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Causal association of plasma *n*-3 PUFA with peptic ulcer disease: a two-sample Mendelian randomisation study

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Abstract

Dietary *n*-3 PUFA may have potential benefits in preventing peptic ulcer disease (PUD). However, data from observational epidemiological studies are limited. Thus, we conducted a Mendelian randomisation analysis to reveal the causal impact of *n*-3 PUFA on PUD. Genetic variants strongly associated with plasma levels of total or individual *n*-3 PUFA including plant-derived α-linolenic acid and marine-derived EPA, DPA and DHA were enrolled as instrumental variables. Effect size estimates of the *n*-3 PUFA-associated genetic variants with PUD were evaluated using data from the UK biobank. Per one SD increase in the level of total *n*-3 PUFA in plasma was significantly associated with a lower risk of PUD (OR = 0·91; 95 % CI 0·85, 0·99; $P = 0·020$). The OR were 0·81 (95 % CI 0·67, 0·97) for EPA, 0·72 (95 % CI 0·58, 0·91) for DPA and 0·87 (95 % CI 0·80, 0·94) for DHA. Genetically predicted α-linolenic acid levels in plasma had no significant association with the risk of PUD (OR = 5·41; 95 % CI 0·70, 41·7). Genetically predicted plasma levels of *n*-3 PUFA were inversely associated with the risk of PUD, especially marine-based *n*-3 PUFA. Such findings may have offered an effective and feasible strategy for the primary prevention of PUD.

Keywords: ***n*-3 PUFA: Plasma level: Peptic ulcer: Mendelian randomisation: Causal relationship**

Peptic ulcer disease (PUD) refers to an acid peptic injury of the gastrointestinal (GI) tract and is mostly located in the stomach or proximal duodenum[1]. Inflammation plays a critical role in the pathophysiology of PUD, which can be caused by *Helicobacter pylori* (HP) infection, consumption of alcohol and use of non-steroidal anti-inflammatory drugs[1,2]. Over the past decades, there has been a rapid decrease in the incidence of PUD, which was partly attributed to the prescribed use of anti-acid drugs and diminished prevalence of HP infection[3,4]. However, the increasing antibiotic resistance of HP and the widespread use of non-steroidal anti-inflammatory drugs in the elderly population may have brought great challenges in preventing PUD[5,6]. Therefore, the identification of more effective approaches to primary prevention of PUD is of paramount importance.

Polyunsaturated fatty acids (PUFAs) have been proposed as important dietary components for long-term health, especially for *n*-3 PUFA that have raised growing concerns. Food *n*-3 PUFA principally include plant-derived α-linolenic acid (ALA) and marine-derived EPA, docosapentaenoic acid (DPA) and DHA, while ALA could act as a precursor and be converted to marine-derived *n*-3 PUFA through an extremely low conversion rate *in vivo*[7]. *n*-3 PUFA cannot be synthesised from other substances in mammals, and dietary supplements are considered as the main source of *n*-3 PUFA, thereby making the plasma level of *n*-3 PUFA a blood-based biomarker to reflect the real intake of food consumption[8].

Increased levels of *n*-3 PUFA in circulation were found to be protective for several GI diseases[9–11], which suggested their beneficial impacts in attenuating the pathological lesion in the GI

Abbreviations: ALA, α-Linolenic acid; DPA, docosapentaenoic acid; GWAS, genome-wide association study; HP, *Helicobacter pylori*; MR, Mendelian randomisation; MR-PRESSO, Mendelian randomisation-pleiotropy residual sum and outlier; PUD, peptic ulcer disease; RCT, randomised controlled trial.

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Table 1. Detailed information on data sources

	Phenotype	Year	Data origin	Country	Population	Number of SNP	Measurements
Exposure	Total *n*-3 PUFA and DHA	2016	Six studies integrated*	Finland	13 544	11 401 623	NMR metabolomics platform
	ALA, EPA and DPA	2011	Cohorts for Heart and Aging Research in Genomic Epidemiology Consortium	USA	8866	2 613 087	GC
Outcome	Peptic ulcer disease	2021	UK Biobank	British	16 666 cases/ 422 995 controls	8 546 065	International Classification of Diseases 9-codes

Abbreviations: ALA, α-Linolenic acid; DPA, docosapentaenoic acid.
* Studies include 'Northern Finland Birth Cohort 1966', 'The Cardiovascular Risk in Young Finns Study', 'Helsinki Birth Cohort Study', 'Health2000 GenMets Study', 'The Dietary, Lifestyle, and Genetic Determinants of Obesity and Metabolic Syndrome' and 'FinnTwin12 (FT12) and FinnTwin16 (FT16) cohort studies'.

mucosa. In laboratory studies, *n*-3 PUFA showed a protective effect on GI mucosa through modulating leucocyte–endothelial adhesive interactions, leucocyte chemotaxis and expression of adhesion molecules[12]. In two randomised controlled trials (RCT), supplementations of EPA and DHA could heal duodenal ulcer by inhibiting the inflammation in the gastric mucosa[13], and the clinical efficacy was equivalent to the use of famotidine (an anti-ulcer drug)[14]. However, few epidemiological studies focused on the protective effect of *n*-3 PUFA on PUD, and their results remained inconclusive. A putative comment was presented in prior observational research that a rising consumption of PUFA might have contributed to a declining risk of PUD[15], but this hypothesis is not currently validated in the relevant cohorts linking diet exposure to the incident PUD. At the biomarker level, a case–control study indicated a protective association of EPA levels in gastric mucosa with the pathological development of PUD[16], but this benefit was not seen in another case–control study that tested the levels of PUFA in adipose tissues[17]. It is noteworthy that data from observational epidemiological studies might be prone to be biased by reverse causation and interactions with confounding factors. For instance, intestinal flora disorders could increase the susceptibility of patients to PUD while disturbing the intestinal absorption of dietary *n*-3 PUFA, which leads to a lower plasma level[18,19].

Compared with traditional studies of observational epidemiology, the Mendelian randomisation (MR) analysis is a novel and reliable method to investigate the causal effects of exposures on disease outcomes[20]. In MR analysis, genetic variants are used as instrumental variables to connect exposures with study outcomes, which are randomly allocated at conception, thereby minimising the confounding effects and reverse causation. To further address the effect of dietary intake of *n*-3 PUFA on the risk of PUD, we performed the present MR analysis to explore the potential causal association of *n*-3 PUFA with PUD.

Methods

Study design and data sources

We performed a two-sample MR analysis to assess the causal relationship between plasma *n*-3 PUFA and PUD and reported according to the STROBE-MR (online Supplementary Table S1)[21]. The SNP were identified from a genome-wide association study (GWAS) as instrumental variables to genetically explain a specific exposure, and their effects on the outcome were analysed using another GWAS. To be screened as eligible instrumental variables for the study exposure, all the SNP were restricted by three assumptions as follows[22]: (1) there is a strong link between an SNP and the exposure, (2) SNP should not be associated with confounding factors and (3) SNP should be linked to outcomes only through the exposure.

In the present study, three databases were used, and their origin details were listed in Table 1. Specifically, total *n*-3 PUFA and four individual *n*-3 PUFA (ALA, EPA, DPA and DHA) were included as exposures. SNP for plasma level of total *n*-3 PUFA and DHA (quantified using NMR metabolomics platform) were obtained from a recent GWAS including 13 544 individuals from Finland[23]. SNP for ALA, EPA and DPA (quantified using GC) were obtained from another GWAS data from the USA[24]. These two GWAS have been wildly used in previous MR analyses on plasma/serum *n*-3 PUFA[25–27]. For data on outcome, we considered a recently published GWAS based on data from UK Biobank, which included 16 666 PUD cases (defined based on the International Classification of Diseases 9-codes) and 439 661 controls from the UK[28]. These GWAS for both exposure and outcome were adjusted for sex, age and the top several principal components generated based on population ancestry (principal components are statistical variables used to address and correct the ancestral or geographic differences among individuals[29]). There was no sample overlap between the GWAS for exposures and the GWAS for outcomes. Information about the above-mentioned GWAS in the present study is summarised in Table 1. All original studies have been ethically approved and have obtained informed consent from the participants.

Selection of instrumental variables

We summarised the selection process of SNP in a flow chart (Fig. 1). First, SNP with a minor allele frequency of at least 0·01 and the significant genome-wide association levels ($P < 5e\text{-}06$) were included from a GWAS. Second, the palindromic SNP with ambiguous allele frequencies (i.e. minor allele frequency > 0·42) were excluded to minimise the potential bias in strand alignment. Third, we used a clustering process ($r^2 < 0{\cdot}01$ and clumping distance = 2000 kb) to eliminate linkage disequilibrium between SNP. Fourth, the included SNP were matched and harmonised in the GWAS data of outcome. Besides, Mendelian

British Journal of Nutrition

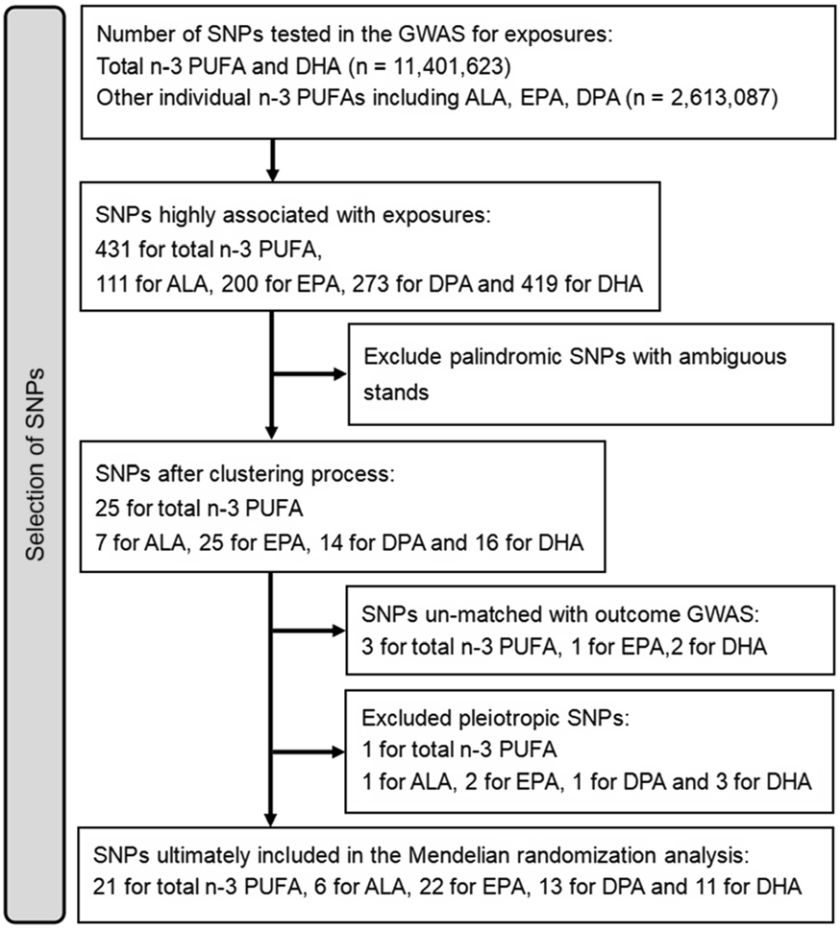

Fig. 1. The flow chart of the selection process of SNP. Abbreviations: GWAS, genome-wide association study; ALA, α-Linolenic acid; DPA, docosapentaenoic acid.

randomisation-pleiotropy residual sum and outlier (MR-PRESSO) and MR-Egger intercept tests were performed to evaluate the horizontal pleiotropy[30,31], and pleiotropic SNP were mainly identified by outlier test in MR-PRESSO (based on a type I error $\alpha = 0{\cdot}05$). If the pleiotropy was still significant after removing outliers in MR-PRESSO, the SNP that has the most significant effect on the outcome while explaining a small variance of exposure (< 0·3 %) would be considered as a pleiotropic SNP and removed. Rs174547 was found to be significantly associated with both ALA ($P = 3{\cdot}47e\text{-}64$) and DPA ($P = 3{\cdot}79e\text{-}154$); we therefore retained it in the more significant exposure (i.e. DPA) to avoid pleiotropy. The potential confounding factors for the pleiotropic SNP were summarised in online Supplementary Table S2.

Statistical analysis

In the main analysis, we used the inverse-variance weighted method to combine the effect of SNP on the outcome with modified second-order weights[32]. In sensitivity analyses, methods with different assumptions were used to further demonstrate the main MR results, including MR-Egger and weighted median estimator. The MR-Egger method provides estimates after adjusting for pleiotropy, with the assumption that the association of each genetic variant with the exposure is independent of the pleiotropic effect[30]. The WM estimator estimates the causal effect as the median of the weighted ratio estimates, which provides robust estimates even if up to 50 % of the included SNP are invalid[33]. OR for the outcome in all analyses were calculated based on per one SD increase in the plasma level of *n*-3 PUFA. Besides, we performed the leave-one-out analysis to determine if the association was driven by any specific SNP, which might be attributed to pleiotropy. We further conducted searches in the PhenoScanner database (available at http://www.phenoscanner.medschl.cam.ac.uk/) to identify potential confounding factors that may explain pleiotropic SNP (online Supplementary Table S2)[34,35]. Cochran's *Q* statistic was calculated to evaluate the heterogeneity among SNP, and a *P*-value < 0·1 was considered as a threshold of high heterogeneity among the SNP, in which case the inverse-variance weighted method should be performed in the random effect model[36]. In addition, *F*-statistics was used to evaluate the strength of the instrumental variable $\left(F = \frac{N-k-1}{k} \times \frac{R^2}{1-R^2}\right)$[37]. *N* refers to the sample size of the GWAS, k refers to the number of instrumental variables[38] and R^2 describes the percentage of the variation explained by SNP in the exposure which is calculated according to the method proposed by Tom *et al.*[39]. The type I error for the present MR analysis was set to $\alpha = 0{\cdot}05$, and we

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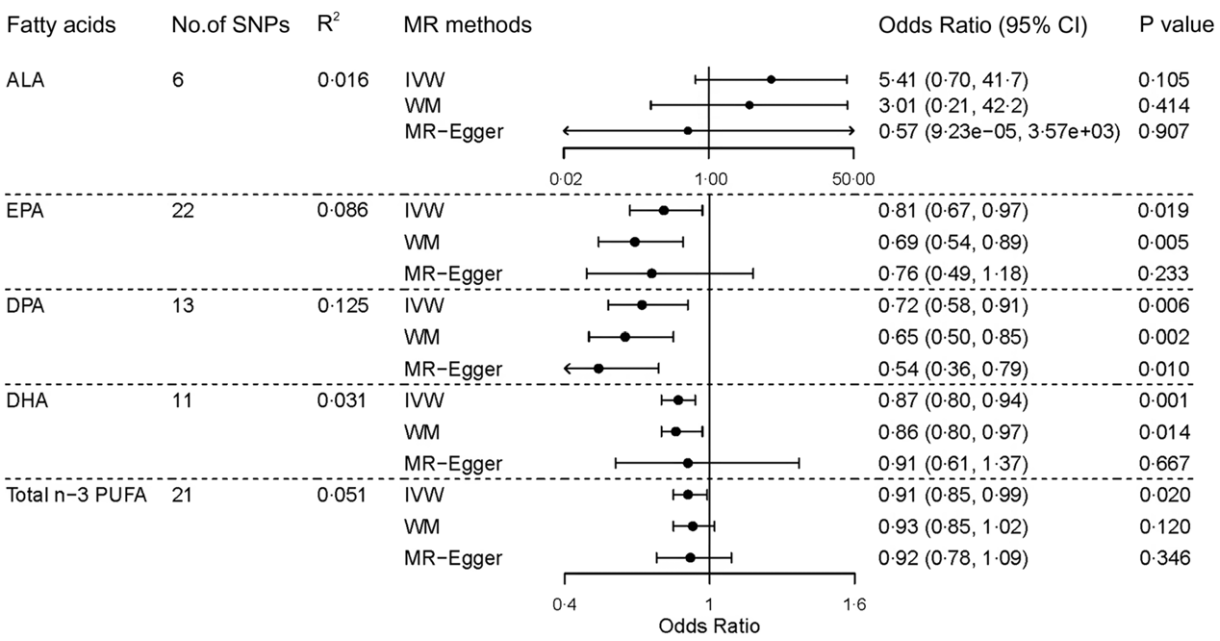

Fig. 2. Mendelian randomisation estimates of associations between plasma levels of *n*-3 PUFA and peptic ulcer disease. R^2 represents the percentage of variation explained by SNP in the exposure. OR are calculated based on per one SD increase in plasma *n*-3 PUFA. Abbreviations: MR, Mendelian randomisation; ALA, α-Linolenic acid; DPA, docosapentaenoic acid; IVW, inverse-variance weighted; WM, weighted median.

calculated statistical power using an online tool available at https://shiny.cnsgenomics.com/mRnd/[40]. We performed a Benjamini-Hochberg correction to adjust for multiple comparisons (online Supplementary Table S3)[41].

All statistical analyses were conducted in R version 4·2·2 (R Foundation for Statistical Computing) using the 'TwoSampleMR' package. Data used in the present MR study are publicly available.

Results

Instrumental variables

Totally, there were twenty-one SNP screened as instrumental variables for total *n*-3 PUFA, and six, twenty-two, thirteen and eleven SNP screened for ALA, EPA, DPA and DHA, respectively. These SNP explained 1·6–12·5 % of the total variance in the *n*-3 PUFA (Fig. 2). All exposures had *F*-statistics above the threshold of 10, which indicates a strong instrument strength. Online Supplementary Table S4 summarised the basic information of the SNP included in the present study.

Main analysis

Genetically predicted plasma levels of total *n*-3 PUFA were inversely associated with the risk of PUD (OR = 0·91; 95 % CI 0·85, 0·99; $P = 0·020$) (Fig. 2). For individual *n*-3 PUFA, the inverse association was more significant with marine-based EPA (OR = 0·81; 95 % CI 0·67, 0·97; $P = 0·019$), DPA (OR = 0·72; 95 % CI 0·58, 0·91; $P = 0·006$) and DHA (OR = 0·87; 95 % CI 0·80, 0·94; $P = 0·001$), but not with plant-based ALA (OR = 5·41; 95 % CI 0·70, 41·7; $P = 0·105$). The inverse associations for total *n*-3 PUFA and marine-based subtypes remained statistically significant in the Benjamini-Hochberg correction (online Supplementary Table S3). The forest plots and scatter plots of the association between *n*-3 PUFA and PUD risk were presented in online Supplementary Fig. S1–S4.

Sensitivity analysis

In MR-Egger and WM methods, total *n*-3 PUFA, EPA, DPA and DHA performed consistent results with the main analysis (Fig. 2). Both MR-PRESSO (global test) and MR-Egger regression (intercept test) found no significant pleiotropy in selected SNP of total or individual *n*-3 PUFA (online Supplementary Table S5). In leave-one-out analyses (online Supplementary Fig. S5 and S6), rs174538 in EPA and rs174547 in DPA showed a significant impact on the results. No significant heterogeneity was found among the SNP by Cochran's *Q* test.

Discussion

To our knowledge, this is the first MR analysis evaluating the effect of *n*-3 PUFA on the risk of PUD, which provides reliable causal estimates that are less susceptible to confounding factors. Our major findings suggested that higher levels of plasma *n*-3 PUFA were significantly associated with a lower risk of PUD, and the inverse association was more pronounced with marine-based subtypes (i.e. EPA, DPA and DHA), but not with the plant-based subtype (i.e. ALA). Such findings provided new evidence for the causal effect of *n*-3 PUFA on PUD risk, which may have offered an effective and feasible strategy for the primary prevention of PUD.

Our MR results found a preventive effect of plasma *n*-3 PUFA on PUD, especially marine-based *n*-3 PUFA. Consistent with our present study, accumulative evidence from pre-clinical studies have revealed the protective effect of marine-based *n*-3 PUFA on PUD[42–45]. In a case–control study, the plasma level of DHA was found lower in PUD patients compared with healthy volunteers and back to normal after PUD recovery, suggesting a protective role that DHA might play in the pathological development of PUD[46]. Another case–control study also indicated a protective association of EPA levels in gastric mucosa with PUD, but not total *n*-3 PUFA or other individual *n*-3 PUFA (ALA, DPA and DHA), and this inconsistency might be attributed to the low statistical power with a limited number of participants (eleven cases and nineteen non-cases) in the study[16]. Besides, two previous RCT have revealed that supplementations of EPA and DHA tended to alleviate the inflammation in the gastric mucosa[13] and could heal duodenal ulcer[47]. The present study extended and confirmed the previous evidence by providing reliable causal estimates with data from a large population (16 666 PUD cases and 439 661 controls) on the association between plasma level of *n*-3 PUFA and the risk of PUD.

The present MR study found that ALA had no causal association with PUD risk. In support of this finding, similar results were also seen in the previous human-based studies. A case–control study tested ALA concentration in adipose tissue, which reflects a long-term consumption of fatty acids, and no difference was found between PUD patients and patients with other diseases[17]. In an RCT based on HP-infected patients with duodenal ulcer, a supplement of ALA failed to reduce HP density in mucosa or modulate inflammatory cytokines including PGE_2 and leukotriene B_4[48]. In contrast, several pre-clinical studies have suggested a protective role of ALA in the development of PUD[49–51], but the concentrations of ALA used are often high in animal models and difficult to achieve through dietary supplementation in humans. Of note, ALA is preferentially oxidised among all of the individual *n*-3 PUFA, with over 60 % of its consumption partitioned to β-oxidation[52], which might minimise the utilisation of ALA involved in the prevention of PUD. Further large cohorts and well-designed RCT are warranted to reconfirm the ALA-based associations.

Several biological mechanisms have been revealed underlying the protective impacts of marine *n*-3 PUFA on PUD. First, EPA and DHA may inhibit GI inflammation by decreasing pro-inflammatory lipid mediators to interfere with the signalling cascade related to NF-κB[12,53] and increasing the production from *n*-3 PUFA-derived specialised pro-resolving mediators such as resolvins, protectins and maresins[54–56]. Second, the supplement of EPA could reduce the generation of reactive oxygen species, inhibit lipid peroxidation and normalise mucosal glutathione, thus protecting the gastric mucosal cells[57]. Third, marine *n*-3 PUFA help balance the secretion of alkaline mucus and gastric acid to reduce the peptic damage to the gastric mucosa[58,59], which might partly be attributed to the down-regulation of gastrin[57]. Fourth, a high plasma level of EPA could stimulate blood flow to improve the delivery of oxygen and nutrients to the gastric mucosa and help prevent and heal peptic ulcers[60]. It is noteworthy that the gastro-protective effect of marine *n*-3 PUFA may not be through mediation of PGE_2, a cyclooxygenase catalysed product with an essential effect on mucous secretion and gastric blood flow[61,62], and some physiological changes resulting from high level of *n*-3 PUFA, including a down-regulation of leukotrienes and thromboxanes, are considered as possible functional substitution for PGE_2[59,60].

Evidence from an experimental study demonstrated the bactericidal effect of *n*-3 PUFA on HP (the most important risk factor of PUD) *in vitro*[63]. Numerous clinical studies have used *n*-3 PUFA as a medication therapy in patients with HP infection and have proved their effectiveness in inhibiting HP colonisation[64,65]. However, our data did not support the beneficial association between plasma *n*-3 PUFA and HP infection (online Supplementary Table S6). Given that evidence from present numerous clinical studies found that *n*-3 PUFA may dose-dependently affect GI microbiota[66], a possible explanation is that variations in plasma level of PUFA (1·6–12·5 %) explained by genetic variants might not be sufficient to inhibit HP colonisation. Therefore, further randomised clinical trials were needed to explore the dose–response effects of supplemental *n*-3 PUFA on HP infection. Moreover, in the previous clinical trials, after treatment of anti-acid drugs, including lansoprazole (a proton pump inhibitor)[46] and famotidine (an H_2 blocker)[47], PUD patients showed a significant increase in plasma level of *n*-3 PUFA, which strongly suggested that use of the anti-acid drugs might have acted partly through beneficial changes of *in vivo* *n*-3 PUFA. Similar to the treatment with PUFA, the improvements of pathobiology changes in peptic ulcers, including free radicals, nitric oxide and anti-oxidants, were also observed in the famotidine treatment[67].

The MR design is the first strength of the present study, which minimises mixed effects from confounding factors as well as reverse causality. Besides, results of total *n*-3 PUFA and marine-based subtypes remain statistical in the Benjamini-Hochberg correction, which lessened the likelihood of type I error. Moreover, the main results of total *n*-3 PUFA and marine-based subtypes were consistent with sensitivity analyses, and no significant pleiotropy was detected by MR-PRESSO and MR-Egger regressions, which enhanced the reliability of the results.

Several limitations should be pointed out in this study. First, we used a compromised significance threshold for genome-wide association level (i.e. $P < 5e\text{-}06$) to better explain the variance of exposure, which was more likely to violate the MR assumptions and bring weak instrument bias compared with the stringent significance threshold (i.e. $P < 5e\text{-}08$). Second, only six eligible SNP were included for ALA, explaining a relatively low variance (1·55 %) of the exposure, which might have diminished statistical power and contributed to the instability of the results. Therefore, a larger GWAS with more detected SNP is needed to build a more robust evaluation model for ALA. Third, rs174538 and rs174547 showed a significant impact on the results of EPA and DPA, respectively. These SNP highly explained the variation in the plasma level of these PUFA (online Supplementary Table S4), which might be a possible explanation for the significant impact on the results, but a possible impact of potential horizontal pleiotropy cannot be ruled out. Fourth, given that the GWAS database provides information only on the whole population with no available data on individuals, it was unable to perform

stratified analysis. Finally, we used GWAS of European ancestry for both exposure and outcome to avoid the population stratification bias, which might limit the generalisability of the results to other ancestry groups.

Conclusions

This MR study based on data of a large population from UK Biobank provided evidence supporting a protective association of higher plasma levels of *n*-3 PUFA with PUD, especially marine-based *n*-3 PUFA. Encouraging increased consumption of *n*-3 PUFA-rich food to improve their levels in plasma may be an effective and feasible strategy for the primary prevention of PUD. Nevertheless, large-scale and well-designed RCT are further needed to confirm the protective effect of *n*-3 PUFA on PUD.

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All authors substantially contributed to the present study and approved the submitted version of the manuscript. The authors' contributions were as follows: Z. D. conceived the idea, designed the study, acquired and performed analysis on data acquisition, drafted and revised the manuscript in considering suggestions of all the co-authors and had primary responsibility for the final content. B. H. and Q. W. advised on the data processing and analysis. W. C. and Q. J. performed the visualisation of the results. B. Y., F. S. and D. Z. provided critical revisions of the manuscript. B. Y., D. Z. and Z. X. provided supervision, administration and funding support.

The authors declare none.

Publicly available GWAS on PUD were obtained from https://www.ebi.ac.uk/gwas/publications/33608531. GWAS data for total *n*-3 PUFA (ID = met-c-855) and DHA (ID = met-c-852) were available in the IEU GWAS database (https://gwas.mrcieu.ac.uk/). GWAS data for ALA, EPA and DPA were obtained from the Supporting Information of the original study published by Lemaitre *et al.* (https://dx.plos.org/10.1371/journal.pgen.1002193). We also used the following web-based resources: PhenoScanner (http://www.phenoscanner.medschl.cam.ac.uk/) and power calculation (https://shiny.cnsgenomics.com/mRnd/).

Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114524001752.

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