Effect of n-3 polyunsaturated fatty acids on ischemic heart disease and cardiometabolic risk factors: a two-sample Mendelian randomization study

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Abstract

Background: The cardioprotective ability of n-3 polyunsaturated fatty acids (PUFAs) is controversial. Most studies suggest a specific role for PUFAs in cardioprotection from ischemic heart disease (IHD). However, few studies have used genetic biomarkers of n-3 PUFAs to examine their potential relationships with IHD. This study aimed to use Mendelian randomization to evaluate whether genetically-predicted n-3 PUFAs affect IHD and cardiometabolic risk factors (CRFs).

Methods: Genetic variants strongly (p < 5 × 10⁻⁸) and independently (r² > 0.1) associated with n-3 PUFAs were derived from the CHARGE Consortium (including 8,866 subjects of European ancestry) and were used as instrumental variables (IVs) for evaluating the effect of n-3 PUFAs, including α-linolenic acid (ALA), docosapentaenoic acid (DPA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA). Data on the associations between the IVs and IHD, myocardial infarction, and CRFs (including diabetes, lipids, blood pressure, body mass index, and waist-to-hip ratio (WHR)) were obtained from the UK Biobank SOFT CAD GWAS with the CARDIoGRAMplusC4D 1000 Genomes-based GWAS (113,937 IHD cases and 339,115 controls), the Myocardial Infarction Genetics and CARDIoGRAM Exome consortia (42,335 MI cases and 78,240 controls), the DIAbetes Genetics Replication And Meta-analysis consortium (26,676 diabetes mellitus cases and 132,532 controls), the Global Lipids Genetics Consortium (n = 196,475), the International Consortium for Blood Pressure (n = 69,395), and the meta-analysis of GWAS for body fat distribution in the UK Biobank and Genetic Investigation of Anthropometric Traits (n = 694,649).

Results: Genetically-predicted higher ALA was associated with lower risk of IHD, type 2 diabetes (T2D), and lower serum lipids. The effect size per 0.05-unit increase (about 1 standard deviation) in plasma ALA level was −1.173 (95% confidence interval −2.214 to −0.133) for IHD. DPA and EPA had no association with IHD but were associated with a higher risk of T2D, higher levels of lipids or WHR. DHA had no association with IHD or CRFs.

Conclusions: Our study suggests a benefit of ALA for IHD and its main risk factors. DHA, DPA, and EPA had no association with IHD but were partly associated with increasing cardiometabolic risk factors.

Keywords: N-3 polyunsaturated fatty acids, Mendelian randomization, Ischemic heart disease, Cardiometabolic risk factors
Background
N-3 polyunsaturated fatty acids (PUFAs) include plant-derived α-linolenic acid (ALA) and marine-derived docosahexaenoic acid (DHA), docosapentaenoic acid (DPA), and eicosapentaenoic acid (EPA). DHA, DPA, and EPA are long-chain n-3 fatty acids. Evidence from observational, experimental studies, and randomized controlled trials (RCT) show that n-3 PUFAs from diet or supplements confer protection against cardiovascular disease (CVD) and relevant risk factors, including cardiac death, ischemic heart disease (IHD), ischemic stroke, heart failure, and blood pressure [1, 2]. However, integrated analyses of these studies have found null, little, or inconsistent results, no matter whether in primary or in secondary prevention of CVD [3–5]. Recent evidence from high-quality large RCTs also suggests that n-3 PUFA intake probably makes little or no difference for coronary heart mortality or events [6–9]. The nutrition recommendations for n-3 PUFA supplements or seafood for cardiovascular benefits have been debated in recent years, but without consensus being reached [10]. These controversies may be confounded by background dietary consumption of fish, health status, medical treatment of IHD, socioeconomic position, lifestyle, different study populations, and different definitions of CVD and study endpoints [1, 10].

Mendelian randomization (MR) studies use germline genetic variants as intermediate instrumental variables (IVs) to assess causal relationships in a non-experimental setting. As genetic variants are determined at conception, MR studies are less susceptible to confounders than observational studies and are not affected by disease status, thereby avoiding reverse causation bias. MR studies can be regarded as “natural” RCTs and have been applied to examine genetic predisposition conferred by several genes on IHD [11]. In the present study, we conducted a two-sample MR study to assess the effect of genetically predicted n-3 PUFAs on IHD, using genetically instrumented n-3 PUFAs from previous studies and a very large case-control dataset of IHD from public consortia. In addition, cardiometabolic risk factors (CRFs) of IHD, including type 2 diabetes (T2D), hyperlipidemia, hypertension, and abdominal obesity were similarly assessed.

Methods
Genetic instruments for n-3 PUFAs
Genetic instruments for n-3 PUFAs were obtained from published genome-wide association studies (GWAS) conducted by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) consortium [12]. The CHARGE Consortium is a design of prospective meta-analyses of GWAS from five population-based cohorts comprising 8,866 subjects of European ancestry and can be used for proxy plasma levels of n-3 fatty acids. The different n-3 PUFAs share a common metabolic pathway and single nucleotide polymorphisms (SNPs) known to influence one n-3 PUFA typically also have strong effects on the others [12]. All SNPs chosen as IVs were associated with the relevant n-3 PUFAs (ALA, DHA, DPA, and EPA) and reached genome-wide significance ($p < 5 \times 10^{-8}$) (Additional file 1: Tables S1–S4). The linkage disequilibrium (LD) between instrumental SNPs was obtained using LDlink [13], a web-based LD analysis tool designed to easily query pair-wise LD between SNPs in specific population groups.

Genetic associations with IHD, T2D, lipids, blood pressure, body mass index (BMI), and waist-to-hip ratio (WHR)
Genetic associations with IHD were obtained from the published meta-analysis of UK Biobank SOFT CAD GWAS with the CARDIoGRAMplusC4D 1000 Genomes-based GWAS and the Myocardial Infarction Genetics and CARDIoGRAM Exome, which is the most up-to-date GWAS of IHD applied to the UK Biobank and involved 113,937 IHD cases and 339,115 controls in total. The UK Biobank SOFT CAD GWAS comprised 10,801 cases and 137,371 controls, and 94% of the participants were of self-reported European ancestry [14]. SOFT CAD phenotypes in UK Biobank encompassed individuals with fatal or nonfatal myocardial infarction (MI), percutaneous transluminal coronary angioplasty (PTCA), or coronary artery bypass grafting (CABG), chronic IHD, and angina. Controls were those who were free from case status. The CARDIoGRAMplusC4D 1000 Genomes-based GWAS consortium comprised 60,801 cases and 123,504 controls and most of the participants (77%) were of European descent [15]. IHD status was determined from clinical diagnosis, medical records and self-reports of medication usage, procedures such as revascularization, and other evidence of stenosis such as from coronary angiography. The Myocardial Infarction Genetics and CARDIoGRAM Exome consortium comprised 42,335 cases and 78,240 controls and all of the participants were of European descent [16].

Genetic associations with diabetes were obtained from the DIAbetes Genetics Replication and Meta-analysis (DIAGRAM) consortium. The DIAGRAM consortium is a grouping of researchers with shared interests in performing large-scale studies to characterize the genetic basis of T2D. The stage 1 analyses comprised a total of 26,676 T2D cases and 132,532 control participants of European descent [17]. Genetic associations with lipids, including high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, total cholesterol (TC), and triglycerides (TG), were obtained from the Global Lipids Genetics Consortium (GLGC), which
included 188,577 European-ancestry individuals and 7,898 non-European ancestry individuals [18]. Genetic associations with blood pressure, systolic and diastolic blood pressure (SBP and DBP), were obtained from the International Consortium for Blood Pressure (ICBP), which included 69,395 individuals of European ancestry [19]. Genetic associations with BMI and WHR were obtained from the meta-analysis of GWAS for body fat distribution in UK Biobank and Genetic Investigation of Anthropometric Traits (GIANT) and included 694,649 individuals of European ancestry [20].

**Statistical analysis**

In this study, we conducted a two-sample MR analysis using R version 4.0.3 (R Foundation for Statistical Computing, Vienna, Austria) and the R package for Mendelian randomization (version 0.5.1). The main methods included inverse-variance weighted (IVW), Mendelian randomization-Egger (MR-Egger), MR-PRESSO (Mendelian Randomization Pleiotropy Residual Sum and Outlier), and weighted median (WM) methods [21–23]. These methods have their advantages and can complement each other, providing a more reliable causal inference for our study. The MR-PRESSO method can identify and exclude SNPs that most likely display pleiotropic effects [21]. All statistical tests were two-sided, and \( p < 0.05 \) was considered statistically significant.

As shown in Fig. 1, MR analysis relies on three stringent assumptions [24]: (1) the genetic instruments (SNPs) used as IVs are strongly predictive of n-3 PUFA, (2) the association of genetic instruments with IHD is not confounded by measured or unmeasured factors, (3) the effect of the genetic instrument on IHD should be fully mediated via n-3 PUFAs and not through any alternative causal pathways. Pleiotropic genetic instruments violate the MR assumptions and may lead to false-positive results, interfering causal inference. We identified potential pleiotropic genetic instruments in the following ways. First, we examined the latest representative GWAS results (the DIAGRAM consortium, the GLGC consortium, the ICBP consortium, the UK Biobank, and the GIANT) to identify associations between our genetic instruments and potential biological confounding factors, including diabetes, lipids, blood pressure, BMI, and WHR. Secondly, potential pleiotropic effects of the genetic instruments were investigated by searching the

![Fig. 1 Assumptions of a Mendelian randomization analysis and data sources. Mendelian randomization analysis rests on 3 assumptions. First, the genetic instruments used as instrumental variables are strongly predictive of exposure (this study specifically refers to n-3 PUFAs, Assumption 1). Second, the association of genetic instruments with outcome (for example, IHD in this study) is not confounded by measured or unmeasured factors (Assumption 2). Third, the effect of the genetic instrument on outcome (IHD) should be fully mediated via exposure (n-3 PUFAs) and not through any direct or alternative causal pathways (Assumption 3). ALA α-linolenic acid, DHA docosahexaenoic acid, DIAGRAM DIAbetes Genetics Replication and Meta-analysis, DPA docosapentaenoic acid, EPA eicosapentaenoic acid, GIANT Genetic Investigation of Anthropometric Traits, GLGC Global Lipids Genetics Consortium, GWAS genome-wide association studies, ICBP International Consortium for Blood Pressure, IHD ischemic heart disease, PUFAs polyunsaturated fatty acids, SNP single nucleotide polymorphism]
According to these 31 instrumental SNPs, genetic associations with IHD and CRFs were found from the corresponding GWAS database (Additional file 1: Table S5). According to these 31 instrumental SNPs, genetic associations with IHD and CRFs were found from the corresponding GWAS database (Additional file 1: Table S5). According to methods of a previous study [25, 26], summary statistics from a previous n-3 PUFA GWAS [12, 27] were used to calculate the F-statistic. The strength of the genetic instruments was evaluated by the F-statistic. According to methods of a previous study [25, 26], summary statistics from a previous n-3 PUFA GWAS [12, 27] were used to calculate the F-statistic. Our results showed the F-statistic of all instrumental SNPs was >10 (mean 85, from 13 to 322) (Table 1), indicating strong genetic instruments for n-3 PUFAs [28].

### Table 1: Characteristics of the SNPs associated with plasma levels of n-3 polyunsaturated fatty acids (PUFAs)

| n-3 PUFA | Nearest gene | SNP | Chr. position | EA/NEA | EAF | Effect | SE | p-value | VE (%) | F |
|----------|--------------|-----|---------------|--------|-----|--------|----|---------|--------|---|
| ALA      | FEN1         | rs412334 | 11:61316387   | t/c    | 0.16 | −0.0118 | 0.0016 | 9.72E−14 | 0.342 | 30 |
|          | C11orf9      | rs198464 | 11:61278197   | a/g    | 0.50 | −0.0057 | 0.0009 | 2.48E−11 | 0.148 | 13 |
|          | C11orf10     | rs740006 | 11:61314444   | t/c    | 0.90 | 0.0163  | 0.0025 | 1.32E−10 | 0.440 | 39 |
|          | C11orf9      | rs17762402 | 11:61309777   | a/g    | 0.07 | −0.0186 | 0.0029 | 1.56E−10 | 0.411 | 37 |
| DHA      | ELOVL2       | rs2236212 | 6:10995015    | a/c    | 0.41 | 0.01132 | 0.0141 | 1.26E−15 | 0.646 | 58 |
| DPA      | ELOVL2       | rs373498  | 6:10928973    | t/c    | 0.57 | −0.0404 | 0.0029 | 9.61E−44 | 2.667 | 243|
|          | FEN1         | rs412334 | 11:61560261   | t/c    | 0.16 | 0.0554  | 0.0052 | 1.40E−26 | 2.750 | 100|
|          | C11orf10     | rs740006 | 11:61557868   | t/c    | 0.9  | −0.0764 | 0.0079 | 4.50E−22 | 3.502 | 322|
|          | FADS3        | rs7394871 | 11:61562514    | a/c    | 0.05 | −0.0637 | 0.0078 | 3.56E−16 | 1.285 | 115 |
|          | FADS2        | rs498793 | 11:61624705   | t/c    | 0.43 | 0.0307  | 0.0238 | 8.47E−13 | 0.141 | 221|
|          | SYCP2L       | rs12199131 | 6:10932569    | a/g    | 0.26 | 0.0267  | 0.0033 | 1.27E−10 | 0.184 | 42 |
|          | RAB3L1       | rs174472  | 11:61671956   | a/g    | 0.58 | 0.0274  | 0.0037 | 7.54E−14 | 1.219 | 109|
|          | C11orf9      | rs17762402 | 11:61553201   | a/g    | 0.06 | 0.0805  | 0.0109 | 1.42E−13 | 2.437 | 221|
|          | SYCP2L       | rs6928281 | 11:6098917    | t/c    | 0.72 | 0.0226  | 0.0032 | 8.04E−13 | 0.866 | 61 |
|          | C11orf9      | rs198464 | 11:61521621   | a/g    | 0.49 | 0.0191  | 0.0028 | 7.47E−12 | 0.608 | 54 |
|          | FADS2        | rs17156442 | 11:61614023   | t/c    | 0.05 | −0.0513 | 0.0077 | 2.09E−11 | 0.833 | 74 |
|          | FADS2        | rs206435  | 11:61722645   | a/c    | 0.01 | −0.0403 | 0.0068 | 5.09E−09 | 0.694 | 32 |
|          | BEST1        | rs1109748 | 11:61535972   | t/c    | 0.84 | 0.0244  | 0.0043 | 1.34E−08 | 0.533 | 48 |
|          | ELOVL2       | rs6936315 | 11:61033927   | t/c    | 0.84 | 0.0244  | 0.0043 | 1.34E−08 | 0.533 | 48 |
|          | FTH1         | rs10792320 | 11:61746291  | a/c    | 0.65 | 0.0160  | 0.0030 | 8.49E−08 | 0.388 | 35 |
|          | BEST1        | rs272766  | 11:61704334   | a/g    | 0.93 | 0.0308  | 0.0058 | 8.86E−08 | 0.412 | 37 |
| EPA      | FADS3        | rs7394871 | 11:61652514   | a/c    | 0.05 | −0.0912 | 0.0128 | 1.13E−12 | 0.494 | 44 |
|          | ELOVL2       | rs3798713 | 6:11008622    | c/g    | 0.42 | 0.0350  | 0.0050 | 1.93E−12 | 0.373 | 33 |
|          | BEST1        | rs1109748 | 11:61722645   | a/c    | 0.07 | −0.0535 | 0.0092 | 5.46E−09 | 0.233 | 21 |
|          | FEN1         | rs142334  | 11:61560261   | t/c    | 0.16 | 0.0440  | 0.0081 | 4.59E−08 | 0.325 | 29 |
|          | FADS2        | rs498793  | 11:61624705   | t/c    | 0.43 | 0.0351  | 0.0064 | 5.11E−08 | 0.377 | 34 |
| MAT2B    | rs1145652    | 5:164764087 | a/g    | 0.87 | 0.0356  | 0.0066 | 8.39E−08 | 0.179 | 16 |

**Table 1** Characteristics of the SNPs associated with plasma levels of n-3 polyunsaturated fatty acids (PUFAs).

**References:**
- ALA: α-linolenic acid, Chr: chromosome, DHA: docosahexaenoic acid, DPA: docosapentaenoic acid, EAF: effect allele frequency, EPA: eicosapentaenoic acid, F: F-statistic, NEA: non-effect allele, PUFAs: polyunsaturated fatty acids, SE: standard error, SNP: single nucleotide polymorphism, VE: variance explained.
- F-statistic is a measure of the strength of the genetic instrument and is calculated as: \( F = \frac{R^2 \times (n - 1 - k)}{(1 - R^2) \times k} \), where \( R^2 = VE(%) \), \( n = \) sample size, \( k = \) number of instrumental variables.
Associations of n-3 PUFAs with IHD

Figure 2 and Table S17 (Additional file 1) showed higher ALA was associated with lower IHD risk, based on IVW ($p = 0.027$) and WM ($p = 0.006$), but with inconsistent results from MR-Egger ($p > 0.05$) and MR-PRESSO ($p < 0.05$). ALA was not associated with MI, with consistent results, based on all 4 methods (IVW, WM, MR-Egger, and MR-PRESSO) ($p > 0.05$). DPA and EPA were not significantly associated with IHD or MI, with consistent results from all 4 methods ($p > 0.05$). MR-Egger intercepts $p > 0.05$ suggested little evidence of directional pleiotropy in all analyses. As WM, MR-Egger and MR-PRESSO methods require a minimum of 3 IVs, the associations of DHA with IHD, MI and CRFs were estimated by IVW only, and the results showed that DHA was not associated with IHD or MI ($p > 0.05$).

Associations of n-3 PUFAs with T2D, lipids, blood pressure, BMI, and WHR

We also tested the association of n-3 PUFAs with CRFs. As shown in Fig. 2 and Table S17 (Additional file 1), ALA was associated with a lower risk of T2D (IVW, MR-PRESSO) and lower HDL (IVW, WM, MR-PRESSO), LDL (IVW, WM), TG (IVW, WM), and TC (IVW, WM) levels ($p < 0.05$ or $0.001$). ALA had no association with SBP, DBP, BMI, or WHR ($p > 0.05$). There was little evidence of directional pleiotropy based on the MR-Egger intercept ($p > 0.05$).

DPA was associated with a higher risk of T2D (IVW, WM, MR-PRESSO) and higher HDL (IVW), LDL (IVW, WM, MR-PRESSO), and WHR (IVW, WM, MR-PRESSO) ($p < 0.05$ or $0.001$). DPA had no association with TG, SBP, DBP, or BMI ($p > 0.05$), and there was little evidence of directional pleiotropy based on the MR-Egger intercept ($p > 0.05$). EPA was associated with higher LDL (IVW, WM), TC (IVW, WM), WHR (IVW), and lower SBP (MR-Egger), DBP (MR-Egger). As for TG, there were inconsistent results: WM indicated TG was a risk factor whereas MR-Egger indicated TG was a protective factor (both $p < 0.05$). EPA had no association with T2D, HDL, or BMI ($p > 0.05$). Based on the MR-Egger intercept, there was potential pleiotropy between EPA and LDL ($p = 0.021$), TC ($p = 0.044$), TG ($p = 0.003$), SBP ($p = 0.012$), and DBP ($p < 0.001$). In this case, the MR-PRESSO results should prevail and we conclude that EPA had no association with LDL, TC, TG, SBP, or DBP ($p > 0.05$). DHA had no association with CRFs.

Discussion

This MR study showed that a genetic predisposition toward higher plasma ALA level is associated with a lower risk of IHD, but not MI. The effect size (beta coefficient) per 0.05-unit increase (about 1 SD) in plasma ALA level was $-1.173$ (95% confidence interval $-2.214$ to $-0.133$) for IHD. In contrast, genetically-predicted levels of marine-derived n-3 PUFAs (DHA, DPA, and EPA) had no association with IHD or MI. On primary prevention of CVD, Abdelhamid et al. [3] found increased ALA may slightly reduce the risk of cardiovascular events, coronary heart disease (CHD) mortality, and arrhythmia, and Pan et al. [29] found dietary ALA is associated with a moderately lower risk of fatal CHD, with each 1 g/d increment of ALA intake being associated with a 10% lower risk of CHD death. By assessing the primary incidence of CHD in generally healthy, free-living populations around the world, Del Gobbo et al. [30] also found ALA to be associated with a 9% lower risk of fatal CHD. From the data extracted from the UK Biobank SOFT CAD GWAS and the CARDIoGRAMplusC4D 1000 Genomes-based GWAS consortia, our results also demonstrate beneficial primary health outcomes for ALA. However, for secondary prevention of CVD, there is little or no effect of ALA, as previously suggested [31], or the evidence is scarce.

Our results, combined with previous findings, support the favorable effects of ALA specifically for the primary prevention of IHD. Mechanistically, these findings are supported by the effects of ALA on improving lipid profile (TC, TG, LDL) [32] and cholesterol homeostasis [33], ameliorating sympathetic heart activity and denervation [34, 35], decreasing fasting free fatty acid and inhibiting inflammation and platelet activation [36]. A meta-analysis of 18 observational studies in generally healthy populations found that ALA may be associated with modestly lower risk T2D [37]. Our results also show that genetically-predicted higher plasma ALA is associated with a lower risk of T2D and lower LDL, HDL, TG, and TC. It is

(See figure on next page.)

**Fig. 2** Mendelian randomization (MR) analysis testing the effects of n-3 PUFAs on IHD and cardiometabolic risk factors. Results obtained using 4 MR methods (IVW: inverse-variance weighted, WM: weighted median, MR-Egger: Mendelian randomization-Egger, and MR-PRESSO: Mendelian Randomization Pleiotropy Residual Sum and Outlier) are presented as a heat map representing causal estimates (CE). CE of SBP and DBP exceeded that of the others a lot. In order to show the others CE better, CE of SBP and DBP were not included in the heat map. ALA α-linolenic acid, BMI body mass index, DHA docosahexaenoic acid, DPA docosapentaenoic acid, EPA eicosapentaenoic acid, HDL high-density lipoprotein, IHD ischemic heart disease, LDL low-density lipoprotein, MI myocardial infarction, T2D type 2 diabetes, TC total cholesterol, TG triglycerides, WHR waist-to-hip ratio.

*Indicates $p < 0.05$ for a particular MR approach.
|        | IVW | WM | MR-Egger | MR-PRESSO |
|--------|-----|----|----------|-----------|
| **ALA**|     |    |          |           |
| IHD    | *   |    | *        |           |
| MI     |     |    |          |           |
| T2D    | *   |    | *        |           |
| HDL    | *   |    | *        |           |
| LDL    |     |    |          |           |
| TC     | *   |    | *        |           |
| TG     |     |    |          |           |
| BMI    |     |    |          |           |
| WHR    |     |    |          |           |
| **DPA**|     |    |          |           |
| IHD    |     |    |          |           |
| MI     |     |    |          |           |
| T2D    |     |    |          |           |
| HDL    |     |    |          |           |
| LDL    | *   |    | *        |           |
| TC     |     |    |          |           |
| TG     |     |    |          |           |
| BMI    |     |    |          |           |
| WHR    |     |    |          |           |
| **EPA**|     |    |          |           |
| IHD    |     |    |          |           |
| MI     |     |    |          |           |
| T2D    |     |    |          |           |
| HDL    | *   |    | *        |           |
| LDL    |     |    |          |           |
| TC     | *   |    | *        |           |
| TG     |     |    |          |           |
| BMI    |     |    |          |           |
| WHR    | *   |    |          |           |
| **DHA**|     |    |          |           |
| IHD    |     |    |          |           |
| MI     |     |    |          |           |
| T2D    |     |    |          |           |
| HDL    |     |    |          |           |
| LDL    |     |    |          |           |
| TC     |     |    |          |           |
| TG     |     |    |          |           |
| BMI    |     |    |          |           |
| WHR    |     |    |          |           |

**Fig. 2** (See legend on previous page.)
well known that LDL is the initiator of IHD [38], hypertriglyceridemia is the residual risk of IHD [39, 40], while diabetes could negatively affect clinical outcomes of IHD, in patients admitted for ST-elevation myocardial infarction (STEMI) [41–43], non-STEMI [44] or stable IHD [45, 46]. From this perspective, ALA can reduce the risk of IHD in many ways. N-3 PUFA do not affect atherosclerotic progression, plaque stability, plaque rupture, or thrombosis [10]. This may be related to the ineffectiveness of ALA on MI. Clinically, the phenotypes of MI are not equal to the presence of coronary atherosclerosis. Coronary atherosclerosis may progress as acute coronary thrombocytic occlusion or MI, often due to the rupture of an unstable plaque [47–49], usually occurring in plaques with a thin, eroded fibrous cap, regardless of the degree of stenosis [47, 50]. Many patients live to advanced age with stable, significant IHD and never suffer an MI.

The clinical research on marine-derived n-3 PUFA (DHA, EPA, and EPA) has been full of twists and turns. Before the use of statins, most of the studies on the cardioprotection of marine-derived n-3 PUFA were positive [51]. However, after statins became widely used, most studies reported neutral effects [52, 53]. In recent years, the cardioprotective role of marine-derived n-3 PUFA, especially EPA and DHA, has become increasingly disputed [54, 55]. Dietary recommendations of EPA and DHA have also been downgraded from Class I to Class II [53], is the reason being that a large number of more recent RCTs [6, 7] and integration analyses have found little or no effect of EPA or DHA on cardioprotection [4, 5], particularly for primary prevention of CVD [3, 8, 9]. Even when there is an effect, the effect is only seen in studies with a moderate to high risk of bias [56]. Some researchers even think fish oil has disappointing therapeutic benefits [5].

In contrast, other researchers still have hope for marine-derived n-3 PUFA, especially EPA. They acknowledge that, in over-the-counter formulations (EPA + DHA or fish oil) at common dosages, primary prevention of CVD by marine-derived n-3 PUFA, is ineffective and that secondary prevention is controversial [10]. They attribute the failure of the previous trials to the low dose and impure formulation of marine-derived n-3 PUFA, short intervention duration, high background of fish intake, and inappropriate participants [51, 52, 55]. With the large success of the REDUCE-IT trial [57], proponents put their hopes on highly purified EPA (icosapent ethyl), which will lower plasma TG levels, and have given some constructive suggestions for future clinical trials [55]. At this time, the AHA has also given more affirmative recommendations to support the use of marine-derived n-3 PUFA for reducing the residual risk of CVD that remains after statin therapy [58]. However, as fibrates [59] and PCSK9 inhibitors [60] not only reduce TG, similar to EPA, but also increase HDL and reduce LDL, a new debate arises as to whether we should use fibrates instead of EPA or PCSK9 inhibitors instead of a statin/EPA combination [61]. So, it seems that the debate on the cardioprotection of marine-derived n-3 PUFA will continue.

Our research explores the role of marine-derived n-3 PUFA from another perspective and finds individual marine-derived n-3 PUFA have no association with IHD or MI in generally healthy populations. As for CRF, DPA is associated with a higher risk of T2D and higher HDL, LDL, TC, and WHR; EPA is associated with higher WHR, and DHA does not affect CRF. Most prior studies showed that, except for reducing TG [57, 61], marine-derived n-3 PUFA do not affect most CRF or intermediate outcomes [3, 9], including high CAD risk factors, i.e., LDL and T2D [9, 37]. Only a few studies have shown that marine-derived n-3 PUFA significantly reduce blood pressure [62], with the greatest reductions in untreated hypertension. Some studies even suggest that marine-derived n-3 PUFA may increase LDL [52, 58], which may negate any cardiovascular benefits [61]. Recently, a large general-practice RCT show that for patients with multiple cardiovascular risk factors (the criterion was defined as at least four of the following, or for patients with diabetes, at least one of the following: age of 65 years or older, male sex, hypertension, hypercholesterolemia, current smoker, obesity, family history of premature cardiovascular disease), treatment with n-3 PUFA (1 g DHA + EPA daily, with a median of 5 years of follow-up) did not reduce cardiovascular mortality and morbidity [63].

There are some limitations to our study. First, in our study, both IVs and outcomes come from Europe. This avoids population stratification and conforms to the homogenous principle of an MR study [11]. However, as few n-3 PUFA GWAS are available for African Americans, Chinese, or other races, the potential effects of n-3 PUFA by race remains to be explored. Similarly, as there are ethnic differences in the risk of cardiovascular disease [64], we need to be cautious in applying our findings to other populations. Second, n-3 PUFA can be detected in many components of the body (e.g., serum, plasma, phospholipids, cholesterol esters, and adipose tissue) and affect many CVD subtypes (e.g., sudden cardiac death, congestive heart failure, arrhythmia, acute coronary syndrome, and stroke), but our study did not analyze this one by one. A comprehensive analysis of n-3 PUFA in different components and their association with different disease subtypes may help to reduce potential bias and provide a better understanding of the effect of n-3 PUFA on cardiovascular health. Third, most instrumental SNPs explain a small proportion variance of n-3 PUFA. This may
reduce the power to detect small effects of n-3 PUFA on IHD risk in our MR framework. Fourth, the effect of n-3 PUFA on IHD and CRFs found in this study represent a lifelong cumulative effect and are not directly comparable to those derived from conventional observational or clinical studies. Finally, we could not assess whether the effect of n-3 PUFA on IHD and CRFs varied by sex, age, or the baseline level of n-3 PUFA as these data are not freely available.

Conclusions
In summary, this study indicates there are favorable effects of plant-derived ALA on IHD and CRFs, but there is no causal association between marine-derived n-3 PUFA (DHA, DPA, EPA) and the risk of IHD. Combined with the more affordable, globally accessible, and sustainable plant sources of ALA, compared to marine-derived n-3 PUFA, our study emphasizes the need to further explore the benefits of ALA on IHD. The benefits of marine-derived n-3 PUFA supplements for cardioprotection remain uncertain and require testing in randomized clinical trials.

Abbreviations
ALA: α-Linolenic acid; BMI: Body mass index; CHD: Coronary heart disease; CRFs: Cardiometabolic risk factors; DBP: Diastolic blood pressure; DHA: Docosahexaenoic acid; DPA: Docosapentaenoic acid; EPA: Eicosapentaenoic acid; GWAS: Genome-wide association studies; HDL: High-density lipoprotein; IHD: Ischemic heart disease; IVs: Instrumental variables; IVW: Inverse variance weighted; LD: Linkage disequilibrium; LDL: Low-density lipoprotein; MI: Myocardial infarction; MR: Mendelian randomization; MR-Egger: Mendelian Randomization Pleiotropy Residual Sum and Outlier; PUFAs: Polyunsaturated fatty acids; RCT: Randomized controlled trial; SBP: Systolic blood pressure; SNP: Single nucleotide polymorphism; STEMI: ST-elevation myocardial infarction; T2D: Type 2 diabetes; TC: Total cholesterol; TG: Triglycerides; WHR: Waist-to-hip ratio; WM: Weighted Median.

Supplementary Information
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Authors’ contributions
BX and XT conceived and designed the study. BX acquired all data in the study and was responsible for the integrity of the data. ZX analyzed the data and took responsibility for the accuracy of the statistical analysis. DX interpreted the results. BX and ZX completed the first draft of the manuscript. XT critically revised the manuscript. All authors reviewed the manuscript and approved the final version.

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Availability of data and materials
All datasets generated and/or analyzed during this study are publicly available.

Declarations
Ethics approval and consent to participate
This study involved analysis of publicly available data and no individual-level data were used. So ethical approval was not required.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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