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Title

Polyunsaturated fatty acids and risk of melanoma: A Mendelian randomisation analysis

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Key words

Mendelian randomisation, Polyunsaturated fatty acids, n-3 fatty acids, n-6 fatty acids, Melanoma

Abbreviations

MR - Mendelian randomisation

SNPs - Single nucleotide polymorphisms

GWAS - Genome-wide association studies

RCT - Randomised controlled trial

OR - Odds Ratio

CI - Confidence Interval

IVW - Inverse variance weighted method

PUFA - Polyunsaturated fatty acids

DPA - Docosapentaenoic acid

DHA - Docosahexaenoic acid

EPA - Eicosapentaenoic acid

ALA - α-linolenic acid

AA - Arachidonic acid

LA - Linoleic acid

UVR - Ultra-violet radiation

Article category

Cancer epidemiology

Novelty/Impact

Polyunsaturated fatty acids (PUFAs) have been proposed to play a role in the risk of various cancers. For cutaneous melanoma, observational epidemiological studies suggest an association with PUFAs but the evidence is inadequate. Hence, we conducted the first ever Mendelian randomisation study to assess if PUFA levels are causally related to melanoma risk. Our results suggest that the effect of PUFA levels on melanoma risk is either zero or very small.

Abstract

Melanoma is the deadliest form of skin cancer, mainly affecting populations of European ancestry. Some observational studies suggest that particular diets reduce melanoma risk - putatively through an increase in polyunsaturated fatty acid (PUFA) consumption. However, interpretation of these observational findings is difficult due to residual confounding or reverse causality. To date, a randomised controlled trial has not been carried out to examine the relationship between PUFAs and melanoma. Hence, we performed a Mendelian randomisation (MR) study to evaluate the link between PUFAs and melanoma.

To perform MR we used summary results from the largest risk genome-wide association study (GWAS) meta-analysis of melanoma, consisting of 12,874 cases and 23,203 controls. As instrumental variables we selected SNPs associated with PUFA levels from a GWAS meta-analysis of PUFA levels, from the CHARGE consortium. We used the inverse variance weighted method to estimate a causal odds ratio. To aid interpretation, we established a benchmark "large" predicted change in PUFAs in which, for example, an increase in docosahexaenoic acid (DPA) of 0.17 units (equal to 1 standard deviation) moves a person from the 17th percentile to the median.

Raising PUFA levels by a large amount (increasing DPA by 0.17 units) only negligibly changed melanoma risk - Odds Ratio [OR] = 1.03 (95% Confidence Interval [CI] = 0.96 - 1.10). Other PUFAs yielded similar results as DPA. Our MR analysis suggests that the effect of PUFA levels on melanoma risk is either zero or very small.

Introduction

Melanoma, the most aggressive form of skin cancer, has an incidence of approximately 132,000 cases worldwide each year[1]. Melanomas arise due to malignant transformation of melanocytes, the cells responsible for pigmentation of the skin. Global incidence of melanoma is continuously rising, leading to a significant burden on health care systems[1]. Currently, the risk factors for melanoma are incompletely understood. Therefore, it is crucial to discover the role of modifiable risk factors on melanomagenesis to strengthen primary prevention strategies, allowing early intervention and subsequently reducing mortality, morbidity and health care costs.

The aetiology of melanoma is complex. Fair skin, red hair[2], a higher number of atypical naevi, a tendency to freckle[3], intermittent or increased exposure to ultra-violet radiation (UVR)[4-6] and a family history of melanoma[7] are well known risk factors. Exposure to UVR is the principal environmental risk factor for cutaneous melanoma[8] and UVR induces the vast majority of melanoma-initiating somatic mutations[9]. One of the biological pathways through which UVR is hypothesized to promote melanomagenesis is by immunosuppression[8]. Recent research has shown that dietary modification has potential to mitigate UVR-induced immunosuppression[10]. For example, a recent randomised controlled trial (RCT) has shown that n-3 polyunsaturated fatty acid (PUFA) supplementation has a beneficial effect on skin immunity and reduces the harmful effects from sun damage[11]. We therefore hypothesized that nutrition may play a role in melanoma risk.

Identification of nutritional interventions is potentially of high importance as they can easily be integrated into primary prevention[12]. Of particular interest is the Mediterranean diet, which includes abundant consumption of legumes, vegetables, fruits, cereals, and olive oil, a moderate intake of fish and alcohol (mostly wine), a moderate to low intake of dairy products, and a low intake of processed meat[13]. A traditional Mediterranean diet is protective for all-cause mortality[14] and many individual diseases**,** including prostate cancer [15], colorectal cancer[16], Alzheimer's disease[17], coronary heart disease[18], diabetes mellitus[19], Parkinson's disease[20] .

It has been proposed that the protective effect on cancers is related to the optimal balance between n-6 and n-3 fatty acids in the Mediterranean diet[21, 22] These n-3 and n-6 fatty acids reduce tumour growth by inhibiting cell growth, apoptosis, angiogenesis and inflammation [23, 24]. Observational studies have discovered that higher levels of dietary n-3 fatty acid intake are inversely associated with melanoma risk[25-27]. A hospital-based case-control study conducted in Italy showed that weekly consumption of fatty fish that was rich in n-3 fatty acids was associated with a reduced risk of melanoma (Odds Ratio [OR] = 0.52, 95% Confidence interval [CI] = 0.34–0.78). A study performed on a mouse model identified that Eicosapentaenoic acid (EPA) (n-3 fatty acid) protected against UVR induced carcinogenesis [28]. *In vitro* experimental studies have shown that EPA and DHA (n-3 fatty acid) inhibit proliferation of cultured human melanoma cells[29]. Collectively, these results suggest that modifying PUFAs may have a role in reducing melanoma incidence.

Although observational studies make a significant contribution to the field of medical research, outcomes from many such studies have failed to validate in randomised controlled trials[30-33]. This lack of concordance is likely due to confounding and/or reverse causation in observational studies[34]. Confounding occurs when an unmeasured risk factor/variable is associated with both the measured risk factor and outcome, distorting the true association of the measured risk factor and outcome. Reverse causation masks the true effect by causally relating the outcome to the risk factor. Although the RCT approach is the gold standard for accessing causality, their use is frequently constrained by ethical and practical issues of administering some interventions. Further, performing a RCT may be time consuming; sometimes it is a challenge to retain the participants until the end of the study and bias may be introduced if participants do not adhere to the intervention.

Mendelian randomisation (MR) is a method in which genetic variants, usually single nucleotide polymorphisms (SNPs), are used to test whether a modifiable exposure (risk factor) is causally related to an outcome (disease). Furthermore, the magnitude and direction of any causal relationship can be determined. MR can be regarded as a “natural” RCT, in that genetic variants are used as instrumental variables for the risk factors in order to infer whether the risk factors are causal for the disease[35]. The random allocation of alleles during meiosis is conceptually similar to a RCT design, and is independent of confounding from environmental exposures. Reverse causation is avoided by the unidirectional flow from

gene to phenotype to disease; the disease cannot alter the gene. MR also has some advantages over RCTs in terms of ethical issues, feasibility, time and cost.

MR makes three key assumptions, and violations of these assumptions will lead to biases. Firstly, there should be strong evidence for the association of the genetic instrument with the risk factor **(strong instrument assumption)**. Secondly, any confounding variables which are associated with risk factor and outcome should not be associated with the genetic variant **(independence assumption)** The third assumption is that the outcome/disease of interest is only associated with the genetic instrument through the risk factor/exposure of interest which acts as a proxy **(exclusion restriction assumption)** (Figure 1)[34].

We performed MR to explore the possible causal relationship between genetically predicted PUFA exposures and melanoma. In order to draw strong conclusions using MR, power must be high; the size of our large melanoma GWAS and the high proportion of variance explained by SNPs associated with PUFA suggests our study is highly powered for MR. We calculated the power using mRnd software[36]. With an OR of 0.52 for the observational study result, and using the instrument with the least variance explained (0.65% variance, rs2236212 for DHA) the power was 99%. Unusually among complex traits, the few SNPs associated with PUFA levels explain a large proportion of the variance in the trait. For example, SNP rs174547 explains 8.6% of variance in docosapentaenoic acid (DPA) [37] and 32.6% of variance in arachidonic acid (AA)[38, 39]. The different PUFAs share a common metabolic pathway (Figure 2) and SNPs known to influence one PUFA typically also have strong effects on the others. We examined the causality of melanoma using n-3 fatty acids docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), α-linolenic acid (ALA) and n-6 fatty acids linoleic acid (LA) and arachidonic acid (AA).

Methods

Study population

We used summary data from the largest melanoma risk GWAS meta-analysis published to date (in 2015), including 12,874 cases and 23,203 controls from Australia, USA and Europe[2]. Details of the study population and GWAS quality control measures have been described previously[2].

Instrumental variables

SNP effect sizes for plasma phospholipid n-3 and n-6 fatty acids levels were derived from a study of 8,866 individuals of European ancestry from the cohorts for heart and aging research in genomic epidemiology (CHARGE) consortium[37, 39]. DPA was selected as an example to illustrate the MR analysis and interpret the results, with other PUFAs considered subsequently (Table 1). The genes *FADS1* and *FADS2* (desaturates) on chromosome 11, *ELOVL2* (elongase) on chromosome 6, and *GCKR* (glucokinase regulator) on chromosome 2 have been associated with the regulation of DPA metabolism (Supplementary Table 6)[37, 40]. The most significant SNP, rs3734398 in *ELOVL2* (allele C), is associated with higher levels of EPA, higher levels of DPA (C), and lower levels of DHA. rs174547 (allele C), in the *FADS1* gene is associated with higher ALA, and lower levels of EPA, and DPA. rs174547(C) is also associated with higher levels of LA and lower levels of AA. Furthermore, rs780094 in the *GCKR* gene is associated with higher levels of DPA (allele T)[37]. However, rs780094 exhibits considerable pleiotropy (Supplementary Tables 1-5) and is also associated with melanoma ($P = 1.3 \times 10^{-2}$; Supplementary Table 13). As this is likely to violate the assumptions underlying MR we have excluded this SNP from the set of instrument variables used.

These two SNPs (rs3734398, and rs174547) were used as instrumental variables in our analysis of DPA. Given the correlations between DPA and the other PUFAs, most of these SNPs were commonly used with other PUFAs as instrumental variables - the specific SNPs are listed in (Supplementary Tables 6 and 13). All variants selected exceeded the genome-wide significant threshold ($P < 5 \times 10^{-8}$) for their association with each PUFA, satisfying the strong instrument criteria and were not in LD with each other ($r^2<0.1$).

MR analysis

R version 3.3.3 was used for the main analysis. Additionally, MR analyses for the confounding traits (FBS, BMI, height) were performed using MR-Base [41].

Statistical Analysis

Two sample Mendelian randomisation analysis was performed using summary data from two different studies. Both studies were comprised of populations of European ancestry. The causal inference on melanoma risk by PUFAs was established using Wald-type estimator ratio method for individual SNP instruments (Table 1). For each SNP the Wald-type estimator divides their effect on the outcome by their exposure effect size; this allows the

resultant effect sizes across multiple SNPs to be meta-analysed (IVW method) to obtain a single causal effect estimate. (Figure 3) [35].

Relationship between PUFAs and possible confounding factors

PUFA levels are associated with other potential confounding factors. We focused on height, based on the linking evidence of height associated with melanoma[42]. If a genetic increase in PUFA levels leads to a proportional increase in height but the converse is not true (i.e. genes influencing height have no clear effect on PUFA levels) then this is consistent with a causal relationship of PUFA levels on height. We drew scatter plots with R version 3.3.3 to visualize the correlation in per SNP effect sizes between PUFAs and height. Our aim was to assess if changes in height is more likely to be the cause or the consequence of changes in PUFAs (or if they are inter-related). Firstly, publicly available genome-wide significant PUFA SNPs were selected ($P < 5 \times 10^{-8}$; pruned for linkage disequilibrium $r^2 < 0.1$; DPA 61 SNPs, EPA 39 SNPs, ALA 32 SNPs, DHA 6 SNPs, LA 197 SNPs, AA 200 SNPs) and we plotted their PUFA effect sizes against height effect sizes. We then performed the reverse using SNPs which were genome-wide significantly associated with height.

Results

Mendelian Randomisation analysis of DPA

The MR analysis results of the association between DPA concentration and melanoma are shown in Table 1. The estimated magnitude of association between DPA level and melanoma was performed firstly for each individual SNP using the (Wald type estimator ratio method)[43]. Subsequently a meta-analysis was performed, combining each individual Wald type estimator ratio, weighted in inverse proportion to its variance (Figure 3). The results are expressed in terms of a “large change” (0.17 units = one standard deviation in DPA). This change was predicted to only negligibly increase melanoma risk (OR = 1.03, 95% CI = 0.96–1.10).

Mendelian Randomisation analysis of non-DPA PUFAs

The results obtained for the magnitude of association between other PUFAs and risk of melanoma is illustrated in Table 2. We obtained similar results for all the PUFAs in our study. This is unsurprising as there is overlap between the set of SNPs influencing the measures of

different PUFAs. The results indicate no association between any PUFA and melanoma risk. In each case we considered a "large change" in each trait (1 SD change): For ALA with a 0.05 unit change (OR = 0.92, 95% CI = 0.82–1.03), for EPA 0.3 unit change (OR = 0.92, 95% CI = 0.82–1.04) and DHA for 0.88 unit change (OR = 1.16, 95% CI = 0.90–1.49). The results for n -6 fatty acids were: LA with 4 units change (OR = 0.94, 95% CI = 0.86–1.02) and AA with 1.9 units change (OR = 1.03, 95% CI = 0.99–1.07).

Checking for violations of MR assumptions

Validation of instrument strength

How each poly-unsaturated fatty acid is converted into the next metabolite in the bio synthesis pathway is illustrated in figure 2[44]. These fatty acids cannot be synthesized in the human body, hence are sourced from the diet. ALA goes through a sequence of reactions forming EPA, DPA and DHA. This process is catalyzed by elongases (encoded by the *ELOVL2* gene) and desaturases (encoded by the *FADS1* and *FADS2* genes). Similarly, conversion of n-6 fatty acids LA to AA is also regulated by the same enzymes (Figure 2). The SNPs used as our genetic instruments are in or near the genes that encode the rate-limiting enzymes for fatty-acid conversion. Thus each of the genetic instruments we employ in our study to find the association between PUFA and melanoma directly regulates some aspect of the PUFA metabolism. This suggests the robust association of the instrumental variables to the relevant PUFAs. Furthermore, we selected SNPs for use as instrumental variables from the largest PUFA GWAS performed so far, investigating levels of n-3 fatty acids EPA, DPA, DHA and ALA (Supplementary Table 6). SNPs used as instrumental variables for n-6 fatty acids (AA, LA) were derived from a large scale meta- analysis of GWAS performed from CHARGE consortium data[39]. All SNPs chosen as instrumental variables were associated with the relevant PUFA at the level of genome-wide significance ($P < 5 \times 10^{-8}$) - this is more stringent than the traditional MR criteria for a strong instrument (F-statistic > 10)[38, 45]. Furthermore, we used multiple genetic variants combined as instrumental variables instead of using individual genetic variants to assess causality. This explained more variance than using single instrumental variables. As an example, for DPA, if we use rs174547 as a single instrumental variable, it would explain 8.3% of the variance in DPA. When we use rs174547 and rs3734398 together as an instrumental variable, they explain 11.1% of the variance in

DPA[38]. For each PUFA, these combined instruments clearly satisfy the usual criteria for strong instruments in MR.

Population Stratification

One potential cause of violations of the exclusion restriction assumption and the independence assumption is population stratification. In our study both exposure and outcome population consist of participants from ethnically homogenous population (European ancestry). For the melanoma GWAS meta-analysis, principal components (PCs) were used to remove ancestry outliers as well as to model subtle stratification effects by including them as covariates in the association analysis. After including these PCs, the genomic inflation factor was minimal (1.03)[2]. Similarly, population substructure control using PCs has been performed in the CHARGE consortium data used to identify the instrumental variable SNPs for the PUFAs[37].

Pleiotropy assessment

If the genetic instrument is pleiotropic (has more than one phenotypic effect) and any of the secondary phenotypes modify the outcome, this violates the MR assumptions and we cannot be certain about the reliability of our findings. These pleiotropic associations may either introduce false positive associations or mask the true causal effect estimate of the exposure on the outcome. Firstly, potential pleiotropic associations of the various genetic instruments were investigated by searching the literature[46].

Then we examined previous GWAS findings to identify associations between the SNPs used as instrumental variables and potential biological and socioeconomic confounding factors including BMI, height, educational attainment, waist circumference and fasting blood sugar (FBS) – (Supplementary Tables 1 to 5). Some SNPs showed associations with height, BMI and FBS after Bonferroni correction (Supplementary Tables 1 to 5). We calculated the causal effect estimate using the SNPs which are not associated with confounding factors (rs3734398 for DPA and rs3798713 for EPA). When this is done our conclusions regarding the effect of PUFAs on melanoma are unchanged; For DPA a 0.17 unit change confers a causal OR close to 1 (OR = 0.93, 95% CI = 0.81–1.06). Similarly for EPA for a 0.30 unit change (OR = 0.88, 95% CI = 0.67–1.16). Furthermore, the SNPs associated with confounding factors do not show any association with melanoma except rs174538 (Supplementary Table 13).

We conducted a supplementary analysis, to identify how these putatively confounding factors (BMI, height, educational attainment, waist circumference and fasting blood sugar) may influence the association of PUFA levels and melanoma. We first looked at height. Using an inverse weighted method we found that a 10cm increase in height was associated with a small but significant increase in the risk of melanoma (OR= 1.08, 95% CI= 1.01–1.16, Supplementary Figure 15). It is hence possible that SNPs which increase height through a pathway independent of PUFAs may affect melanoma risk, violating our MR assumptions. We investigated whether our selected IV SNPs affect height only via changes in PUFAs by generating scatter plots of the SNPs effect sizes to assess the likely causal pathway (Methods). For height the SNPs which are associated with DPA had an effect on height (P = 2.1×10^{-3}, r^2 = 0.15), EPA (P = 2.9×10^{-2}, r^2 = 0.13) and LA (P = 1.8×10^{-4}, r^2 = 0.09) (Supplementary Figures 01, 03, 09). Conversely, the reverse was not true where the SNPs which are associated with height did not show a strong effect on PUFAs (DPA: P = 0.2, r^2 = 0.003; EPA: P = 0.89, r^2 = 2.8×10^{-5}; LA: P = 0.96, r^2 = 3.3×10^{-6}; Supplementary Figures 02, 04, 10). These results suggest that height shows vertical pleiotropy, with changes in DPA (and EPA, LA) causing changes in height. Conversely, for ALA a bidirectional association was observed; changes to ALA had consequential effects on height (P = 3.8×10^{-3}, r^2 = 0.25, Supplementary Figure 05) and vice versa (P = 2.3×10^{-2}, r^2 = 0.008, Supplementary Figure 06). Hence for ALA (but not for DPA, EPA and LA), it is possible that some of our SNP instruments affect melanoma risk through a pathway which is independent of the putative pathway through ALA. However, the SNPs effect on height is tiny (which are only significant due to the very large sample sizes in the height GWAS), and combined with the small effect of changes in height on melanoma (OR=1.08 per 10cm increase in height), it is very unlikely that this would lead to a violation of the MR assumptions to any substantial extent.

We then examined whether the SNPs associated with fasting blood sugar (FBS) may violate the MR assumptions. We first examined the relationship between FBS and melanoma by performing a MR analysis with GWAS significant FBS SNPs on melanoma risk. We found there was no causality observed for FBS on melanoma risk (OR = 1.23, 95% CI = 0.77–1.95; inverse variance weighted method) (Supplementary Figure 13). It is hence unlikely that the effect the SNP IVs have on FBS has any bearing on melanoma risk.

We then considered BMI. As for FBS, we found that BMI was not causally related to melanoma risk (OR= 1.03, 95% CI = 0.88–1.21) (Supplementary figure 14). It is hence unlikely that the effect the SNP IVs have on BMI is relevant to melanoma risk. Finally, although we cannot be certain regarding unmeasured confounders, it is unlikely that the genetic variants we selected affect some unmeasured trait which subsequently affects melanoma via pleiotropy.

Discussion

There has been much work done on the potentially causal role of modifiable risk factors on cancer. Diet is particularly attractive, as proven causal links would motivate the adoption of relatively easily integrated life style changes. Observational epidemiological studies conducted to date have not provided clear guidance on the role of PUFAs in melanoma risk. Hence, we explored the causal association of PUFA levels with melanoma risk using a Mendelian randomisation approach. If we take DPA as an example, our results identified that a very large (0.17 unit change - 1 SD) increase in DPA levels had little or no effect on the risk of melanoma (OR = 1.03, 95% CI = 0.96 –1.10). Hence, it is unlikely that DPA (or any other PUFAs) play an important role in determining the risk of melanoma. Usually null association results are considered stronger than positive results in MR because while a positive result can be driven by an unmeasured confounder, this is less likely to occur with a negative finding. To get a negative result solely due to confounding effects would require a similar magnitude of both (true) positive and negative confounding effects (which cancel the effect estimates on each other out exactly), which is very unlikely [47].

Our result is inconsistent with the observation of Fortes *et al.,* in which consumption of fish containing high n-3 was found to be protective for melanoma (OR = 0.52, 95% CI = 0.34–0.78)[25]. There are several possible reasons the results from the two studies are inconsistent. Unlike MR studies, these observational study findings are susceptible to confounding effects (or incomplete correction for confounding), such as socio-economic status and sun exposure. Furthermore, the hospital-based case control study data in the study by Fortes *et al.* were collected retrospectively using questionnaires. It is likely that information biases

such as recall bias and imperfect measures on food portion size adversely affected their results.

The complex metabolic pathways of these PUFAs, their inter-relations, and the influence of different SNPs are well understood (Figure 2). In MR analysis validity of the causal inference is determined by satisfying the prior mentioned assumptions. Our chosen genetic variants have well-established specific roles in the PUFA biological pathway, making them highly suitable instruments for MR analysis. The main strength of our study was the very large sample size used for analyses. We derived the causal effect estimates of SNPs and the outcomes from the largest melanoma risk GWAS to date[2]. Similarly, we had causal effect estimates for the SNP-risk factor association from the largest PUFA GWAS to date, conducted using 8,826 individuals. We used two different samples to generate the summary data for our analyses. Using a two sample MR approach has an advantage over one sample MR, because effect estimates are more accurately measured than from a single study because of the larger sample sizes which leads to increase the statistical power[48]. One of the limitations in the MR approach is that it requires a large sample size because most genetic instruments explain very little of the variation in the exposure of interest. However, the instrument used here explained relatively large amounts of the variance in some of the PUFA levels (AA = 33.1%, LA = 8.3-21.3%). Moreover, we used independent SNPs combined together as instrumental variables, rather than a single variant, which further increased the variance explained and thus the statistical power to discern the true relationship between exposure and outcome.

One of the limitations of our study is that we cannot rule out the possible effect of other, unmeasured confounders. Although we tested the SNP instruments to check for potential pleiotropic effects for BMI, height, level of education, waist circumference and fasting glucose level, there may be potentially confounding effects from other variables, such as UV exposure, number of atypical moles and phenotypes (hair, eye and skin colour), for which we did not have data. Presence of directional pleiotropy can be identified using MR-Egger regression and drawing a funnel plot[43] although in our case we did not have enough SNP instruments for such approaches to be informative. Most genetic instruments explained a high proportion of the variance for the trait, for some PUFAs a smaller fraction of variance (ALA = 1.0%, DHA = 0.7%, EPA = 2.1%) was explained, reducing our power to detect small

effects of these PUFAs on melanoma risk in our MR framework. Furthermore, identifying the effects of individual PUFAs on melanoma risk was difficult due to shared instrumental variables among the PUFAs.

Conclusion

We used multiple SNP genetic instruments to examine the effect of raising PUFA levels (percentage of total fatty acids). Even large changes in genetically determined PUFA levels were not found to be associated with melanoma risk. Whilst some observational studies have suggested that the Mediterranean diet reduces the risk of melanoma, our results from analysing one constituent of this diet – PUFAs - suggest that the effect of increased PUFA levels on melanoma risk are either zero or very small. We used an analytically robust MR approach, which negates the issues of residual confounding and reverse causality, two issues that adversely affect the interpretation of results from observational studies.

Thus, we therefore conclude that any protective role of the Mediterranean diet on melanoma risk is not due to PUFAs. Further studies are needed to explore the causality of other components of the Mediterranean diet for possible effects on melanoma risk.

Acknowledgements

Acknowledgements are in the supplementary material**.**

Conflict of interest

The authors have declared that no conflicts of interests exist.

Tables

Table 1: **Mendelian randomisation results: DPA concentration and melanoma**

SNP	Gene	CHR	EA/NEA	R^2	β DPA	σ DPA	β melanoma	σ melanoma	EAF	β IVW	σ IVW
rs174547	*FADS1*	11	T/C	8.4%	0.075	0.0028	0.027	0.018	0.67	0.36	0.24
rs3734398	*ELOVL2*	6	C/T	2.8%	0.040	0.0029	-0.017	0.017	0.43	-0.42	0.43
Combined				11.2%						0.17	0.21

EA - Effect allele, NEA - Non-effect allele, R^2 – Percentage of variance of DPA explained by the SNP(s), β DPA - Magnitude of the association of SNP(s) and modifiable exposure (DPA). σ DPA - Standard error of the magnitude of association between SNP(s) and DPA, β melanoma - Magnitude of the association between SNP(s) and outcome (melanoma), σ melanoma - Standard error of the magnitude of the association between SNP(s) and melanoma, EAF - Effect allele frequency, β IVW - in log (OR) scale , magnitude of association between DPA and melanoma (for a 1unit of DPA change), σ IVW - Standard error of the magnitude of association between DPA and melanoma. Note: β DPA estimates were directly taken from Lemaitre *et al*., [37] and β melanoma estimates were taken from Law *et al*., [2] The percentage variance of DPA explained were obtained from Khankari *et al*., [38]

Table 2: Mendelian randomisation results: PUFA and melanoma

Trait	Scale	OR	95%CI
LA	4	0.94	0.86–1.02
AA	1.9	1.03	0.99–1.07
ALA	0.05	0.92	0.82–1.03
EPA	0.30	0.92	0.82–1.04
DPA	0.17	1.03	0.96–1.10
DHA	0.88	1.16	0.90–1.49

Scale - Units of PUFA change which is equal to 1SD deviation in CHARGE cohort, DPA - Docosapentaenoic acid, DHA - Docosahexaenoic acid, EPA - Eicosapentaenoic acid, ALA - α - linolenic acid, AA - Arachidonic acid, LA - Linoleic acid

Figure Legends

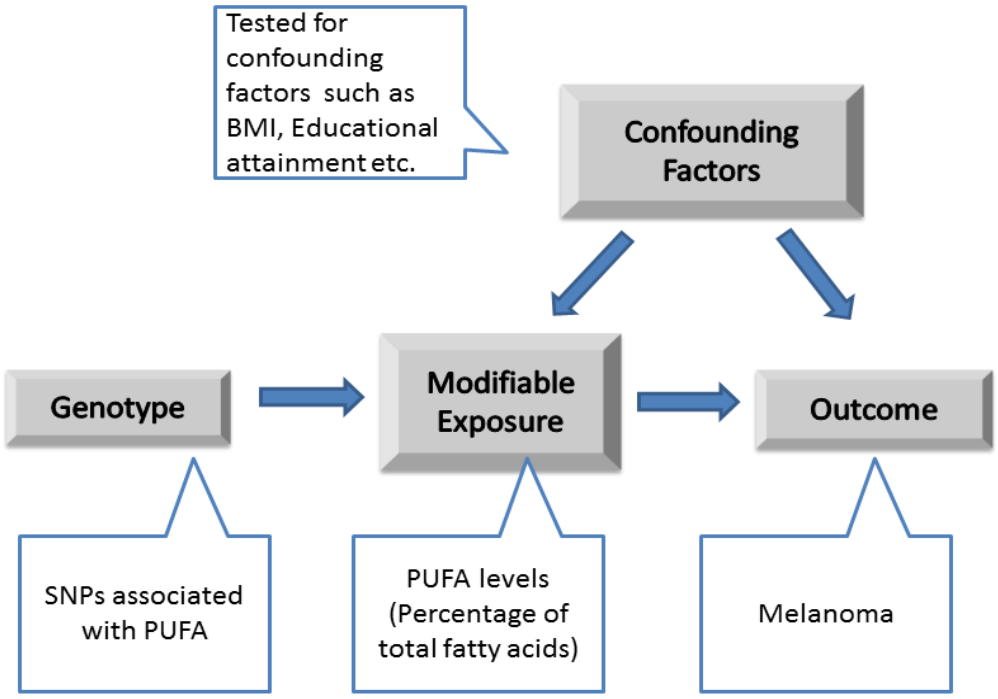

Figure 1: Directed Acyclic Graph (DAG) depiction of our study of PUFA and risk of melanoma

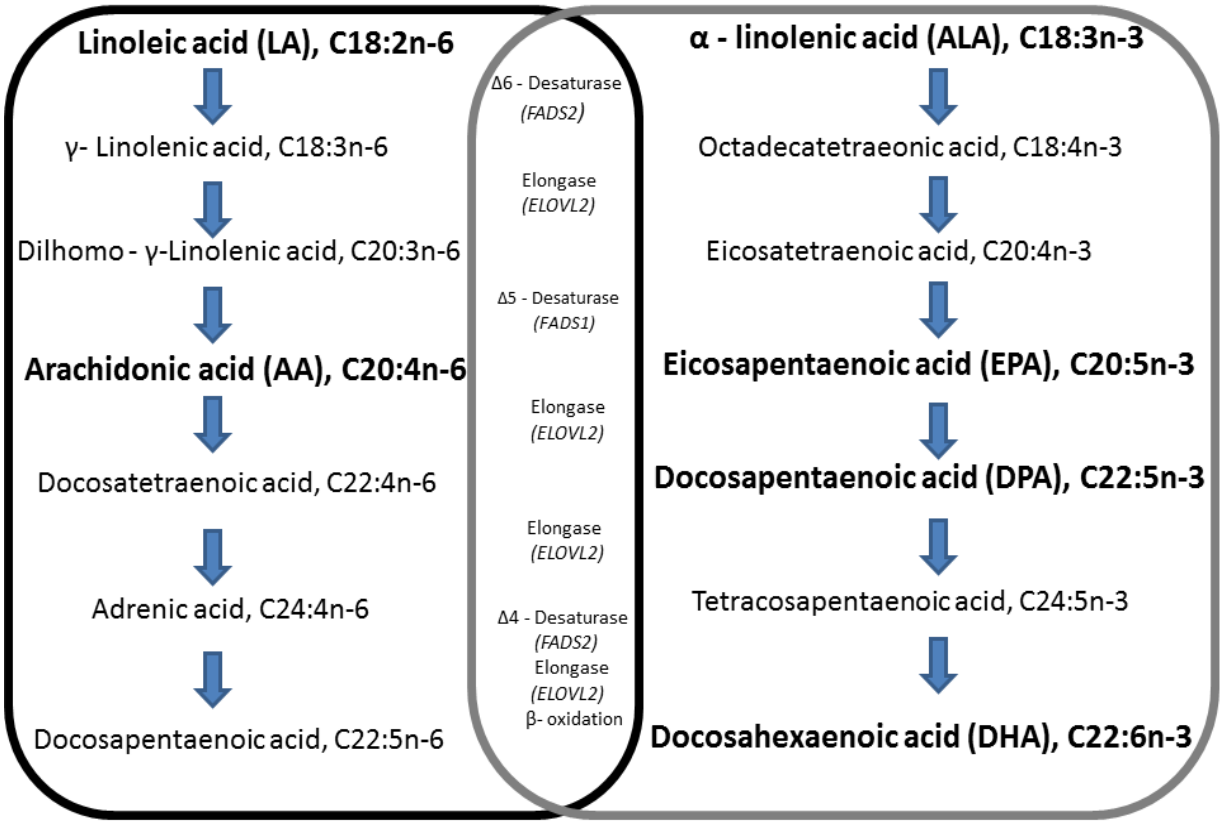

Figure 2: PUFA metabolic pathway, annotated with loci associated with PUFA metabolism: n-6 rounded rectangle (left), n-3 grey rounded rectangle (right)

$$\hat{\beta}_{IVW} = \frac{\sum_z \hat{\beta}_{zx}\,\hat{\beta}_{zy}\,\sigma_{zy}^{-2}}{\sum_z \hat{\beta}_{zx}^{2}\;\sigma_{zy}^{-2}}$$

$$\sigma_{IVW} = \sqrt{\frac{1}{\sum_z \hat{\beta}_{zx}^{2}\,\sigma_{zy}^{-2}}}$$

Figure 3: Equation - Inverse variance weighted method[35]

$\hat{\beta}_{IVW}$ - Estimated magnitude of effect of the modifiable exposure (PUFA) on outcome (melanoma)

σ_{IVW} - Standard deviation of the effect of the modifiable exposure on outcome

$\hat{\beta}_{zx}$ - Estimated magnitude of the effect of the instrumental variables (SNP(s)) on the modifiable exposure

$\hat{\beta}_{zy}$ - Estimated magnitude of the effect of the instrumental variables on outcome

σ_{zy} - Standard deviation of the effect of the instrumental variables on outcome

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