions were observed for short-chain PUFAs, linoleic (ORLA=0.95, 95%CI=0.92,0.98) and α-linolenic acids (ORALA=0.96, 95%CI=0.93,0.98), among men <62 years; whereas increased risk was found among men ≥62 years for LA (ORLA=1.04, 95%CI=1.01,1.07). For long-chain PUFAs (i.e., arachidonic, eicosapentaenoic, and docosapentaenoic acids), increased risks were observed among men <62 years (ORAA=1.05, 95%CI=1.02,1.08; OREPA=1.04, 95%CI=1.01,1.06; ORDPA=1.05, 95%CI=1.02,1.08).

**Conclusion**: Results from this study suggest that circulating ω-3 and ω-6 PUFAs may play a different role in the etiology of early- and late-onset prostate cancer.

**Keywords:** Polyunsaturated fatty acids, prostate cancer, Mendelian randomization, polygenic risk score, linoleic acid, arachidonic acid, alpha-linolenic acid, eicosapentaenoic acid, docosapentaenoic acid, docosahexaenoic acid

**MANUSCRIPT word count** (Intro, Methods, Results, Discussion) = 3,715/5,000

**Number of References**= 25

**Introduction**

Prostate cancer is the most common cancer among Caucasian men worldwide (Torre *et al*, 2015). Identifying modifiable prostate cancer risk factors could help to alleviate the burden of prostate cancer. However, little is known about modifiable factors for this common cancer.

Several previous epidemiologic studies have examined the relation between polyunsaturated fatty acids (PUFAs) and prostate cancer risk (Zock & Katan, 1998; Carayol *et al*, 2010; Sakai *et al*, 2012; Alexander *et al*, 2015). Given the possible role that PUFAs may play in prostate carcinogenesis, with suggested anti-inflammatory effects for ω-3 PUFAs and inflammatory effects for ω-6 PUFAs (Berquin *et al*, 2011), an examination of these nutritional factors may be warranted. Specifically, metabolism of ω-6 PUFAs via the cyclooxygenase-2 enzyme results in the production of inflammatory mediators including prostaglandin E2 that has been reported to affect prostate carcinogenesis (Sobolewski *et al*, 2010). Others include the lipoxygenase and cytochrome p450 pathways producing leukotrienes and hydroxyeicosatetraenoic acids, which have also been implicated in cancer development (Panigrahy *et al*, 2010; Wang & Dubois, 2010). On the contrary, products of ω-3 PUFA metabolism via these same biologic pathways have demonstrated anti-inflammatory properties (Chapkin *et al*, 2009). However, the association between PUFAs and prostate cancer risk is not supported by a recent meta-analysis summarizing prospective studies of long-chain ω-3 PUFA intake and prostate cancer incidence that reported null results for both self-reported dietary intakes and biomarker measures of PUFAs (Alexander *et al*, 2015). Observational studies of dietary factors and cancer risk are prone to biases, including confounding, selection bias, measurement error, and reverse causation. Measurement error is an important limitation for studies examining diet via food frequency questionnaires. While biomarker PUFA measurements may provide an objective measure of intake, depending on the biomarker used (i.e., serum vs. red blood cell) the time period of exposure will vary (Arab, 2003), and thus an objective PUFA measurement may not represent the relevant etiologic time period. As a result, reverse causation in studies of prostate cancer and diet (regardless of whether diet was measured via food frequency questionnaire or biomarkers) may be of particular concern, given the slow growth of most prostate tumors and the prospect that men diagnosed with low risk (i.e., low volume and grade) disease may not be treated for several years in accord with current treatment guidelines. Given these potential limitations of observational studies the estimation of an unbiased (potentially causal) association may be difficult.

Mendelian randomization is based on the principle of random assortment of alleles at conception, and may identify causal risk factors for disease by utilizing a number of genetic variants (also known as the genetic instrument) as a proxy for an exposure. Previous genome-wide association studies (GWAS) have identified several variants that together explain a large proportion of variation in PUFA levels, thus making them a potential candidate for Mendelian randomization analysis.

We sought to identify potentially causal associations between genetically-predicted plasma PUFA levels and risk of developing prostate cancer using case-control data from a large consortium. In our Mendelian randomization analysis we examined the main ω-3 and ω-6 PUFAs, including: (1) ω-6 PUFAs: linoleic acid (LA) and arachidonic acid (AA); and (2) ω-3 PUFAs: α-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA).

**MATERIALS AND Methods**

*Study population*

We used the resources of the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL), a large consortium of prostate cancer genetic association studies (Eeles *et al*, 2013). In our analysis, we excluded those individuals who were not of European ancestry (n=1,189) and all individuals from the Washington University Genetics Study (WUGS) case-only study (n=944) and the Prostate Cancer Mechanisms of Progression and Treatment (PrOMPT) study which had only 2 controls (n=168). The final analytic dataset consisted of 45,755 individuals (22,721 cases and 23,034 controls).

*Instrumental variables*

We used results from published GWAS conducted by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium examining plasma levels of ω-6 (Guan *et al*, 2014) and ω-3 (Lemaitre *et al*, 2011) PUFAs in order to identify genetic variants associated with plasma PUFA levels. We also considered several variants identified from the metabolomics literature; however, many of these SNPs were either the same or in high linkage disequilibrium with those reported in the two CHARGE GWAS. Therefore, in total we identified 23 SNPs associated with any PUFA trait from these two published GWAS. Of these, 14 were associated with the essential PUFAs [i.e., linoleic acid (LA), arachidonic acid (AA), alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA)]. Finally, nine of these 14 SNPs were independent (r2 < 0.1), and thus were used in the genetic instrument for the Mendelian randomization analyses. Please refer to **Supplement Figure 1** for a summary of SNP selection.

For each variant selected, the allele that was associated with increased levels of plasma PUFAs was considered the effect allele, and the summary statistics for these effect alleles were obtained from published PUFA GWAS (Lemaitre *et al*, 2011; Guan *et al*, 2014). Two of these selected variants (rs174547 and rs16966952) were associated with multiple PUFAs and, thus, were not exclusive to any particular genetic instrument.

*Genotyping and Imputation*

The PRACTICAL study samples were genotyped using a custom Illumina Infinium array (iCOGS) as part of the Collaborative Oncological Gene-environment Study (COGS), including more than 85,000 prostate cancer-related single nucleotide polymorphisms (SNPs) selected from four previous GWAS (UKGPCS, CGEMS, BPC3, and CAPS), fine mapping of known prostate cancer susceptibility regions at the time of custom chip design, and from candidate gene studies examining important biologic pathways (including hormone metabolism, cell cycle, and DNA repair) (Eeles *et al*, 2013). Standard quality control protocols were followed by excluding individuals with genotyping call rates <95%, heterozygosity greater than or less than 4.89 standard deviations from the ethnicity-specific mean, duplicates, and relative pairs (Eeles *et al*, 2013; Al Olama *et al*, 2014). SNPs with call rates <95% were excluded, as well as those deviating from Hardy-Weinberg Equilibrium in the controls at *p* value <1x10-7 (Eeles *et al*, 2013; Al Olama *et al*, 2014). Of the nine SNPs associated with PUFAs included in our analysis, three were directly genotyped (rs780094, rs2236212, and rs174538) and six were imputed (rs3734398, rs3798713, rs1074011, rs174547, rs2727270, and rs1696695) with high quality (r2 > 0.76). SNPs were imputed in two stages; first using SHAPEIT (http://www/shapeit.fr/) by chromosome and chunk, and then data was phased with the haplotypes from 1000 Genomes Phase 3 (March 2012 release) which were then used for imputation using IMPUTE.V2 (https://mathgen.stats.ok.ac.uk/impute/impute\_v2.html) (Eeles *et al*, 2013; Al Olama *et al*, 2014).

*Weighted-polygenic risk scores (wPRSs)*

For analyses using individual-level data, an externally weighted-polygenic risk score (wPRS) was constructed for each PUFA separately using the SNPs associated with that fatty acid. Allele dosage was used for imputed SNPs. Using this information PUFA-specific wPRSs were constructed per individual where effect alleles were weighted according to their published associations from PUFA GWAS (Lemaitre *et al*, 2011; Guan *et al*, 2014), as follows:

where *SNPi* represents the effect allele dosage and *βi* represents the increase in PUFA levels (as a percentage of total plasma fatty acids) for that specific variant, summed across each of the *n* variants used in the PUFA-specific wPRSs. Thus, the wPRS represents an increase in PUFA levels measured as percentage of total plasma fatty acids. The GWAS summary statistics for the association between each variant and PUFA trait are listed in **Table 1**. The theoretical maximum value for each PUFA-specific wPRS was 5.53, 3.78, 0.03, 0.24, 0.26, 0.23, for LA, AA, ALA, EPA, DPA, and DHA, respectively. The theoretical maximum value for each PUFA-specific wPRS per individual was calculated by taking the sum of the product of the GWAS effect allele summary estimate and the maximum number of effect alleles per SNP included in each PUFA-specific instrument [e.g., maximum wPRS for AA = (1.691\*2) + (0.199\*2) = 3.78].

*Statistical analyses*

Unconditional logistic regression was used to estimate associations between genetically-predicted PUFA levels (wPRSs) and risk of prostate cancer per one standard deviation increase in predicted fatty acid levels. All models were adjusted for age, eight principal components for European ancestry, and PRACTICAL study site. We further assessed the relation between wPRS and prostate cancer risk using restricted cubic splines for those polygenic risk scores including more than one variant (LA, AA, EPA, DPA). **Supplement Figures 2-5** display the shape of the dose-response between the wPRS and log-odds of prostate cancer from restricted cubic spline models suggesting non-linearity (Desquilbet & Mariotti, 2010).

We also conducted stratified analyses to explore the relation between PUFAs and prostate cancer risk among subgroups, including smoking status (ever vs. never smokers), median age at diagnosis (<62 vs. ≥62 years), disease status (advanced vs. non-advanced prostate cancer), and method of detection (screen- vs. clinically-detected prostate cancer). Polytomous regression was used to estimate adjusted stratum-specific ORs and 95% CIs for the associations between PUFA-specific wPRSs and disease status and method of prostate cancer detection. Statistically significant differences between strata of each potential effect measure modifier were assessed using the likelihood ratio test for the multiplicative interaction term (for smoking status and age at diagnosis), and using the test for homogeneity (for disease status and method of detection). Advanced prostate cancer included those cases with either Gleason score ≥8, died from prostate cancer, had metastatic disease, or prostate-specific antigen levels >100 ng/ml at diagnosis. We also compared the results for the associations between the PUFA-specific wPRS and prostate cancer from the pooled analysis using individual level data to the summary associations derived from meta-analyses of each PRACTICAL study (**Supplement Figures 6-11**). Analyses were conducted using SAS version 9.4 (Cary, NC), and STATA version 12.1 (College Station, TX).

*Sensitivity analyses*

Several sensitivity analyses were conducted to assess the robustness of our results. First, we assessed whether the PUFA-specific wPRSs were associated with prostate cancer risk factors, namely age, body mass index, prostate specific antigen levels, smoking, alcohol intake, family history of prostate cancer, history of benign prostatic hyperplasia, history of prostatitis, and physical activity levels. Only age was significantly associated with most PUFA-specific wPRSs (with the exception of DHA), and physical activity was associated with the wPRSs for DPA and DHA. We compared models adjusting for different covariates; however our results did not change appreciably after controlling for age, eight principal components for European ancestry, PRACTICAL study site, or physical activity (**Supplement Table 1**).

Summary statistics from the previous PUFA GWAS (Lemaitre *et al*, 2011; Guan *et al*, 2014) were used in tandem with the summary estimates from the PRACTICAL consortium to calculate the Mendelian randomization estimate using an inverse-variance weighted meta-analysis approach (Burgess *et al*, 2013). We further standardized the Mendelian randomization ORs and 95% CIs to represent an increase in prostate cancer risk per one standard deviation increase for each PUFA-specific wPRS, thus representing a standard deviation increase in percentage of PUFA levels per total plasma fatty acids (**Supplement Table 2**).

*Assessing pleiotropy*

Two data-driven approaches were used to formally assess the impact of genetic pleiotropy on our results using summary statistics. First, we assessed the impact of genetic pleiotropy and potentially invalid instruments using Egger regression (Bowden *et al*, 2015). This approach assesses the validity of the genetic instrument and provides an estimate of the average pleiotropic effect across genetic instruments used in the instrument (**Supplement Table 2**).

Second, given several variants were included in the different PUFA-specific genetic instruments, we conducted a sensitivity analysis to account for this potential pleiotropy. This method also further evaluated the strength of the genetic instruments used in our analysis. Using a weighted-regression based approach, the association (Yg) between variant (g) and prostate cancer (Y) were regressed on the association (Xg) between that same variant (g) and the PUFA trait of interest (X), weighted by the inverse-variance (σYg-2) (Burgess *et al*, 2015; Burgess & Thompson, 2015). This approach accounts for the potential pleiotropy of variants used in each instrument on other PUFA traits. Results from this sensitivity analysis to account for potential pleiotropy and causal associations between PUFA subtypes are presented in **Supplement Table 3**.

**Results**

In **Table 1**, we provide a list of PUFA-associated genetic variants and their GWAS-reported results that were used to create the PUFA-specific wPRSs. Each PUFA-specific instrument explanatory variation ranged from 0.65% (for DHA) to approximately 33% (for AA). Due to the large size of the PRACTICAL consortium, the F-statistic for all the genetic variants was large (all F-statistics were >10) indicating a strong genetic instrument for the PUFA exposures of interest (Stock *et al*, 2002).

The associations between one standard deviation increase wPRSs with prostate cancer risk for the majority of PUFA-specific wPRSs were null (**Table 2**). When stratified by age, modest increases in prostate cancer risk were observed for AA (OR=1.05, 95% CI=1.02, 1.08), EPA (OR=1.04, 95% CI=1.01, 1.06), and DPA (OR=1.05, 95% CI=1.02, 1.08) among men less than 62 years of age. Whereas a modest risk reduction was observed for LA (OR=0.95, 95% CI=0.92, 0.98), and ALA (OR=0.96, 95% CI=0.93, 0.98) among this same age group. No differences were observed when stratified by smoking status (ever vs. never smokers), disease status (advanced vs. non-advanced prostate cancer), or method of detection (screen-detected vs. clinically-detected prostate cancer). When modeled using the restricted cubic splines, the associations between the wPRS and prostate cancer risk were also null (data not shown). The pooled results for the association between PUFA-specific wPRSs and prostate cancer risk were nearly identical to the summary estimate derived from fixed- and random-effects meta-analyses of the wPRSs and prostate cancer risk across studies included in the PRACTICAL consortium (**Supplement Figures 6-11**). Furthermore, our results did not change after adjusting for different covariates, including age and physical activity which were found to be associated with the PUFA-specific wPRSs (**Supplement Table 1**). We also conducted a Mendelian randomization analysis via the two-sample method using summary statistics scaled per one standard deviation unit increase (**Supplement Table 2**), and the results were nearly identical to those obtained from the individual-level analysis using wPRSs.

The impact of pleiotropic variants on the Mendelian randomization estimate was assessed using two different approaches, Egger regression and a weighted-regression based method. With the exception of the wPRS for DPA (β0=0.01304, *p*<0.0001), we did not observe any statistically significant intercepts as an indication of potential pleiotropic effects and an invalid instrument (**Supplement Table 2**). We also assessed the impact of pleiotropic variants on other PUFA traits via the weighted-regression based approach and, in general, observed little difference between the unadjusted models and models adjusted for potential pleiotropic effects on other PUFA traits (**Supplement Table 3**). A 12% risk reduction (95% CI=0.60, 1.29) for AA, and a 10% increased risk (95% CI=0.88, 1.36) for ALA were indicated after adjusting for the potential pleiotropic effects of the instrument on other PUFA traits; however, the confidence intervals were imprecise.

**Discussion**

We examined the association between genetically-predicted plasma PUFA levels (via construction of PUFA-specific wPRSs) using individual-level data and summary statistics for PUFAs in relation to prostate cancer risk. Our findings suggest no overall association between plasma PUFA levels and risk of developing prostate cancer. However, a potential interaction with age (<62 vs. ≥62 years of age) was observed.

Meta-analysis results from previous studies of Caucasian populations reported a null association for studies examining self-reported dietary intakes of long-chain ω-3 PUFAs (summary RR=1.00, 95% CI=0.93, 1.09), and a modest, but not statistically significant, increased risk for studies examining biomarkers (summary RR=1.07; 95% CI=0.94, 1.20) (Alexander *et al*, 2015). The meta-analysis also suggested prostate cancer risk reductions from studies that examined DPA intake via self-report (summary RR=0.92; 95% CI=0.71, 1.19) and biomarkers (summary RR=0.85, 95% CI=0.72, 0.99). Results from another meta-analysis of prospective studies reported null associations with high intake of ALA in relation to prostate cancer risk (Carayol *et al*, 2010). Although our results for the overall null association were consistent with findings from previous studies as summarized in the two meta-analyses described above, we found that the association between PUFAs and prostate cancer risk may be modified by age at onset. Stratification by age at onset may have revealed the cumulative effect of PUFAs on prostate cancer risk. Given germline genetic variation will not vary over time, and if we assume that the wPRS is representative of a cumulative lifetime exposure to PUFAs, then it is possible that a higher magnitude of the effect would have been revealed for older men (e.g., increased risk for ω-6 would have been stronger and reduced risk would have been lower for ω-3 PUFAs among older men). However, our results indicate modest increases in risks for LA and modest reduced risks for long-chain ω-3 PUFAs (EPA, DPA, and DHA) among older men (≥62 years of age) relative to younger men (<62 years). It is also possible that prostate cancer cases diagnosed at less than 62 years of age could reflect a more aggressive form of disease. However, when we considered stratification by disease severity the increased risks were not observed. Thus, additional research may be needed to disentangle the effects of screening and the potential for outcome misclassification of aggressive versus indolent prostate cancer cases. For ω-6 PUFAs, a systematic review reported no strong positive association for AA (either dietary or biomarker) in relation to prostate cancer risk (Sakai *et al*, 2012), nor was an association observed in a meta-analysis of dietary LA intake and prostate cancer risk (Zock & Katan, 1998).

Although our study was sufficiently large to detect associations between PUFAs and prostate cancer incidence, several limitations remain. First, Mendelian randomization assumes the genetic instrument is: (1) associated with the exposure; (2) not associated with any confounders of the exposure-outcome association; and (3) independent of the outcome given the exposure and confounders (i.e., the genetic instrument only affects the outcome via the exposure of interest) (Burgess *et al*, 2015; Burgess & Thompson, 2015). The validity of the Mendelian randomization estimate hinges on these assumptions. In our study, the F-statistics for all the genetic instruments were large (>10) indicating strong genetic instruments that are associated with the exposure. However, for many of the PUFA-specific instruments the percentage of variation explained was low (<3%), and future research investigations should identify additional variants to incorporate into the genetic instruments to further improve the instrument strength. Furthermore, the PUFA-specific genetic instruments were not associated with potential confounders, with the exception of physical activity for DPA and DHA. However, adjustment for physical activity did not alter our conclusions, thus providing additional evidence that the genetic instruments utilized in this analysis are independent of confounders. The only potential concern regarding the validity of the genetic instrument is the possibility of unknown pleiotropic effects, which would violate the aforementioned third assumption. Even though this analysis used several common GWAS-identified variants in the PRS, there are likely additional rare variants that were not included in this analysis and have yet to be discovered. However, even with the inclusion of potential rare variants, the percent variation explained by the genetic instrument may not be vastly improved unless these rare variants are found to have large effects. Further replication by others is required to elucidate the true associations for other PUFAs, including the long-chain ω-3 PUFAs for which anti-inflammatory action has been suggested by laboratory studies (Berquin *et al*, 2011). Although we examined stratification by disease status, the possibility for misclassification of aggressive versus low-risk prostate cancer cases remains. Future advancements in prostate cancer screening, via serum (i.e., prostate health index or Kallikrein protein levels) or urinary (i.e., *PCA3* or *TMPRSS2-ERG* fusion) markers (Cuzick *et al*, 2014), may help to better separate aggressive prostate cancer from low-risk indolent cases, which may help to potentially reveal the benefits of long-chain ω-3 PUFAs among truly aggressive prostate cancers.

Our analysis has several strengths. First, we conducted analyses using individual level data, which allowed us to control for potential confounders of the association between the wPRS and prostate cancer risk, including principal components for European ancestry. The individual-level analysis also allowed us to examine effect measure modification by conducting stratified analyses. Second, we conducted our analysis using a large sample of data from the PRACTICAL consortium. Furthermore, we utilized available summary statistics data from this large PRACTICAL consortium and effect estimates from previous PUFA GWAS to conduct a two-sample Mendelian randomization analysis. Given large sample sizes of these studies and the use of independent variants in each genetic instrument, the Mendelian randomization estimate from the two-sample approach using summary statistics will be equivalent to the Mendelian randomization estimate from a one-sample approach (via two-stage least-squares regression) with available genetic and biomarker information (Haycock *et al*, 2016). Although, we did not observe any substantial pleiotropic effects when we conducted the weighted-regression based method (Burgess *et al*, 2015; Burgess & Thompson, 2015) nor via Egger regression (Bowden *et al*, 2015), we are unable to completely rule out the impact of unknown pleiotropic effects which could reduce the validity of the Mendelian randomization estimate (in particular for DPA, for which the Egger’s *p* value was statistically significant). Lastly, the proportion of variation explained by the SNPs included in the genetic instrument for several PUFAs (AA, LA, and DPA) was relatively high compared to other Mendelian randomization studies examining other traits (Ehret *et al*, 2011; Ahmad *et al*, 2015). Thus, the Mendelian randomization association may reflect the true null association, but requires confirmation by others, using instruments that include additional variants and explain an even higher percentage of variation in fatty acid levels (especially for those PUFAs for which the percentage of variation explained was low).

In conclusion, using data from a large consortium, we report an overall null association between PUFAs (both ω-3 and ω-6) and prostate cancer risk. Specifically, we report no association for AA in relation to prostate cancer incidence, for which the strength of the instrument and proportion of variation explained, was high. However, increased risks were indicated for men less than 62 years of age for genetically-predicted increases in long-chain ω-6 (AA). Similar increases were observed for long-chain ω-3 PUFAs (EPA and DPA) among this age group, which is contrary to what would be expected, given the hypothesized anti-inflammatory action of long-chain ω-3 PUFAs. Future investigations into these different associations by age at onset could help to elucidate the roles of PUFAs in the etiology of prostate cancer.

**References**

Ahmad OS, Morris JA, Mujammami M, Forgetta V, Leong A, Li R, Turgeon M, Greenwood CMT, Thanassoulis G, Meigs JB, Sladek R, Richards JB (2015) A Mendelian randomization study of the effect of type-2 diabetes on coronary heart disease. *Nat Commun* **6**: 7060, doi:10.1038/ncomms8060.

Alexander DD, Bassett JK, Weed DL, Barrett EC, Watson H, Harris W (2015) Meta-Analysis of Long-Chain Omega-3 Polyunsaturated Fatty Acids (LCω-3PUFA) and Prostate Cancer. *Nutr Cancer* **67**: 543–554, doi:10.1080/01635581.2015.1015745.

Arab L (2003) Biomarkers of fat and fatty acid intake. *J Nutr* **133 Suppl 3**: 925S – 932S.

Berquin IM, Edwards IJ, Kridel SJ, Chen YQ (2011) Polyunsaturated fatty acid metabolism in prostate cancer. *Cancer Metastasis Rev* **30**: 295–309, doi:10.1007/s10555-011-9299-7.

Bowden J, Davey Smith G, Burgess S (2015) Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol* **44**: 512–525, doi:10.1093/ije/dyv080.

Burgess S, Butterworth A, Thompson SG (2013) Mendelian Randomization Analysis With Multiple Genetic Variants Using Summarized Data: Mendelian Randomization Using Summarized Data. *Genet Epidemiol* **37**: 658–665, doi:10.1002/gepi.21758.

Burgess S, Dudbridge F, Thompson SG (2015) Re: ‘Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects’. *Am J Epidemiol* **181**: 290–291, doi:10.1093/aje/kwv017.

Burgess S, Thompson SG (2015) Multivariable mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. *Am J Epidemiol* **181**: 251–260, doi:10.1093/aje/kwu283.

Carayol M, Grosclaude P, Delpierre C (2010) Prospective studies of dietary alpha-linolenic acid intake and prostate cancer risk: a meta-analysis. *Cancer Causes Control CCC* **21**: 347–355, doi:10.1007/s10552-009-9465-1.

Chapkin RS, Kim W, Lupton JR, McMurray DN (2009) Dietary docosahexaenoic and eicosapentaenoic acid: emerging mediators of inflammation. *Prostaglandins Leukot Essent Fatty Acids* **81**: 187–191, doi:10.1016/j.plefa.2009.05.010.

Cuzick J, Thorat MA, Andriole G, Brawley OW, Brown PH, Culig Z, Eeles RA, Ford LG, Hamdy FC, Holmberg L, Ilic D, Key TJ, Vecchia CL, Lilja H, Marberger M, Meyskens FL, Minasian LM, Parker C, Parnes HL, Perner S, Rittenhouse H, Schalken J, Schmid H-P, Schmitz-Dräger BJ, Schröder FH, Stenzl A, Tombal B, Wilt TJ, Wolk A (2014) Prevention and early detection of prostate cancer. *Lancet Oncol* **15**: e484–e492, doi:10.1016/S1470-2045(14)70211-6.

Desquilbet L, Mariotti F (2010) Dose-response analyses using restricted cubic spline functions in public health research. *Stat Med* **29**: 1037–1057, doi:10.1002/sim.3841.

Eeles RA, Olama AAA, Benlloch S, Saunders EJ, Leongamornlert DA, Tymrakiewicz M, Ghoussaini M, Luccarini C, Dennis J, Jugurnauth-Little S, Dadaev T, Neal DE, Hamdy FC, Donovan JL, Muir K, Giles GG, Severi G, Wiklund F, Gronberg H, Haiman CA, Schumacher F, Henderson BE, Le Marchand L, Lindstrom S, Kraft P, Hunter DJ, Gapstur S, Chanock SJ, Berndt SI, Albanes D, Andriole G, Schleutker J, Weischer M, Canzian F, Riboli E, Key TJ, Travis RC, Campa D, Ingles SA, John EM, Hayes RB, Pharoah PDP, Pashayan N, Khaw K-T, Stanford JL, Ostrander EA, Signorello LB, Thibodeau SN, Schaid D, Maier C, Vogel W, Kibel AS, Cybulski C, Lubinski J, Cannon-Albright L, Brenner H, Park JY, Kaneva R, Batra J, Spurdle AB, Clements JA, Teixeira MR, Dicks E, Lee A, Dunning AM, Baynes C, Conroy D, Maranian MJ, Ahmed S, Govindasami K, Guy M, Wilkinson RA, Sawyer EJ, Morgan A, Dearnaley DP, Horwich A, Huddart RA, Khoo VS, Parker CC, Van As NJ, Woodhouse CJ, Thompson A, Dudderidge T, Ogden C, Cooper CS, Lophatananon A, Cox A, Southey MC, Hopper JL, English DR, Aly M, Adolfsson J, Xu J, Zheng SL, Yeager M, Kaaks R, Diver WR, Gaudet MM, Stern MC, Corral R, Joshi AD, Shahabi A, Wahlfors T, Tammela TLJ, Auvinen A, Virtamo J, Klarskov P, Nordestgaard BG, Røder MA, Nielsen SF, Bojesen SE, Siddiq A, Fitzgerald LM, Kolb S, Kwon EM, Karyadi DM, Blot WJ, Zheng W, Cai Q, McDonnell SK, Rinckleb AE, Drake B, Colditz G, Wokolorczyk D, Stephenson RA, Teerlink C, Muller H, Rothenbacher D, Sellers TA, Lin H-Y, Slavov C, Mitev V, Lose F, Srinivasan S, Maia S, Paulo P, Lange E, Cooney KA, Antoniou AC, Vincent D, Bacot F, Tessier DC, COGS–Cancer Research UK GWAS–ELLIPSE (part of GAME-ON) Initiative, Australian Prostate Cancer Bioresource, UK Genetic Prostate Cancer Study Collaborators/British Association of Urological Surgeons’ Section of Oncology, UK ProtecT (Prostate testing for cancer and Treatment) Study Collaborators, PRACTICAL (Prostate Cancer Association Group to Investigate Cancer-Associated Alterations in the Genome) Consortium, Kote-Jarai Z, Easton DF (2013) Identification of 23 new prostate cancer susceptibility loci using the iCOGS custom genotyping array. *Nat Genet* **45**: 385–391, 391e1–e2, doi:10.1038/ng.2560.

Ehret GB, Munroe PB, Rice KM, Bochud M, Johnson AD, Chasman DI, Smith AV, Tobin MD, Verwoert GC, Hwang S-J, Pihur V, Vollenweider P, O’Reilly PF, Amin N, Bragg-Gresham JL, Teumer A, Glazer NL, Launer L, Hua Zhao J, Aulchenko Y, Heath S, Sõber S, Parsa A, Luan J, Arora P, Dehghan A, Zhang F, Lucas G, Hicks AA, Jackson AU, Peden JF, Tanaka T, Wild SH, Rudan I, Igl W, Milaneschi Y, Parker AN, Fava C, Chambers JC, Fox ER, Kumari M, Jin Go M, van der Harst P, Hong Linda Kao W, Sjögren M, Vinay DG, Alexander M, Tabara Y, Shaw-Hawkins S, Whincup PH, Liu Y, Shi G, Kuusisto J, Tayo B, Seielstad M, Sim X, Hoang Nguyen K-D, Lehtimäki T, Matullo G, Wu Y, Gaunt TR, Charlotte Onland-Moret N, Cooper MN, Platou CGP, Org E, Hardy R, Dahgam S, Palmen J, Vitart V, Braund PS, Kuznetsova T, Uiterwaal CSPM, Adeyemo A, Palmas W, Campbell H, Ludwig B, Tomaszewski M, Tzoulaki I, Palmer ND, Aspelund T, Garcia M, Chang Y-PC, O’Connell JR, Steinle NI, Grobbee DE, Arking DE, Kardia SL, Morrison AC, Hernandez D, Najjar S, McArdle WL, Hadley D, Brown MJ, Connell JM, Hingorani AD, Day INM, Lawlor DA, Beilby JP, Lawrence RW, Clarke R, Hopewell JC, Ongen H, Dreisbach AW, Li Y, Hunter Young J, Bis JC, Kähönen M, Viikari J, Adair LS, Lee NR, Chen M-H, Olden M, Pattaro C, Hoffman Bolton JA, Köttgen A, Bergmann S, Mooser V, Chaturvedi N, Frayling TM, Islam M, Jafar TH, Erdmann J, Kulkarni SR, Bornstein SR, Grässler J, Groop L, Voight BF, Kettunen J, Howard P, Taylor A, Guarrera S, Ricceri F, Emilsson V, Plump A, Barroso I, Khaw K-T, Weder AB, Hunt SC, Sun YV, Bergman RN, Collins FS, Bonnycastle LL, Scott LJ, Stringham HM, Peltonen L, Perola M, Vartiainen E, Brand S-M, Staessen JA, Wang TJ, Burton PR, Soler Artigas M, Dong Y, Snieder H, Wang X, Zhu H, Lohman KK, Rudock ME, Heckbert SR, Smith NL, Wiggins KL, Doumatey A, Shriner D, Veldre G, Viigimaa M, Kinra S, Prabhakaran D, Tripathy V, Langefeld CD, Rosengren A, Thelle DS, Maria Corsi A, Singleton A, Forrester T, Hilton G, McKenzie CA, Salako T, Iwai N, Kita Y, Ogihara T, Ohkubo T, Okamura T, Ueshima H, Umemura S, Eyheramendy S, Meitinger T, Wichmann H-E, Shin Cho Y, Kim H-L, Lee J-Y, Scott J, Sehmi JS, Zhang W, Hedblad B, Nilsson P, Davey Smith G, Wong A, Narisu N, Stančáková A, Raffel LJ, Yao J, Kathiresan S, O’Donnell CJ, Schwartz SM, Arfan Ikram M, Longstreth Jr WT, Mosley TH, Seshadri S, Shrine NRG, Wain LV, Morken MA, Swift AJ, Laitinen J, Prokopenko I, Zitting P, Cooper JA, Humphries SE, Danesh J, Rasheed A, Goel A, Hamsten A, Watkins H, Bakker SJL, van Gilst WH, Janipalli CS, Radha Mani K, Yajnik CS, Hofman A, Mattace-Raso FUS, Oostra BA, Demirkan A, Isaacs A, Rivadeneira F, Lakatta EG, Orru M, Scuteri A, Ala-Korpela M, Kangas AJ, Lyytikäinen L-P, Soininen P, Tukiainen T, Würtz P, Twee-Hee Ong R, Dörr M, Kroemer HK, Völker U, Völzke H, Galan P, Hercberg S, Lathrop M, Zelenika D, Deloukas P, Mangino M, Spector TD, Zhai G, Meschia JF, Nalls MA, Sharma P, Terzic J, Kranthi Kumar MV, Denniff M, Zukowska-Szczechowska E, Wagenknecht LE, Gerald R. Fowkes F, Charchar FJ, Schwarz PEH, Hayward C, Guo X, Rotimi C, Bots ML, Brand E, Samani NJ, Polasek O, Talmud PJ, Nyberg F, Kuh D, Laan M, Hveem K, Palmer LJ, van der Schouw YT, Casas JP, Mohlke KL, Vineis P, Raitakari O, Ganesh SK, Wong TY, Shyong Tai E, Cooper RS, Laakso M, Rao DC, Harris TB, Morris RW, Dominiczak AF, Kivimaki M, Marmot MG, Miki T, Saleheen D, Chandak GR, Coresh J, Navis G, Salomaa V, Han B-G, Zhu X, Kooner JS, Melander O, Ridker PM, Bandinelli S, Gyllensten UB, Wright AF, Wilson JF, Ferrucci L, Farrall M, Tuomilehto J, Pramstaller PP, Elosua R, Soranzo N, Sijbrands EJG, Altshuler D, Loos RJF, Shuldiner AR, Gieger C, Meneton P, Uitterlinden AG, Wareham NJ, Gudnason V, Rotter JI, Rettig R, Uda M, Strachan DP, Witteman JCM, Hartikainen A-L, Beckmann JS, Boerwinkle E, Vasan RS, Boehnke M, Larson MG, Järvelin M-R, Psaty BM, Abecasis GR, Chakravarti A, Elliott P, van Duijn CM, Newton-Cheh C, Levy D, Caulfield MJ, Johnson T (2011) Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* **478**: 103–109, doi:10.1038/nature10405.

Guan W, Steffen BT, Lemaitre RN, Wu JHY, Tanaka T, Manichaikul A, Foy M, Rich SS, Wang L, Nettleton JA, Tang W, Gu X, Bandinelli S, King IB, McKnight B, Psaty BM, Siscovick D, Djousse L, Ida Chen Y-D, Ferrucci L, Fornage M, Mozafarrian D, Tsai MY, Steffen LM (2014) Genome-wide association study of plasma N6 polyunsaturated fatty acids within the cohorts for heart and aging research in genomic epidemiology consortium. *Circ Cardiovasc Genet* **7**: 321–331, doi:10.1161/CIRCGENETICS.113.000208.

Haycock PC, Burgess S, Wade KH, Bowden J, Relton C, Davey Smith G (2016) Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. *Am J Clin Nutr* doi:10.3945/ajcn.115.118216.

Lemaitre RN, Tanaka T, Tang W, Manichaikul A, Foy M, Kabagambe EK, Nettleton JA, King IB, Weng L-C, Bhattacharya S, Bandinelli S, Bis JC, Rich SS, Jacobs DR, Cherubini A, McKnight B, Liang S, Gu X, Rice K, Laurie CC, Lumley T, Browning BL, Psaty BM, Chen Y-DI, Friedlander Y, Djousse L, Wu JHY, Siscovick DS, Uitterlinden AG, Arnett DK, Ferrucci L, Fornage M, Tsai MY, Mozaffarian D, Steffen LM (2011) Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. *PLoS Genet* **7**: e1002193, doi:10.1371/journal.pgen.1002193.

Al Olama AA, Kote-Jarai Z, Berndt SI, Conti DV, Schumacher F, Han Y, Benlloch S, Hazelett DJ, Wang Z, Saunders E, Leongamornlert D, Lindstrom S, Jugurnauth-Little S, Dadaev T, Tymrakiewicz M, Stram DO, Rand K, Wan P, Stram A, Sheng X, Pooler LC, Park K, Xia L, Tyrer J, Kolonel LN, Le Marchand L, Hoover RN, Machiela MJ, Yeager M, Burdette L, Chung CC, Hutchinson A, Yu K, Goh C, Ahmed M, Govindasami K, Guy M, Tammela TLJ, Auvinen A, Wahlfors T, Schleutker J, Visakorpi T, Leinonen KA, Xu J, Aly M, Donovan J, Travis RC, Key TJ, Siddiq A, Canzian F, Khaw K-T, Takahashi A, Kubo M, Pharoah P, Pashayan N, Weischer M, Nordestgaard BG, Nielsen SF, Klarskov P, Røder MA, Iversen P, Thibodeau SN, McDonnell SK, Schaid DJ, Stanford JL, Kolb S, Holt S, Knudsen B, Coll AH, Gapstur SM, Diver WR, Stevens VL, Maier C, Luedeke M, Herkommer K, Rinckleb AE, Strom SS, Pettaway C, Yeboah ED, Tettey Y, Biritwum RB, Adjei AA, Tay E, Truelove A, Niwa S, Chokkalingam AP, Cannon-Albright L, Cybulski C, Wokołorczyk D, Kluźniak W, Park J, Sellers T, Lin H-Y, Isaacs WB, Partin AW, Brenner H, Dieffenbach AK, Stegmaier C, Chen C, Giovannucci EL, Ma J, Stampfer M, Penney KL, Mucci L, John EM, Ingles SA, Kittles RA, Murphy AB, Pandha H, Michael A, Kierzek AM, Blot W, Signorello LB, Zheng W, Albanes D, Virtamo J, Weinstein S, Nemesure B, Carpten J, Leske C, Wu S-Y, Hennis A, Kibel AS, Rybicki BA, Neslund-Dudas C, Hsing AW, Chu L, Goodman PJ, Klein EA, Zheng SL, Batra J, Clements J, Spurdle A, Teixeira MR, Paulo P, Maia S, Slavov C, Kaneva R, Mitev V, Witte JS, Casey G, Gillanders EM, Seminara D, Riboli E, Hamdy FC, Coetzee GA, Li Q, Freedman ML, Hunter DJ, Muir K, Gronberg H, Neal DE, Southey M, Giles GG, Severi G, Breast and Prostate Cancer Cohort Consortium (BPC3), PRACTICAL (Prostate Cancer Association Group to Investigate Cancer-Associated Alterations in the Genome) Consortium, COGS (Collaborative Oncological Gene-environment Study) Consortium, GAME-ON/ELLIPSE Consortium, Cook MB, Nakagawa H, Wiklund F, Kraft P, Chanock SJ, Henderson BE, Easton DF, Eeles RA, Haiman CA (2014) A meta-analysis of 87,040 individuals identifies 23 new susceptibility loci for prostate cancer. *Nat Genet* **46**: 1103–1109, doi:10.1038/ng.3094.

Panigrahy D, Kaipainen A, Greene ER, Huang S (2010) Cytochrome P450-derived eicosanoids: the neglected pathway in cancer. *Cancer Metastasis Rev* **29**: 723–735.

Sakai M, Kakutani S, Horikawa C, Tokuda H, Kawashima H, Shibata H, Okubo H, Sasaki S (2012) Arachidonic acid and cancer risk: a systematic review of observational studies. *BMC Cancer* **12**: 606–2407 – 12–606.

Sobolewski C, Cerella C, Dicato M, Ghibelli L, Diederich M (2010) The role of cyclooxygenase-2 in cell proliferation and cell death in human malignancies. *Int J Cell Biol* **2010**: 215158, doi:10.1155/2010/215158.

Stock JH, Wright JH, Yogo M (2002) A survey of weak instruments and weak identification in generalized method of moments. *J Bus Econ Stat* **20**: 518.

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A (2015) Global cancer statistics, 2012. *CA Cancer J Clin* **65**: 87–108, doi:10.3322/caac.21262.

Wang D, Dubois RN (2010) Eicosanoids and cancer. *Nat Rev* **10**: 181–193.

Zock PL, Katan MB (1998) Linoleic acid intake and cancer risk: a review and meta-analysis. *Am J Clin Nutr* **68**: 142–153.