Polyunsaturated Fatty Acid Levels and the Risk of Keratinocyte Cancer: A Mendelian Randomization Analysis

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ABSTRACT

Background: Keratinocyte cancer is the commonest cancer, imposing a high economic burden on the health care system. Observational studies have shown mixed associations between polyunsaturated fatty acids (PUFA) and keratinocyte cancer, basal cell carcinoma (BCC), and squamous cell carcinoma (SCC). We explored whether genetically predicted PUFA levels are associated with BCC and SCC risks.

Methods: We conducted a two-sample Mendelian randomization study using PUFA level genome-wide association studies (GWAS) from the Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium ($n > 8,000$), and the meta-analysis GWASs from UKB, 23andMe, and Qskin for BCC ($n = 651,138$) and SCC ($n = 635,331$) risk.

Results: One SD increase in genetically predicted levels of linoleic acid [OR = 0.94, 95% confidence interval (CI) = 0.91–0.97, $P = 1.4 \times 10^{-4}$] and alpha-linolenic acid (OR = 0.91, 95% CI = 0.86–0.96, $P = 5.1 \times 10^{-4}$) was associated with a reduced BCC risk, while arachidonic acid (OR = 1.04, 95% CI = 1.02–1.06, $P = 3.2 \times 10^{-4}$) and eicosapentaenoic acid (OR = 1.10, 95% CI = 1.04–1.16, $P = 1.5 \times 10^{-3}$) were associated with an increased BCC risk.

Conclusions: Higher genetically predicted levels of linoleic acid and alpha-linolenic acid were associated with a reduced BCC risk, but arachidonic acid and eicosapentaenoic acid were associated with a higher BCC risk.

Impact: PUFA-related diet and supplementation could influence BCC etiology.

Introduction

Keratinocyte cancers (KC) are the commonest cancers globally, and include two principal types: basal cell carcinoma (BCC) and squamous cell carcinoma (SCC). Because of their frequency, they incur considerable morbidity and very large health expenditures up to AUD $700 million and USD $4.8 billion for their treatment annually in Australia and the United States, respectively (1–3). These cancers are caused by sun exposure, and personal risk is influenced by factors such as fair skin, red hair, and genetic factors (4–6). As a modifiable factor, the role of dietary factors in keratinocyte cancer risk has been contentious. Polyunsaturated fatty acids (PUFA) that include n-6 or omega-6 fats; linoleic acid (LA; 18:2n-6), and arachidonic acid (AA; 20:4n-6), and n-3 or omega-3 fats; alpha-linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), docosapentaenoic acid (DPA, 22:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) have been found to influence the risk of a number of diseases. Previous studies have reported

associations between PUFAs and coronary heart disease (7, 8) and cancers (9, 10).

PUFAs influence biological processes that influence both carcinogenesis and cancer progression (11–14). Omega-6 LA stimulates production of anti-inflammatory products with carcinoprotective properties while AA triggers generation of cytokines which initiate cancer-related inflammation (11–14). In contrast, n-3 PUFAs (e.g., ALA and EPA) are generally metabolized into anti-inflammatory eicosanoids thus halting carcinogenesis (11, 12, 14, 15). The respective PUFA effects are thought to be independent of each PUFA family. For example, a previous study found no interaction between plasma phospholipid levels of LA (n-6) and the levels of DHA or EPA (n-3), but significant interaction of ALA with EPA levels (increase in ALA resulted in increased EPA; ref. 16).

Although the aforementioned biological processes link PUFAs to cancers, observational studies examining the relationship between PUFAs and BCC and SCC have reported mixed findings (17). For example, the Nambour Study in Australia found no association between PUFAs (total omega-6 and omega-3) with the risk of BCC and SCC (18). Specifically, it found no association between higher levels of each of the omega-3 fats; ALA, EPA, DHA, and DPA and the risk of BCC, and SCC (18). Similarly, higher levels of omega-6 fats; LA, and AA were not associated with the risk of BCC and SCC (18). However, recent findings from the Nurses' Health Study and Health Professionals Follow-up Study from the United States, revealed that higher intake of omega-6 fat was associated with higher risks of both BCC and SCC (19). In addition, while higher intake of omega-3 fat was positively associated with the risk of BCC, this was not the case with SCC (19).

However, observational studies are more prone to limitations including; selection and recall bias, confounding, and reverse causation, than randomized control trials (RCT) and Mendelian randomization (MR) studies. RCTs are less prone to error, but are very difficult to conduct for dietary interventions, and consequently are extremely

scarce. Thus, it is desirable to use the MR approach. MR is based on the principle of random allocation of risk alleles and independent assortment of genes at meiosis. Therefore, it is less prone to some of the biases which affect observational studies when the assumptions for a valid instrument variable are met. The MR design utilizes genetic variants as the instrumental variables for the exposure (here PUFA levels). It assumes that the instrumental variables; (i) are associated with the exposure (PUFA levels), (ii) are not associated with any confounders of PUFA-KC association (exposure outcome), and (iii) affect the outcome (here BCC and SCC) only through the exposure (here PUFA levels; ref. 20).

Previous MR studies have found significant associations between genetically predicted levels of particular PUFAs and the risk of cancer including; prostate cancer (21), lung cancer (22), and colorectal cancer (12, 23). However, previous MR studies found no association between PUFAs and the risk of cutaneous melanoma (24) and all cancer (23). No MR study to date has explored a causal relationship between PUFA levels and the risk of BCC or SCC. Therefore, our aim was to assess whether genetically predicted PUFA levels are associated with the risk of BCC and SCC.

Materials and Methods

Study population for PUFA levels

We obtained summary statistics data to identify genetic instruments for PUFA levels from genome-wide association study (GWAS) meta-analysis on n-3 (ALA, EPA, DPA and DHA) and n-6 (AA, and LA) PUFAs that included that included 8,866 and 8,631 participants, respectively, of European ancestry in five studies in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium (16, 25). Details for each cohort including recruitment, quality control, and ethical procedures have been published previously (16, 25–29). The five study cohorts included were Atherosclerosis Risk in Communities (ARIC, $n = 3,268$), Coronary Artery Risk Development in Young Adults (CARDIA, $n = 1,507$), Cardiovascular Health Study (CHS, $n = 2,326$), Invecchiare in Chianti (InCHIANTI, $n = 1,075$), and Multi-Ethnic Study of Atherosclerosis (MESA, $n = 690$). PUFA levels were analyzed by gas chromatographic techniques and reported as percentage of the total plasma fatty acids (16, 30). The mean and SD of each PUFA in the cohorts were reported.

Study population for the BCC and SCC risk

We used summary data from the recently published GWAS meta-analysis that included overall 31,787 BCC cases, and 619,351 controls, and 9,674 SCC cases and 625,657 controls of European descent from Europe, United States, and Australia (31). For BCC, the meta-analysis included the UK Biobank (16,847 cases, 340,302 controls), the 23andMe Research cohort (12,945 cases, 274,252 controls), and the QSkin Sun and Health Study (QSkin) cohort (1,995 cases, 4,797 controls). Similarly, the SCC meta-analysis included the UK Biobank (2,274 cases, 340,302 controls), 23andMe Research cohort (6,579 cases, 280,558 controls), and the Qskin cohort (821 cases and 4,797 controls). All participants were of European ancestry. Details on the respective cohorts have been extensively described previously (4, 32–34).

In summary, the UK Biobank is a population-based cohort of over 500,000 adult participants (40–69 years old) recruited between 2006 and 2010 in the United Kingdom. Detailed phenotypic and genetic data were collected and participants with information on BCC and SCC were included in the meta-analysis. The study was approved by the United Kingdom's National North West Multi-Centre Research

Ethics Committee. 23andMe Research cohort included international participants of European ancestry with self-reported data on BCC and SCC. Validation of the self-reported data showed high accuracy (4). The research protocol was approved by the Ethical and Independent Review Services, an Institutional Review Board accredited by the Association for the Accreditation of Human Research Protection. The QSkin is a population based prospective cohort of adulted participants (40–60 years old, $N \sim 43,000$) from Queensland, Australia recruited between 2011 and 2012, and over 17,000 of them genotyped in 2017 (34). Both clinically validated and self-reported data on BCC and SCC were collected. The study was approved by the Human Research Ethics Committee at QIMR Berghofer Medical Research Institute, Brisbane, Australia. All participants in the three cohorts provided written informed consent. Details on the methods used to conduct the GWAS in each cohort and the meta-analysis published elsewhere (31).

Selection of the instrumental variables

We identified the SNPs that were associated with increased PUFA plasma levels at the genome-wide significant level ($P = 5 \times 10^{-8}$) in published GWAS meta-analysis summary data from the CHARGE Consortium (16, 25, 30). For each PUFA, we extracted the SNP, its PUFA-increasing allele, the estimated SNP-PUFA magnitude of association (beta), and its SE (**Table 1**) for the instrument selection for PUFA and harmonized using the TwoSampleMR package in R (35). The selected instrumental variables (IV) were largely consistent with previous studies that explored the associations between PUFA and other morbidities (21, 22, 24). Next, we retrieved summary data for the selected IVs for each PUFA for both the BCC and SCC analysis from the previous keratinocyte cancer GWAS meta-analysis (31).

MR

We conducted a two-sample MR analysis using the inverse variance weighted (IVW) method (36, 37). IVW utilizes GWAS summary data, a method equivalent to using individual level data (36). For each PUFA, the Wald-type ratio estimator (38) was used to compute the MR estimates for each SNP. Then, the SNP estimates (for multiple IVs) were meta-analyzed using the IVW approach based on random effects. TwoSampleMR package in R was used for the analysis (35). Then, the estimated associations for the genetically determined levels of PUFA and BCC and SCC risk were expressed as OR per SD increase in PUFA levels.

Sensitivity analyses

We conducted "leave-one-out" analyses to assess whether the MR results were being driven or biased by a SNP for each PUFA. Next, we investigated the possibility of directional (horizontal) pleiotropy through MR-Egger regression (39). The intercept term in the MR-Egger regression quantifies evidence for the directional pleiotropy; the magnitude and direction of the effect of the instrumental variables (SNPs) on the outcome (BCC and SCC risk) that are not mediated through the exposure (39). Egger method explores whether the intercept is significantly different from zero (40). The intercept estimate is interpreted as the average pleiotropic effect across all instrument variables used. An intercept estimate, significantly different from zero indicates directional pleiotropy. However, as the MR-Egger regression requires at least three genetic variants, it was only used for PUFAs which showed a significant association and had at least three IVs (LA). For the PUFAs that were significantly associated with the outcomes, we also assessed whether a single dominantly influenced the results.

Table 1. The instrument variables for the PUFAs that were used for the MR study.

PUFA	CHR	Gene	SNP	A1*	A2	A1 Freq	P	Beta	SE	VE per allele (%)
Linoleic acid (LA)	10	NRBF2	rs10740118	G	C	0.56	8.1×10^{-09}	0.248	0.043	0.2–0.7
	11	FADS1	rs174547	C	T	0.32	5.0×10^{-274}	1.474	0.042	7.6–18.1
	16	NTAN1	rs16966952	A	G	0.31	1.2×10^{-15}	0.351	0.044	0.5–2.5
Arachidonic acid (AA)	11	FADS1	rs174547	T	C	0.68	3.3×10^{-971}	1.691	0.025	3.7–37.6
	16	NTAN1	rs16966952	G	A	0.69	2.4×10^{-10}	0.199	0.031	0.1–0.6
Alpha-linolenic acid (ALA)	11	FADS1	rs174547	C	T	0.33	4.0×10^{-64}	0.016	0.001	1.03
Eicosapentaenoic acid (EPA)	6	ELOVL2	rs3798713	C	G	0.43	2.0×10^{-12}	0.035	0.005	0.36
	11	FADS1	rs174538	G	A	0.72	5.0×10^{-58}	0.083	0.005	1.69
Docosapentaenoic acid (DPA)	6	ELOVL2	rs3734398	C	T	0.43	1.0×10^{-43}	0.04	0.003	0.46
	11	FADS1	rs174547	T	C	0.67	4.0×10^{-154}	0.075	0.003	2.74
	2	GCKR	rs780094	T	C	0.41	9.0×10^{-09}	0.017	0.003	8.38
Docosahexaenoic acid (DHA)	6		rs2236212	G	C	0.57	1.3×10^{-15}	0.113	0.014	0.65

A1*-PUFA increasing allele	A2 - other allele	SE - standard error
VE- variance explained	IV - instrument variable	A1 Freq - frequency of PUFA increasing allele

Next, to rule out the possibility of reverse causality for BCC and SCC on PUFAs, we conducted a reverse-MR analysis evaluating whether genetically predicted BCC and SCC risks are associated with each PUFA separately. We used TwoSampleMR package in R (35) for all statistical analyses.

Finally, we explored whether potential confounders such as body mass index (BMI), educational attainment, and vitamin D levels affected the associations between the PUFAs and BCC risk. We obtained IVs using publicly available published data for educational attainment (years and college completion; ref. 41) and BMI (42) and we tested whether the PUFA IVs were causally affected BCC through BMI, educational attainment, and vitamin D using the IVW approach described earlier. Vitamin D GWAS summary data were generated using the UK Biobank data (data field 100021) that included 401,529 participants of European ancestry. Age, sex, the first 10 principal components and monthly vitamin D variations were adjusted for using BOLT-LMM (43). SNPs with minor allele frequency of >1% and imputation score of 0.3 were then selected.
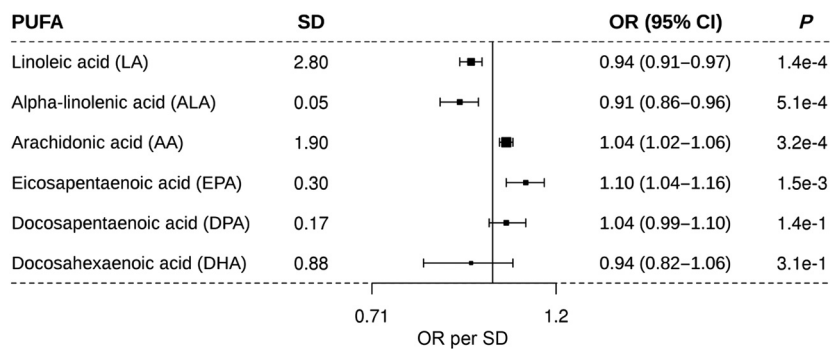
Results

Association between PUFA levels and BCC incidence

MR results for each of the six PUFA traits on BCC incidence are shown in **Fig. 1**. Briefly, among the six PUFAs evaluated, a one SD increase in genetically determined plasma levels of LA, and ALA was associated with a decreased incidence of BCC. Conversely, a one SD increase in genetically predicted levels of AA and EPA was associated with an increased incidence of BCC. However, genetically determined levels of DPA and DHA were not associated with BCC incidence.

Association of PUFA levels and SCC incidence

While the point estimates for the associations between each genetically predicted PUFA levels and the SCC incidence were similar to those of BCC, the 95% confidence intervals (CI) around these estimates were much wider, likely due to the smaller sample size for SCC (**Fig. 2**).

Sensitivity analyses

MR-Egger intercept regression results for LA (−0.005, −0.02 to 0.01; $P = 0.50$) and showed no evidence of directional pleiotropic effects on BCC risk through other pathways independent of serum LA. In a leave-one-out analysis the sensitivity analysis results did not differ materially from the primary results. No single SNP was strongly driving the overall effects of LA (**Fig. 3A**), AA (**Fig. 3B**), and EPA (**Fig. 3C**) on the BCC risk. There was no opposite causal association between PUFA levels and BCC risk; LA (OR = 0.95, 95% CI = 0.80–1.13, $P = 0.596$); ALA (OR = 1.00, 95% CI = 1.00–1.003, $P = 0.935$); AA (OR = 0.99, 95% CI = 0.88–1.12, $P = 0.851$) and EPA (OR = 1.00, 95% CI = 0.98–1.02, $P = 0.855$).

Our assessment for violations of the MR assumptions by body mass index, education attainment, and vitamin D as a potential confounder revealed it was unlikely that PUFA IVs would affect the BCC risk through the aforementioned traits ($P > 0.05$ for all PUFAs; **Table 2**).

Discussion

We used a two-sample MR to investigate the association between specific genetically predicted PUFA levels and the incidence of BCC and SCC among people of European ancestry. Our findings suggest that people with genetically predicted high levels of LA and ALA have
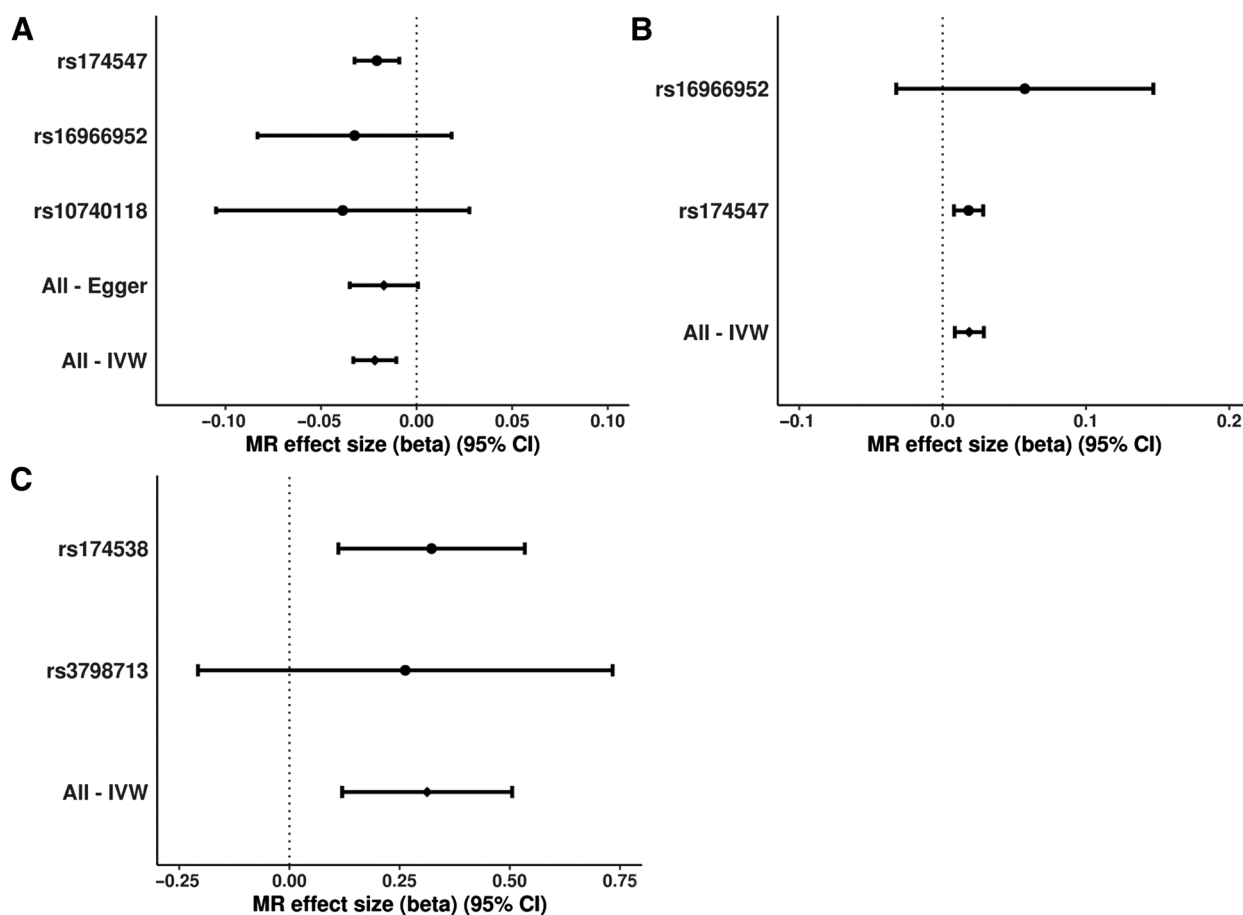
Figure 1.

Association of one SD increase in genetically determined levels of PUFA and the risk of BCC. PUFA, polyunsaturated fatty acid; SD, standard deviation; OR, odds ratio per SD; and 95% confidence interval. The middle line represents the null (OR = 1.00), and the error bars represent 95% CI. The figure shows the MR results for the relationship between the six PUFAs and the risk of BCC.

PUFA	SD	OR (95% CI)	P
Linoleic acid (LA)	2.80	0.94 (0.91–0.97)	1.4e-4
Alpha-linolenic acid (ALA)	0.05	0.91 (0.86–0.96)	5.1e-4
Arachidonic acid (AA)	1.90	1.04 (1.02–1.06)	3.2e-4
Eicosapentaenoic acid (EPA)	0.30	1.10 (1.04–1.16)	1.5e-3
Docosapentaenoic acid (DPA)	0.17	1.04 (0.99–1.10)	1.4e-1
Docosahexaenoic acid (DHA)	0.88	0.94 (0.82–1.06)	3.1e-1

0.71   1.2
OR per SD

PUFA	SD		OR (95% CI)	P
Linoleic acid (LA)	2.80		0.95 (0.90–1.00)	6.4e-2
Alpha-linolenic acid (ALA)	0.05		0.91 (0.83–1.00)	5.1e-2
Arachidonic acid (AA)	1.90		1.04 (1.00–1.07)	4.8e-2
Eicosapentaenoic acid (EPA)	0.30		1.07 (0.91–1.25)	4.0E-1
Docosapentaenoic acid (DPA)	0.17		1.02 (0.95–1.09)	5.4e-1
Docosahexaenoic acid (DHA)	0.88		1.07 (0.86–1.35)	5.4e-1

0.71   1.4

OR per SD

Figure 2.

Association of one SD increase in genetically determined levels of PUFA and the risk of SCC. PUFA, polyunsaturated fatty acid; SD, standard deviation; OR, odds ratio per SD; and 95% confidence interval. The middle line represents the null (OR = 1.00), and the error bars represent 95% CI. The figure shows the MR results for the relationship between the six PUFAs and the risk of SCC.

lower risks of BCC than those with lower levels of these dietary factors. In contrast, we found that people with high plasma levels of AA and EPA had elevated risks of BCC. Our analyses suggest that one n-3 PUFA is protective for BCC (ALA: OR = 0.91) while the other is associated with increased risk for BCC (EPA: OR = 1.10). Similarly, one n-6 PUFA is protective (LA: OR = 0.94) while another one increases the incidence of BCC (AA: OR = 1.04). These findings suggest that n-3 and n-6 PUFAs may act in opposing ways to influence the risks of BCC. Although results for SCC were overlapping with the null, the magnitude and directions of association for each PUFA were similar to those observed for BCC, but were subject to greater imprecision due to smaller sample sizes.

A recent systematic review and meta-analyses of observational studies reported that combined n-3 PUFAs (ALA, EPA, DHA) were not associated with the risk of BCC (pooled OR = 1.05, 95% CI = 0.86–1.28) and SCC (pooled OR = 0.86, 95% CI = 0.59–1.23; ref. 17). However, this finding is not surprising because all the n-3 PUFAs were considered together, yet our data and other studies suggest that these

Figure 3.

Forest plots of the individual SNP effects (log OR) for genetically predicted LA, AA, and EPA levels on BCC risk. This figure illustrates the contribution of individual IVs used for the association between LA (**A**), AA (**B**), EPA (**C**), and BCC risk. The error bars represent the individual SNP effects (beta or log OR) and the 95% CI derived using the IVW method or MR Egger for all SNPs and the Wald ratio method for instrumental variables. The vertical dotted line is the null (log OR). The figure shows that the results were not driven by a single IV.

Table 2. Causal relationship between the PUFAs and the potential confounders.

Exposure	Potential confounder	Beta*	95% CI		P
LA	BMI	0.0017	−0.0129	0.0163	0.8200
	EA	−0.0029	−0.0150	0.0093	0.6431
	VitD	−0.0011	−0.0126	0.0105	0.8587
AA	BMI	−0.0011	−0.0136	0.0115	0.8658
	EA	0.0012	−0.0022	0.0047	0.4833
	VitD	0.0021	−0.0033	0.0074	0.4516
ALA	BMI	0.1950	−0.0639	0.4538	0.1399
	EA	−0.1258	−0.4956	0.2440	0.5050
	VitD	−0.1805	−0.5196	0.1585	0.2966
EPA	BMI	−0.0392	−0.0844	0.0059	0.0885
	EA	0.0253	−0.0344	0.0849	0.4062
	VitD	0.0026	−0.1192	0.1244	0.9666
DPA	BMI	−0.0575	−0.1769	0.0620	0.3457
	EA	0.0270	−0.0333	0.0873	0.3802
	VitD	−0.0657	−0.4518	0.3204	0.7389
DHA	BMI	0.0080	−0.0267	0.0426	0.6527
	EA	−0.0088	−0.0608	0.0431	0.7389
	VitD	0.0332	−0.0018	0.0682	0.0628

Abbreviations: AA, arachidonic acid; ALA, alpha-linolenic acid; Beta*, beta coefficient for the effect; BMI, body mass index; CI, confidence interval; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EA, educational attainment; EPA, eicosapentaenoic acid; LA, linoleic acid; PUFAs, polyunsaturated fatty acids; VitD, vitamin D or 25-hydroxyvitamin D.

factors likely have heterogeneous effects on cancer development (12, 21). Another observational study found no association between any of the PUFAs with the incidence of BCC and SCC (18). However, findings from two observational longitudinal studies in the United States found that higher intake of both n-6 and n-3 fats was associated with higher risk of BCC (for n-3; HR = 1.08, 95% CI = 1.02–1.14; P_{trend} = 0.01 and for n-3; HR = 1.09, 95% CI = 1.04–1.13, P_{trend} < 0001; ref. 19). Higher intake of n-6 fat was significantly associated with increased risk of SCC (highest vs. lowest quintile, HR = 1.23, 95% CI = 1.08–1.41, $P = 5 \times 10^{-4}$; ref. 19). However, higher intake of n-3 fat was not associated with SCC risk (highest vs. lowest quintile, HR = 0.97, 95% CI = 0.87–1.10, P = 0.78; ref. 19).

Observational studies for dietary factors are prone to biases, especially from confounding and reverse causation (44). A recent RCT of 46 participants showed that supplementation of EPA+DHA given to lung transplant patients was not associated with the risk of KC (OR = 0.34, 95% CI = 0.09–1.32; ref. 45). However, this was a small RCT with very limited power to detect any meaningful associations. Second, participants followed for a short period (1 year), which is not applicable for slow growing tumors. Therefore, in absence of a well-conducted RCT, our MR study offers reliable findings to clarify results from observational studies.

Our results are comparable with other MR PUFA findings for other cancers previously published. They are similar to an MR of PUFA and prostate cancer risk among men < 62 years that revealed that LA (OR = 0.95, 95%CI = 0.92–0.98) and ALA (OR = 0.96, 95% CI = 0.93–0.98) were associated with a reduced risk of prostate cancer (21). In addition, conversely, 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) were associated with an increased risk of prostate cancer (21). A previous MR study also showed that LA (OR = 0.95, 95% CI = 0.93–0.98) and AA (OR = 1.05, 95% CI = 1.02–1.07) were negatively and positively, respectively, associated with the risk of colorectal cancer (12). A recently published MR on PUFA and the risk of melanoma found no significant associations between different PUFAs and melanoma

risk (24). However, the magnitude and direction of the associations (per SD increase in PUFA) are similar to our results for LA (OR = 0.94 for BCC vs. 0.94 for melanoma 95% CI = 0.86–1.02), ALA (OR = 0.91 vs. 0.92, 95% CI 0.82–1.03), and AA (OR = 1.04 vs. 1.03, 95% CI = 0.99–1.07). Therefore, our findings are comparable in terms of both the direction and the magnitude to previous PUFA MR results on prostate cancer, colorectal cancer and melanoma.

Possible biological mechanisms for carcinogenesis

The PUFA metabolic pathways and other biological routes to possible carcinogenesis are summarized in **Fig. 4**. Downstream metabolism of LA via metabolites GLA and DLA through desaturation (using fatty acid desaturase 2 (FADS2) and FADS1) and elongation (using elongase) results into AA and other eicosanoids. On the other hand, downstream metabolism of ALA via metabolites SDA and ETA using FADS2, FADS1, and elongase results in EPA and other eicosanoids. Several pathways in which PUFAs initiate carcinogenesis (which may apply to keratinocyte cancer) have been suggested. For example, it has been suggested that at high concentrations, LA stimulates tumoricidal actions through generation of free radicals within the cancer cell (12–15). It induces mitochondrial dysfunction and oxidative stress which lead to suppression of tumor cell growth and the eventual tumor cell apoptosis (12–15). It also stimulates production of prostaglandin E_1, an anti-inflammatory product (12, 14, 15). However, downstream metabolism of LA to AA leads to production of carcinogenic proinflammatory AA-derived eicosanoids like leukotriene B_4 through lipoxygenase; and prostaglandin E_2 and thromboxane A_2 through cyclooxygenases (12, 14). This potentially explains why AA is associated with increased risk of BCC in our findings. Downstream metabolism of ALA results in production of EPA-derived eicosanoids like leukotriene B_5 and prostaglandin E_3, which have anti-inflammatory actions that are carcinoprotective (12, 14, 15). However, in our study, EPA was associated with increased risk of BCC. This may be a true effect as is the case with prostate cancer EPA (OR = 1.04, 95% CI = 1.01–1.06; ref. 21). However, it is also possible that the results are influenced by residual confounding.
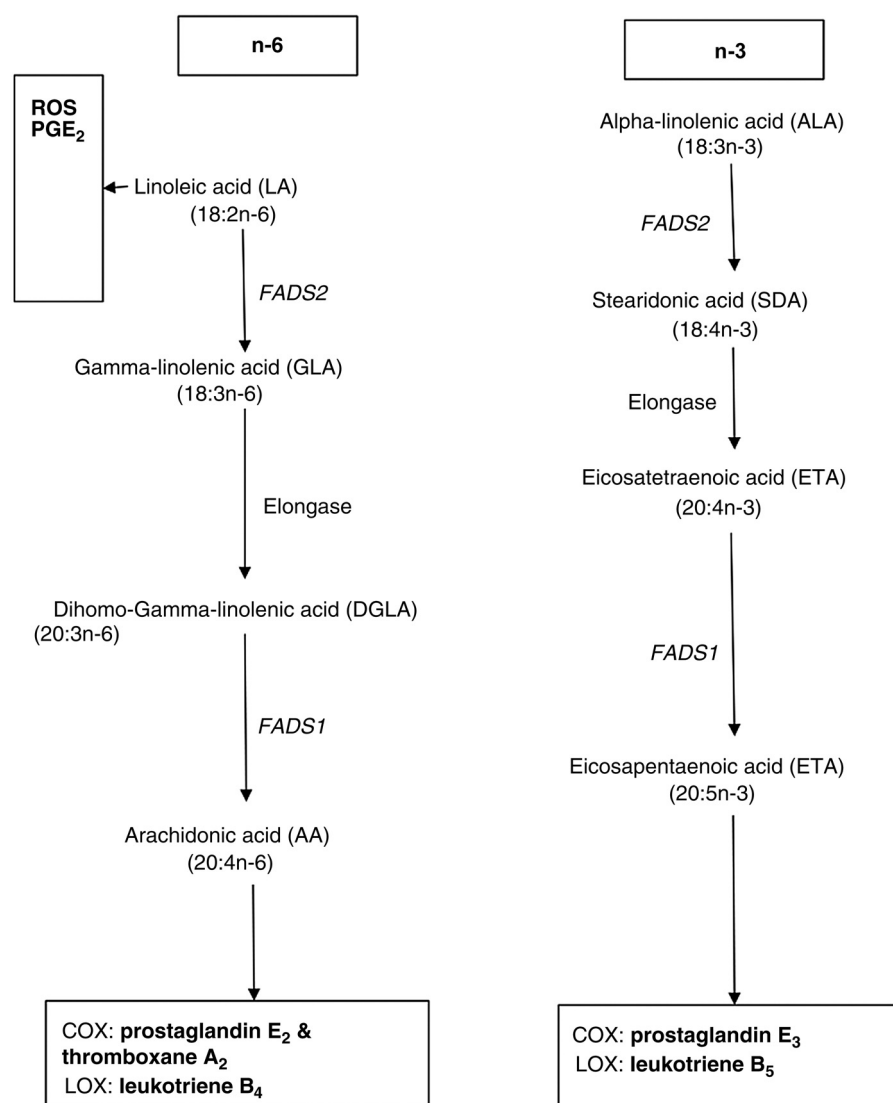
Figure 4.
The PUFA metabolic pathways and other biological routes to carcinogenesis. Schematic diagram showing downstream metabolism of essential fatty acids LA and ALA into proinflammatory and anti-inflammatory eicosanoids and free radicals, such as ROS. *LOX*, lipoxygenase; COX, cyclooxygenases (*COX-1* and *COX-2*); PGE, prostaglandin E; ROS, reactive oxygen species; *FADS2*, fatty acid desaturase 2; *FADS1*, fatty acid desaturase 1.

Strengths and limitations

Our study has several strengths. First, we used strong instrumental variables since they were genome-wide significant with the exposure (PUFA levels); and with some IVs explaining up to 30% of trait variance (**Table 1**). In addition, the F-statistic (a measure of the strength of the genetic instrument) for each PUFA was high i.e., AA (11, 302), EPA (479), LA (1, 104-3, 533), DPA (1, 997) and DHA (299) as indicated previously (21). Therefore, it is unlikely that our results were affected by bias from weak genetic instruments (36, 46). Thus, combination of strength IVs and the large BCC sample size, this allows great precision in our MR findings. Furthermore, we used the IVW method on summary statistics which gives results equivalent to individual level data (36). Our study also used data from the United States, Europe, and Australia and thus making our results broadly generalizable to people of European descent. Finally, MR overcomes reverse causation, a key source of bias in observational studies.

However, the findings have limited specific application to the clinical setting. For example, it is not clear what one SD of any of the PUFAs translates into in terms of diet change (food quantities). Nevertheless, it provides a broad perspective of the associations between specific PUFAs and BCC. Our MR findings pertain to people of European descent; thus, it remains unclear whether they can be generalized to other non-European ancestry populations. Our analysis involved 1–3 SNPs for the PUFAs (**Table 1**). Thus, we were able to assess directional horizontal pleiotropy for only LA using the MR-Egger method, and although it did not influence our MR estimates (39), we cannot rule out residual pleiotropic effects (including for other PUFAs). In addition, due to the limited number of PUFA SNPs, a multivariable MR approach (47) was not appropriate to assess highly polygenic potential confounders including; BMI, vitamin D, and educational attainment in the same model as this would introduce the regression dilution bias toward the null for the PUFA estimates (40). Nevertheless, the PUFA genetic instruments did not affect BCC through potential confounders; BMI, vitamin D, or education attainment (**Table 2**), and have been well studied and have known biology with PUFA metabolism (16, 25). While our findings for BCC were robust, the findings for SCC were less precise, mainly due to the much lower prevalence of the disease. "There is also a possibility for the results for AA and EPA being influenced by the levels of their metabolic precursors LA and ALA, respectively. For example, one

SD unit increase in ALA was found to increase EPA levels by 23% of one SD (16). However, it may not be the case that n-6 PUFAs influence n-3 PUFA results because the associations of *FADS1/2* and *ELOVL2* genes with EPA and DHA were found to be independent of LA levels in a previous study (16).

Clinical and public health implications and future research

This study provides meaningful insights on the possible benefits and risks of PUFA supplementation with respect to BCC and SCC. However, the findings are modest and future widespread RCTs using specific PUFA supplements are needed to understand if PUFAs influence BCC or SCC risk in a clinical setting. A recent pilot RCT that assessed the feasibility of PUFA supplementation (EPA+DHA) in lung transplant recipients showed 88% and 83% retention in the intervention and placebo groups respectively; as well as good adherence to the supplements (45). Therefore, an RCT on supplementation is feasible.

Conclusions

Genetically predicted levels of LA and ALA were causally associated with a reduced incidence of BCC, while AA and EPA were causally associated with an increased incidence of BCC. This MR study provides support for future RCTs to determine whether LA and ALA supplementation will practically reduce the risk of these very common cancers in the population. Thus, supplementation of LA and ALA might be useful in prevention of keratinocyte cancers in high risk groups such as organ transplant recipients.

Authors' Disclosures

Authors' Contributions

M. Seviiri: Conceptualization, data curation, software, formal analysis, investigation, visualization, methodology, writing–original draft, writing–review and editing. **M.H. Law:** Conceptualization, resources, data curation, supervision, funding acquisition, investigation, methodology, project administration, writing–review and editing. **J.S. Ong:** Investigation, methodology, writing–review and editing. **P. Gharahkhani:** Investigation, methodology, writing–review and editing. **D.R. Nyholt:** Investigation, methodology, writing–review and editing. **C.M. Olsen:** Investigation, methodology, writing–review and editing. **D.C. Whiteman:** Funding acquisition, investigation, methodology, writing–review and editing. **S. MacGregor:** Conceptualization, resources, supervision, funding acquisition, investigation, methodology, project administration, writing–review and editing.

Acknowledgments

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