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

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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 randomisation, 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 to 33.07%, were constructed and used to evaluate associations with prostate cancer risk per one standard deviation (s.d.) increase in 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 ($OR_{LA} = 0.95$, 95%CI = 0.92, 0.98) and α-linolenic acids ($OR_{ALA} = 0.96$, 95%CI = 0.93, 0.98), among men <62 years; whereas increased risk was found among men ⩾62 years for LA ($OR_{LA} = 1.04$, 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 ($OR_{AA} = 1.05$, 95%CI = 1.02, 1.08; $OR_{EPA} = 1.04$, 95%CI = 1.01, 1.06; $OR_{DPA} = 1.05$, 95%CI = 1.02, 1.08).

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

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 and Katan, 1998; Carayol *et al*, 2010; Sakai *et al*, 2012; Alexander *et al*, 2015). Given the possible role that PUFAs may have in prostate carcinogenesis, with suggested anti-

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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 and 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 summarising 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. Although 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 aetiologic 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 tumours 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 randomisation is based on the principle of random assortment of alleles at conception, and may identify causal risk factors for disease by utilising 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 randomisation 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 randomisation 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 ($n=1189$) 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 that had only two controls ($n=168$). The final analytic data set 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 single-nucleotide polymorphisms (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., LA, AA, ALA, EPA, DPA, and DHA). Finally, 9 of these 14 SNPs were independent ($r^2 < 0.1$), and thus were used in the genetic instrument for the Mendelian randomisation analyses. Please refer to Supplementary 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 >85 000 prostate cancer-related 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). Single-nucleotide polymorphisms with call rates <95% were excluded, as well as those deviating from Hardy–Weinberg Equilibrium in the controls at *P*-value $<1\times10^{-7}$ (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 ($r^2 > 0.76$). Single-nucleotide polymorphisms were imputed in two stages; first using SHAPEIT (http://www/shapeit.fr/) by chromosome and chunk, and then data were 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. 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:

$$\sum_{i=1}^{n} \beta_i SNP_i,$$

where SNP_i represents the effect allele dosage and β_i 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 a 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). Supplementary 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 and 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^{-1} 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 (Supplementary Figures 6–11). Analyses were conducted using SAS version 9.4 (Cary, NC, USA), and STATA version 12.1 (College Station, TX, USA).

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 (Supplementary 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 randomisation estimate using an inverse-variance weighted meta-analysis approach (Burgess *et al*, 2013). We further standardised the Mendelian randomisation ORs and 95% CIs to represent an increase in prostate cancer risk per one standard

Table 1. Effect estimates for plasma phospholipid levels of polyunsaturated fatty acids (PUFAs, % of total fatty acids) for genome-wide significant ($P < 5 \times 10^{-8}$), independent ($r^2 < 0.1$) genetic variants reported from previous GWAS

Chr	SNP	GRCh37/ hg19 position	Allele[a]	EAF	β	s.e.	*P*-value	% VE[b] per allele	% VE per IV[c]	F-statistic per IV[d]
Linoleic acid (LA, 18:2n6)										
10	rs10740118	65101207	C/**G**	0.56	0.248	0.043	8.08×10^{-9}	0.2–0.7		
11	rs174547	61570783	T/**C**	0.32	1.474	0.042	4.98×10^{-274}	7.6–18.1		
11	rs2727270	61603237	**T**/C	0.44	0.690	0.070	2.60×10^{-21}	0.5–2.4		
16	rs16966952	15135943	A/**G**	0.31	0.351	0.044	1.23×10^{-15}	0.5–2.5	8.8–23.6[e]	1104–3533
Arachidonic acid (AA, 20:4n6)										
11	rs174547	61570783	**T**/C	0.68	1.691	0.025	3.00×10^{-971}	32.63		
16	rs16966952	15135943	A/**G**	0.69	0.199	0.031	2.43×10^{-10}	0.44	33.07	11 302
α-Linolenic acid (ALA, 18:3n3)										
11	rs174547	61570783	T/**C**	0.33	0.016	0.001	3.47×10^{-64}	1.03	1.03	476
Eicosapentaenoic acid (EPA, 20:5n3)										
6	rs3798713	11008622	**C**/G	0.43	0.035	0.005	1.93×10^{-12}	0.36		
11	rs174538	61560081	A/**G**	0.72	0.083	0.005	5.37×10^{-58}	1.69	2.05	479
Docosapentaenoic acid (DPA, 22:5n3)										
2	rs780094	27741237	**T**/C	0.41	0.017	0.003	9.04×10^{-09}	0.46		
6	rs3734398	10982973	T/**C**	0.43	0.040	0.003	9.61×10^{-44}	2.74		
11	rs174547	61570783	**T**/C	0.67	0.075	0.003	3.79×10^{-154}	8.38	11.58	1997
Docosahexaenoic acid (DHA, 22:6n3)										
6	rs2236212	10995015	C/**G**	0.57	0.113	0.014	1.26×10^{-15}	0.65	0.65	299

Abbreviations: EAF = effect allele frequency; IV = instrumental variable; s.e. = standard error; SNP = single-nucleotide polymorphism.
[a]Allele associated with an increase in PUFA levels is in bold, and is considered the effect allele.
[b]% variation explained (VE) = $(2 \times \beta^2 \times EAF \times (1 - EAF)/var(PUFA)) \times 100$.
[c]% VE per IV = sum of the %VE per allele for each SNP included in the IV.
[d]F-statistic is a measure of the strength of the genetic instrument and is calculated as follows: $(R^2 \times (n\text{-}1\text{-}k))/((1\text{-}R^2) \times k)$, where R^2 = % variation explained, *n* = sample size, k = total number of instrumental variables.
[e]Ranges for % VE per SNP and % VE IV as reported in Guan *et al* (2014).

deviation increase for each PUFA-specific wPRS, thus representing a standard deviation increase in percentage of PUFA levels per total plasma fatty acids (Supplementary 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 (Supplementary 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 (Y_g) between variant (g) and prostate cancer (Y) was regressed on the association (X_g) between that same variant (g) and the PUFA trait of interest (X), weighted by the inverse variance (σ_{Yg}^{-2}) (Burgess *et al*, 2015; Burgess and 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 Supplementary 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 ~33% (for AA). Because of 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 in 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 <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 (Supplementary Figures 6–11). Furthermore, our results did not change after adjusting for different covariates, including age and physical activity that were found to be associated with the PUFA-specific wPRSs (Supplementary Table 1). We also conducted a Mendelian randomisation analysis via the two-sample method using summary statistics scaled per one standard deviation unit increase (Supplementary 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 randomisation estimate was assessed using two different approaches, Egger regression and a weighted regression-based method. With the exception of the wPRS for DPA ($\beta_0 = 0.01304$, $P < 0.0001$), we did not observe any statistically significant intercepts as an indication of potential pleiotropic effects and an invalid instrument (Supplementary 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 (Supplementary 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 summarised 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 <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 *vs* 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 and Katan, 1998).

Although our study was sufficiently large to detect associations between PUFAs and prostate cancer incidence, several limitations remain. First, Mendelian randomisation assumes that the genetic

Table 2. ORs[a] and 95% CIs for the overall association between one s.d. increase in PUFA-specific wPRSs and prostate cancer risk, and associations stratified by potential effect measure modifiers using individual-level data from the PRACTICAL consortium

		ω-6 PUFAs						ω-3 PUFAs											
		Linoleic acid (LA)			Arachidonic acid (AA)			α-Linolenic acid (ALA)			Eicosapentaenoic acid (EPA)			Docosapentaenoic acid (DPA)			Docosahexaenoic acid (DHA)		
Subgroup	**Cases/Controls**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
Overall																			
All men	22 721/23 034	1.00	0.98, 1.02	0.65	1.01	0.99, 1.03	0.36	0.99	0.97, 1.01	0.38	1.01	0.99, 1.03	0.31	1.01	0.99, 1.03	0.16	1.00	0.98, 1.02	0.81
Smoking status																			
Ever smokers	4789/4914	0.99	0.95, 1.04	0.81	1.01	0.97, 1.05	0.69	0.99	0.95, 1.03	0.66	1.01	0.97, 1.06	0.65	1.01	0.97, 1.05	0.66	1.00	0.96, 1.04	0.99
Never smokers	3091/2954	1.00	0.95, 1.06	0.98	1.01	0.95, 1.07	0.76	0.99	0.94, 1.05	0.81	0.99	0.93, 1.04	0.62	1.00	0.94, 1.05	0.94	0.98	0.93, 1.04	0.56
$P_{interaction}$[b]			0.87			0.99			0.92			0.50			0.74			0.65	
Age																			
<62 years of age	8259/13 684	0.95	0.92, 0.98	2.6×10^{-4}	1.05	1.02, 1.08	2.0×10^{-3}	0.96	0.93, 0.98	1.7×10^{-3}	1.04	1.01, 1.06	1.7×10^{-2}	1.05	1.02, 1.08	2.0×10^{-3}	1.01	0.98, 1.04	0.43
⩾62 years of age	14 462/9350	1.04	1.01, 1.07	6.0×10^{-3}	0.98	0.95, 1.01	0.11	1.02	0.99, 1.05	0.10	0.99	0.96, 1.02	0.43	0.99	0.96, 1.01	0.28	0.99	0.96, 1.02	0.48
$P_{interaction}$[b]			5.2×10^{-6}			8.5×10^{-4}			6.5×10^{-4}			2.3×10^{-2}			2.8×10^{-3}			0.29	
Disease severity																			
Advanced cancer[c]	4802/23 034	0.99	0.97, 1.01	0.24	1.01	0.99, 1.04	0.58	0.99	0.97, 1.01	0.59	1.01	0.99, 1.03	0.96	1.02	0.99, 1.03	0.69	1.00	0.98, 1.02	0.43
Non-advanced cancer	17 919/23 034	1.02	0.99, 1.05	0.31	0.99	0.96, 1.02	0.19	1.01	0.98, 1.04	0.20	1.00	0.97, 1.03	0.23	1.01	0.97, 1.04	0.13	1.01	0.98, 1.05	0.99
$P_{homogeneity}$[d]			0.10			0.32			0.28			0.59			0.65			0.62	
Method of detection																			
Screen-detected	4414/23 034	0.98	0.94, 1.02	0.25	1.04	0.99, 1.08	0.06	0.97	0.93, 1.00	0.07	1.03	0.99, 1.07	0.08	1.03	0.99, 1.07	0.08	1.00	0.96, 1.04	0.93
Clinically-detected	8597/23 034	1.00	0.97, 1.03	0.86	1.00	0.97, 1.03	0.92	1.00	0.97, 1.03	0.98	0.99	0.96, 1.02	0.58	1.00	0.97, 1.03	0.97	0.99	0.96, 1.02	0.52
$P_{homogeneity}$[d]			0.43			0.15			0.20			0.12			0.24			0.69	

Abbreviations: 95% CIs = 95% confidence intervals; ORs = odds ratios; s.d. = standard deviation; wPRSs = weighted-polygenic risk scores.

[a]ORs and 95% CIs adjusted for age, eight principal components for European ancestry, and PRACTICAL study site, and represent one s.d. increase in each PUFA-specific wPRS (i.e., 1.20 for LA, 1.13 for AA, 0.01 for ALA, 0.06 for EPA, 0.06 for DPA, and 0.08 for DHA).

[b]Interaction assessed on a multiplicative scale using the likelihood ratio test.

[c]Advanced prostate cancer refers to prostate cancer cases with either Gleason score ⩾ 8, death from prostate cancer, metastatic disease, or prostate-specific antigen levels > 100 ng ml^{-1} at diagnosis.

[d]ORs and 95% CIs for advanced *vs* non-advanced and screen-detected *vs* clinically-detected prostate cancers were estimated using polytomous regression. A homogeneity test was conducted to assess statistically significant differences between stratum-specific estimates.

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 and Thompson, 2015). The validity of the Mendelian randomisation 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 utilised 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 *vs* 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 utilised available summary statistics data from this large PRACTICAL consortium and effect estimates from previous PUFA GWAS to conduct a two-sample Mendelian randomisation analysis. Given large sample sizes of these studies and the use of independent variants in each genetic instrument, the Mendelian randomisation estimate from the two-sample approach using summary statistics will be equivalent to the Mendelian randomisation 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 and Thompson, 2015) nor via Egger regression (Bowden *et al*, 2015), we are unable to completely rule out the impact of unknown pleiotropic effects that could reduce the validity of the Mendelian randomisation estimate (in particular for DPA, for which the Egger's *P*-value was statistically significant). Finally, the proportion of variation explained by the SNPs included in the genetic instrument for several PUFAs (AA, LA, and DPA) was relatively high compared with other Mendelian randomisation studies examining other traits (Ehret *et al*, 2011; Ahmad *et al*, 2015). Thus, the Mendelian randomisation 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 were high. However, increased risks were indicated for men <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 hypothesised 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 aetiology of prostate cancer.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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