



The associations of plasma phospholipid arachidonic acid with cardiovascular diseases: A Mendelian randomization study

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ABSTRACT

Background: Arachidonic acid (AA), a major long-chain *n*-6 polyunsaturated fatty acid in animal foods, has been linked to inflammation, coagulation, and testosterone, which might relate to atherosclerotic cardiovascular diseases (ASCVD). We assessed the associations of genetically predicted plasma phospholipid AA with ASCVD and other CVD overall and by sex using Mendelian randomization (MR).

Methods: We conducted two-sample MR, applying eight genetic variants, independent of a highly pleiotropic variant (rs174547), strongly ($p < 5 \times 10^{-8}$) predicting AA, primarily to summary statistics of genetic associations with ASCVD, including ischaemic heart disease (IHD), ischaemic stroke, and peripheral artery disease (PAD) from CARDIoGRAMplusC4D 1000 Genomes (60,801 IHD cases, 123,504 controls), MEGASTROKE (34,217 ischaemic stroke cases, 406,111 controls), and Pan-UK Biobank ($n=420,531$), and secondarily to genetic associations with other CVD from Pan-UK Biobank, Atrial Fibrillation Consortium, HERMES consortium, and FinnGen. We also assessed sex differences.

Findings: Genetically predicted AA was associated with ASCVD (odds ratio (OR) per % of total fatty acids increase 1.03, 95% confidence interval (CI) 1.01 to 1.05) and its subtypes IHD (OR 1.03, 95% CI 1.004 to 1.05), ischaemic stroke (OR 1.03, 95% CI 1.004 to 1.06) and possibly PAD (OR 1.08, 95% CI 1.00 to 1.17), possibly more strongly in men than women. AA was also associated with venous thromboembolism (OR 1.12, 95% CI 1.05 to 1.19). A similar pattern was observed when using rs174547 to genetically predict AA.

Interpretation: Our study suggests positive associations of AA with ASCVD and venous thromboembolism, with possibly stronger associations in men than women.

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1. Introduction

The role of diet in cardiovascular diseases (CVD) has been studied since the 1950s. Despite concerns about sugar [1] and meat consumption [2], the main focus has been on the role of dietary fats [3]. Different types of fats have different effects, with saturated fats mainly raising cholesterol [4]. Dietary guidelines since 1977 have

recommended lowering intake of saturated fatty acids and replacing them with polyunsaturated fatty acids (PUFA) to prevent CVD [5], [6]. However, controversies remain about the effects of specific PUFAs, with recent meta-analyses of randomized controlled trials (RCTs) suggesting that replacing saturated fatty acids with *n*-6 PUFA linoleic acid (LA) has no benefit [7] or possible harm for CVD [8], possibly because different PUFAs potentially have distinct effects on CVD. More recently, icosapent ethyl, a purified *n*-3 eicosapentaenoic acid (EPA) has shown cardiovascular benefit [9], although benefits of combined EPA and docosahexaenoic acid (DHA), the marine long-chain *n*-3 PUFAs recommended for the prevention of CVD [6], are less clear [10]. In contrast, the effect of arachidonic acid (AA), one of the major dietary long-chain *n*-6 PUFA, derived from intake of animal foods (e.g., meat, eggs, and fish, ~200mg/100g) [11] has been less studied, although AA competes with EPA for cyclooxygenase [12] and its metabolites are involved in inflammation and coagulation [13], which are related to atherosclerosis [14] and atherosclerotic CVD (ASCVD).

Abbreviations: AA, arachidonic acid; ASCVD, atherosclerotic cardiovascular disease; CHARGE, Cohorts for Heart and Aging Research in Genomic Epidemiology; CI, confidence interval; CVD, cardiovascular disease; DGLA, dihomogamma linoleic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; GWAS, genome-wide association studies; HERMES, Heart Failure Molecular Epidemiology for Therapeutic Targets consortium; IHD, ischaemic heart disease; IVW, inverse variance weighting; LA, linoleic acid; MR, Mendelian randomization; OR, odds ratio; PAD, peripheral artery disease; PUFA, polyunsaturated fatty acid; RCT, randomized controlled trials; SNP, single-nucleotide polymorphisms; TXA₂, thromboxane A₂; VTE, venous thromboembolism

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Research in Context

Evidence before this study

Arachidonic acid (AA), derived from intake of animal foods (e.g., meat, egg, and fish), is one of the major dietary long-chain *n*-6 PUFA. It is well-established that AA competes with eicosapentaenoic acid (EPA) for cyclooxygenase and lipoxygenase *in vivo* and its metabolites are involved in inflammation and coagulation, which are particularly related to atherosclerosis and atherosclerotic cardiovascular diseases (ASCVD). AA and its metabolites also affect the synthesis and secretion of testosterone in animal studies, which is related to myocardial infarction, stroke, thromboembolism and heart failure in men. Evidence on the effect of AA on ASCVD is limited. Observational studies are open to confounding, while Mendelian randomization (MR) studies suggested some associations largely driven by a highly pleiotropic genetic variant (rs174547) but did not consider sex-specific effects.

Added value of this study

In the present MR study, using genetic variants, independent of the highly pleiotropic variant (rs174547), strongly ($p < 5 \times 10^{-8}$) predicting AA, we provided unconfounded evidence of positive associations of genetically predicted plasma phospholipid AA with ASCVD and venous thromboembolism, independent of the pleiotropic haplotype, with potentially stronger associations in men than women.

Implications of all the available evidence

Our study clarified the role of AA in particularly ASCVD and venous thromboembolism, which may provide insight into the underlying mechanisms and corresponding interventions, as well as potentially elucidating actionable reasons for men being more vulnerable to CVD than women.

Observational findings concerning circulating or tissue levels of AA are mixed, with some studies suggesting no association with CVD [15–17], one showing a positive association with myocardial infarction [18], and another suggesting an inverse association with coronary events [19]. No obvious difference by sex has been observed [15], [18]. Observational studies may be biased by confounding, because factor such as socio-economic position may determine both diet and CVD risk, and can be open to selective reporting [19]. No RCTs to date have examined the effect of AA on CVD [20], with a limited number of short-term trials suggesting no effect of AA supplementation on blood lipids, coagulation or platelet aggregation [21] or a beneficial effect of supplementation of AA plus DHA on coronary circulation [22] which cannot distinguish between their effects.

A small genetic study found that variants from *ALOX5*, likely functionally related to the conversion of AA to proinflammatory leukotrienes, were associated with higher intima-media thickness [23]. More recently, Mendelian randomization (MR), i.e., instrumental variable analysis using genetic variants, has been used to obtain unconfounded effects of some fatty acids, including EPA and DHA, on ischaemic heart disease (IHD) ([24], [25]) and ischaemic stroke [26]. However, many of the findings are driven by a genetic variant (tagged by rs174547 in the *FADS1* gene) [25] homozygous in the people of Flores Island 60 to 100 thousand years ago [27] which encodes a key desaturase in PUFA synthesis and has multiple pleiotropic traits (via fatty acids and other mechanisms) [25], making it difficult to identify specific effects of AA. In addition, previous studies have not considered differences by sex. Using genetic instruments, distinct from the

Flores haplotype, predicting plasma phospholipid AA [28] applied primarily to genome-wide association studies (GWAS) [29–31] of ASCVD (i.e., IHD, ischaemic stroke, and peripheral artery disease (PAD)), and secondarily to associations with other CVD (i.e., rheumatic valve disease, nonrheumatic valve disorder, atrial fibrillation, heart failure, intracranial haemorrhage, aortic aneurysm, and venous thromboembolism (VTE)) ([30], [32], [33]), we used MR to assess the associations of genetically predicted plasma phospholipid AA with ASCVD, other CVD, and their major subtypes. We also assessed whether the associations varied by sex, because AA and its metabolites affect testosterone synthesis and secretion ([34], [35]), which is related to IHD, myocardial infarction, stroke, thromboembolism and heart failure in men [36], [37].

2. Methods

2.1. Genetic instruments for AA

Genetic variants, i.e., single-nucleotide polymorphisms (SNP), strongly ($p < 5 \times 10^{-8}$) associated with plasma phospholipid AA were identified from a GWAS of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium in 8,631 individuals of European ancestry adjusted for age, sex, site and 2–10 principal components [28]. We only included SNPs available for all cohorts with a minor allele frequency $>=0.01$. To ensure genetic instruments specific to AA independent of the Flores haplotype, which affects the fatty acid AA [38] but has many other effects beyond the fatty acid [25], we constructed two sets of instruments for AA, one affecting AA independent of the Flores haplotype (tagged by rs174547) and the other affecting AA only through the Flores haplotype, i.e., rs174547. We assessed instrument strength from the F-statistics calculated using an approximation [39]. An F-statistic of <10 indicates possible weak instruments. We calculated the proportion of AA variance explained by each SNP using an established approximation [40] where variance was obtained from the largest cohort in the GWAS.

For the AA instruments independent of the Flores haplotype, we obtained independent SNPs using MR-Base *ld_clump* with $r^2 < 0.01$ and distance $>10,000$ kb. We checked whether the SNPs were associated with other PUFAs from existing GWAS [28], [41]. We checked for known pleiotropic effects using two comprehensive curated genotype to phenotype cross-references, i.e., *PhenoScanner* and *Ensembl 100* (accessed 2 July 2020). We also checked whether the SNPs were confounded with CVD by assessing their associations with key confounders (i.e., Townsend index, education, alcohol drinking, smoking, and physical activity) in the UK Biobank. We excluded SNPs with known pleiotropic effects or associated with key confounders at $p < 5 \times 10^{-8}$.

2.2. Genetic associations with ASCVD and other CVD and their major subtypes

Genetic associations with ASCVD (i.e., IHD, ischaemic stroke, and PAD) and some other CVD (rheumatic valve disease, nonrheumatic valve disorder, intracranial haemorrhage, and aortic aneurysm), ascertained through linkage to hospital episodes and mortality data, with cases diagnosed according to the International Classification of Diseases (ICD)-9 and/or 10 codes (details in Supplementary Table 1), were taken from the UK Biobank [30] Pan-ancestry summary statistics (Pan-UK Biobank, <https://pan.ukbb.broadinstitute.org/>, released 16 June 2020, assessed 15 August 2020) adjusted for age, sex, age^{*}-sex, age², age²*sex, and 10 principal components. We used summary statistics based on 420,531 individuals of European ancestry only (54% women, age between 40–69 years). IHD cases included myocardial infarction, unstable angina, angina pectoris, coronary atherosclerosis, and other chronic, subacute, and acute IHD. Genetic

associations with IHD ($n_{\text{case}} = 60,801$, $n_{\text{control}} = 123,504$) in individuals of mainly European ancestry (77%) and ischaemic stroke ($n_{\text{case}} = 34,217$, $n_{\text{control}} = 406,111$) in individuals of European ancestry only were also obtained from the CARDIoGRAMplusC4D 1000 Genomes [29] and the MEGASTROKE consortium [31], respectively, adjusted for study specific covariates and genomic control. In CARDIoGRAMplusC4D 1000 Genomes IHD cases included myocardial infarction, acute coronary syndrome, chronic stable angina, and coronary stenosis >50%. The participants in CARDIoGRAMplusC4D 1000 Genomes and MEGASTROKE do not overlap with the UK Biobank. Genetic associations with atrial fibrillation ($n_{\text{case}} = 65,446$, $n_{\text{control}} = 522,744$) in individuals of mainly European ancestry (91%), heart failure ($n_{\text{case}} = 47,309$, $n_{\text{control}} = 930,014$) in individuals of European ancestry only, and VTE ($n_{\text{case}} = 5,403$, $n_{\text{control}} = 130,235$) in individuals of Finnish ancestry only were taken from the Atrial Fibrillation Consortium [33], the Heart Failure Molecular Epidemiology for Therapeutic Targets (HERMES) consortium [32], and FinnGen (<https://finngen.gitbook.io/documentation/>, released 5 June 2020, assessed 25 August 2020), respectively, adjusted for study specific covariates and genomic control. Sex-specific genetic associations with CVD subtypes in white British were taken from the UK Biobank [30] (<http://www.nealelab.is/uk-biobank/>, released 31 July 2018, assessed 10 August 2020) in 167,020 men and 194,174 women aged between 40–69 years. The estimates derived from linear regressions adjusted for age, age², and 20 principal components for all CVDs were transformed to log odds ratios using an established approximation [42].

2.3. Statistical analysis

We aligned effect allele for exposure and outcomes and further aligned on effect allele frequency for palindromic SNPs. For SNPs not available for an outcome, we sought a highly correlated proxy ($r^2 \geq 0.8$) in *LDlink* (Accessed 25 August 2020). We used MR inverse variance weighting (IVW) estimates with multiplicative random-effects [43] to assess the overall and sex-specific associations of genetically predicted AA with each CVD. We obtained an overall estimate for IHD by meta-analyzing estimates from the UK Biobank and CARDIoGRAMplusC4D 1000 Genomes and for ischaemic stroke by meta-analyzing estimates from the UK Biobank and MEGASTROKE with random effects. We obtained sex-specific estimates for ASCVD by meta-analyzing the sex-specific estimates for IHD, ischaemic stroke, and PAD. We assessed sex differences for ASCVD and each CVD subtype using a two-sided z-test [44]. In sensitivity analysis, we used the weighted median which is valid even when up to 50% of the information is from invalid SNPs [45]. We also used MR Egger which is robust to genetic pleiotropy but assumes pleiotropic effects independent of the genetic associations with the exposure [39]. A non-zero intercept indicates directional pleiotropy (some genetic variants act not through AA).

A two-sided p -value of < 0.05 was considered as significant for sex differences. All the analyses were conducted using R version 3.6.3 (The R Foundation for Statistical Computing, Vienna, Austria) and the “MendelianRandomization” and “metafor” R packages. No ethical approval was required because we used only publicly available summary data.

2.3.1. Role of the funding source

No funding.

3. Results

We identified eight uncorrelated SNPs, independent of rs174547 ($r^2 < 0.05$ in *LDlink*) (Supplementary Table 2), strongly ($p < 5 \times 10^{-8}$) predicting plasma phospholipid AA (Supplementary Table 3). Of the eight SNPs, rs2903922, rs2760306 and rs472031 were associated with other n -6 PUFAs ($p < 5 \times 10^{-8}$), including LA and dihomogamma

linoleic acid (DGLA), but these associations were weaker than the associations with AA. Rs1741 was more significantly associated with DGLA and LA (Supplementary Table 4). None of the eight SNPs was associated with any n -3 PUFA at genome-wide significance ($p < 5 \times 10^{-8}$) but six of them were associated with EPA at nominal significance ($p < 0.05$) (Supplementary Table 4). None of the eight SNPs was associated with any known pleiotropic traits or key confounders in the UK Biobank ($p < 5 \times 10^{-8}$) (Supplementary Table 5), all had F -statistics > 10, and together accounted for a total of 5.3% variance in AA. Rs174547 explained more than 30% of variance in AA. All of the SNPs were available for each outcome (Supplementary Figure 1).

Based on eight SNPs independent of rs174547, overall genetically predicted AA was positively associated with ASCVD, and with IHD in the UK Biobank and CARDIoGRAMplusC4D 1000 Genomes combined and ischaemic stroke in the UK Biobank and MEGASTROKE combined but not with PAD (Fig. 1). Based on the eight SNPs, genetically predicted AA was positively associated with VTE but not with other CVDs (Fig. 2). Based on rs174547, overall genetically predicted AA was positively associated with ischaemic stroke and PAD, suggestively associated with ASCVD, but not with IHD. It was also associated with aortic aneurysm, heart failure and VTE, and overall other CVD (Fig. 2). In the UK Biobank, based on eight SNPs independent of rs174547, genetically predicted AA was associated with ASCVD in men but not women but the sex difference was not significant ($p = 0.25$ (z-test)). Based on rs174547, associations were directionally similar for ASCVD (Supplementary Figure 2).

The weighted median and MR Egger gave directionally similar estimates. The intercept of MR Egger generally indicated no pleiotropy, except for IHD in CARDIoGRAMplusC4D 1000 Genomes (p for intercept = 0.043) (Supplementary Table 6). The analyses after excluding rs1741 showed similar results for the associations of AA with CVD in both sexes (Supplementary Figure 3).

4. Discussion

Our MR study suggests genetically predicted plasma phospholipid AA is associated with ASCVD (IHD, stroke and possibly PAD) and VTE, but not with other CVDs, independent of the Flores haplotype rs174547, with possibly a stronger association for ASCVD in men than women. These findings are consistent with some [18], [46] but not all [15–17], [19] observational studies of the association of circulating or tissue AA with ASCVD or its subtypes but add by providing an estimate less open to residual and unmeasured confounding. Our findings add to previous MR studies by using larger GWASs, assessing the associations with a wider range of CVDs, and distinguishing effects of AA from effects of the highly pleiotropic Flores haplotype [25], [26].

Several biological mechanisms might explain the adverse effect of AA on ASCVD and VTE. First, AA-derived eicosanoids by cyclooxygenase, including thromboxane A₂ (TXA₂), prostaglandins (PGE₂, PGF_{2 α} , PGD₂) and prostacyclin (PGI₂), are involved in vessel tone regulation, platelet aggregation and coagulation, and inflammation, which may play roles in atherosclerosis and ASCVD [14]. AA-derived leukotrienes by lipoxygenase could affect the progression of hyperlipidaemia-dependent vascular disease and are related to atherogenesis, IHD, and stroke [13]. 20-hydroxyeicosatetraenoic acid via cytochrome P450 can lead to hypertension and vascular endothelium damage [47]. Furthermore, AA itself can be metabolized to isoprostanes, which are linked to platelet aggregation, smooth muscle cell proliferation, and cardiomyocyte hypertrophy [13]. Moreover, free AA could induce oxidative stress by altering nicotinamide adenine dinucleotide phosphate (NADPH) oxidase mediated production of reactive oxygen species, which may induce insulin resistance and lead to ASCVD [13]. Alternatively, AA competes with EPA for cyclooxygenase and lipoxygenase, which may hinder the production of EPA-derived anti-

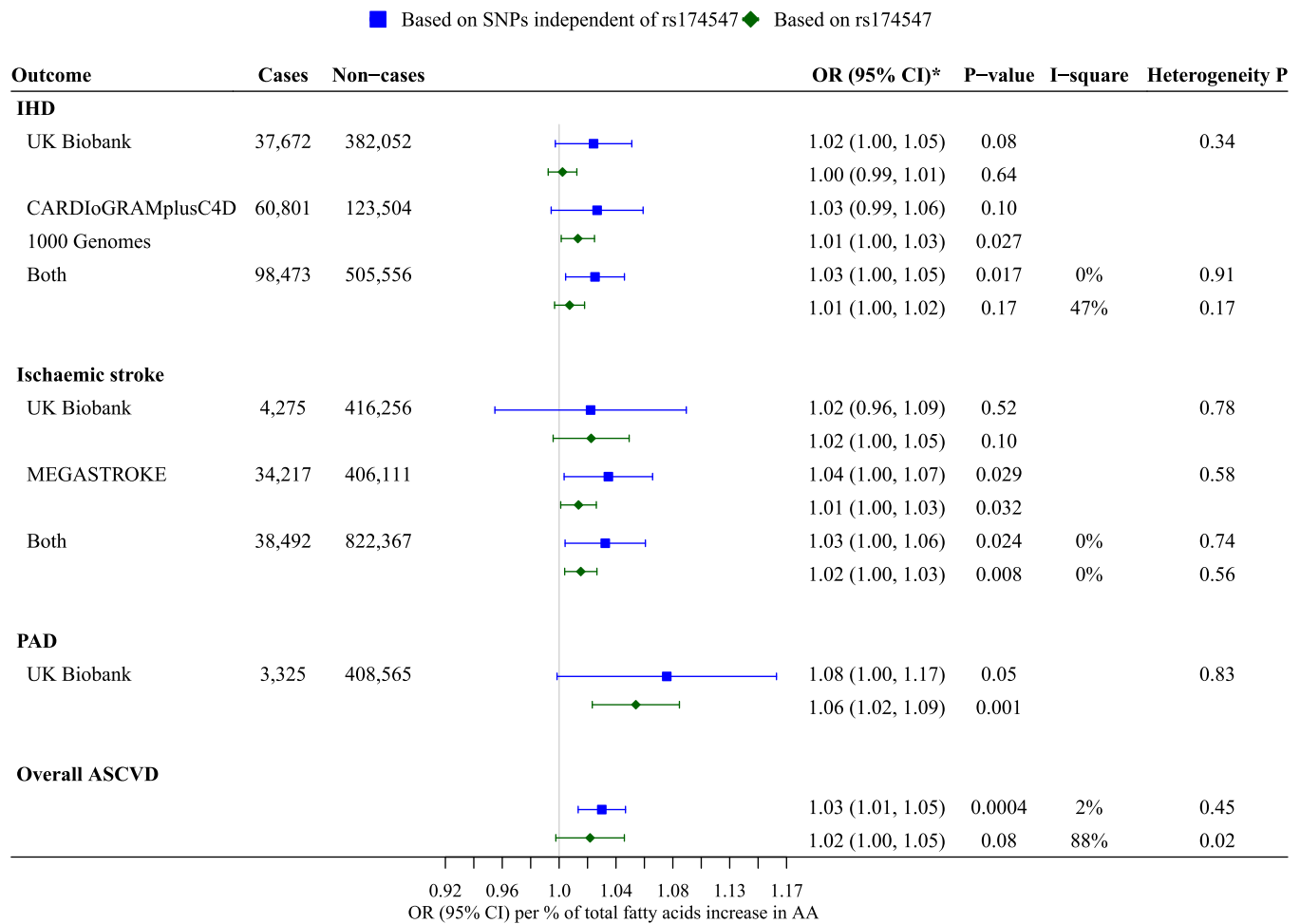


Fig. 1. Associations of genetically predicted plasma phospholipid arachidonic acid with ischaemic heart disease (Pan-UK Biobank: $n_{\text{case}} = 37,672$, $n_{\text{control}} = 382,052$; CARDIoGRAMplusC4D 1000 Genomes: $n_{\text{case}} = 60,801$, $n_{\text{control}} = 123,504$), ischaemic stroke (Pan-UK Biobank: $n_{\text{case}} = 4,275$, $n_{\text{control}} = 416,256$; MEGASTROKE: $n_{\text{case}} = 34,217$, $n_{\text{control}} = 406,111$), peripheral artery disease (Pan-UK Biobank: $n_{\text{case}} = 3,325$, $n_{\text{control}} = 408,565$), and overall atherosclerotic cardiovascular diseases in both sexes.

The error bar indicated the lower and upper limits of 95% confidence interval of the estimate (odds ratio). The scale bar indicated the odds ratio in CVD risk per % of total fatty acids increase in AA.

* For each CVD, the estimates based on eight SNPs independent of rs174547 were obtained from random-effect inverse variance weighting, while the estimates based on rs174547 were obtained from Wald-type estimator. The estimates for overall IHD, overall ischaemic stroke, and overall ASCVD were derived from random-effect meta-analysis.

ASCVD, atherosclerotic cardiovascular disease; CI, confidence interval; IHD, ischaemic heart disease; OR, odds ratio; PAD, peripheral artery disease.

inflammatory eicosanoids [48] thus indirectly leading to ASCVD. AA composition in blood cells might be at the expense of EPA, which may influence blood viscosity and flexibility thus likely increasing thrombosis formation [12]. Nevertheless, available evidence from RCTs on these potential pathways is limited [21].

To our knowledge, this is the first MR study examining the sex-specific effects of a fatty acid on CVD. Our findings suggest a possibly stronger association of genetically instrumented AA with ASCVD in men than women. Although the AA cascade has been linked to inflammation and coagulation [13], and may play a role in testosterone synthesis and release [34], [35], limited evidence of sex-specific associations of AA with ASCVD exists, with observational studies suggesting no obvious sex difference [15], [18]. AA and its metabolite TXA₂ and prostaglandins may stimulate the formation and secretion of testosterone [34], [35] and affect its action [49], which is increasingly recognized as related to thromboembolism, myocardial infarction, and heart failure in men [36]. Evidence from RCTs shows a more pronounced effect of aspirin, a cyclooxygenase inhibitor and thromboxane A₂ blocker, on major CVD events in men than women [50]. In addition, AA-derived 20-hydroxyeicosatetraenoic acid via cytochrome P450 might have a role in testosterone-induced endothelial dysfunction and hypertension, as shown in animal studies [47].

This study has some limitations. First, MR has stringent assumptions of relevance, independence and exclusion restriction. We selected genetic instruments associated with AA at genome-wide significance ($p < 5 \times 10^{-8}$), which had F-statistics > 10. Any slight overlap of the sample for AA with outcome GWAS, including CARDIoGRAMplusC4D 1000 Genomes, MEGASTROKE consortium, HERMES consortium, and Atrial Fibrillation Consortium (~2%), is unlikely to create a bias. We checked that the genetic instruments for AA were independent of key potential confounders (i.e., Townsend index, education, alcohol drinking, smoking, and physical activity) in the UK Biobank. Almost all of the GWAS we used were in people of European descent only, except for CARDIoGRAMplusC4D 1000 Genomes (23% of non-European ancestry) and Atrial Fibrillation Consortium (9% of non-European ancestry) which could be open to bias from population stratification. However, the two GWAS were corrected for genomic control [29], [33], and results for IHD were consistent using Pan-UK Biobank and CARDIoGRAMplusC4D1000 Genomes. We specifically selected genetic instruments independent of the highly pleiotropic Flores haplotype rs174547 [25], [27]. The genetic instruments for AA were not associated with known pleiotropic traits or any n-3 PUFA at genome wide significance. One SNP (rs1741) was more significantly associated with other n-6 PUFAs, but

■ Based on SNPs independent of rs174547 ◆ Based on rs174547

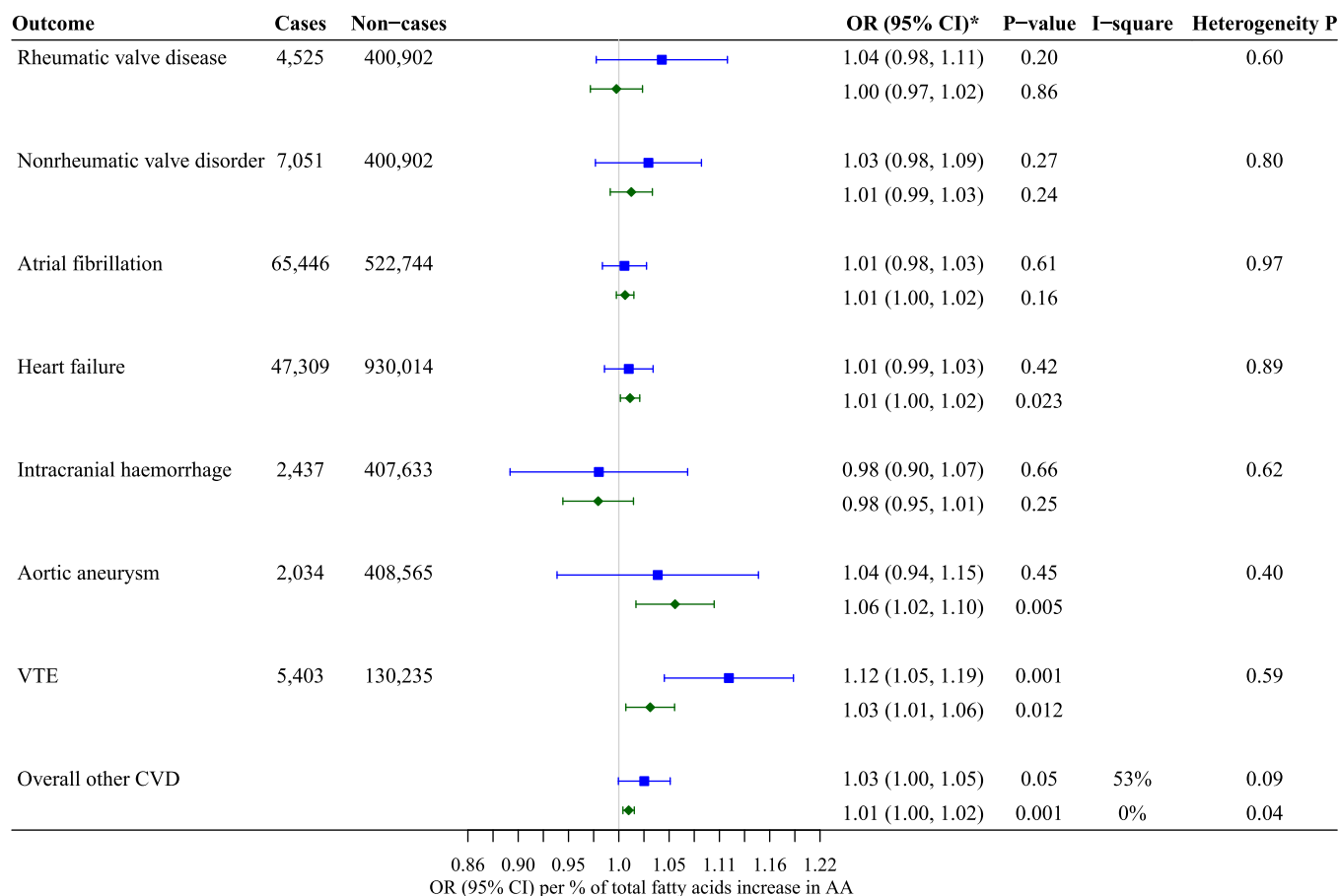


Figure 2. Associations of genetically predicted plasma phospholipid arachidonic acid with rheumatic valve disease (Pan-UK Biobank: $n_{\text{case}} = 4,525$, $n_{\text{control}} = 400,902$), nonrheumatic valve disorder (Pan-UK Biobank: $n_{\text{case}} = 7,051$, $n_{\text{control}} = 400,902$), atrial fibrillation (Atrial Fibrillation Consortium: $n_{\text{case}} = 65,446$, $n_{\text{control}} = 522,744$), heart failure (HERMES: $n_{\text{case}} = 47,309$, $n_{\text{control}} = 930,014$), intracranial haemorrhage (Pan-UK Biobank: $n_{\text{case}} = 2,437$, $n_{\text{control}} = 407,633$), aortic aneurysm (Pan-UK Biobank: $n_{\text{case}} = 2,034$, $n_{\text{control}} = 408,565$), venous thromboembolism (FinnGen: $n_{\text{case}} = 5,403$, $n_{\text{control}} = 130,235$), and overall other cardiovascular diseases in both sexes.

The error bar indicated the lower and upper limits of 95% confidence interval of the estimate (odds ratio). The scale bar indicated the odds ratio in CVD risk per % of total fatty acids increase in AA.

* For each CVD, the estimates based on eight SNPs independent of rs174547 were obtained from random-effect inverse variance weighting, while the estimates based on rs174547 were obtained from Wald-type estimator. The estimates for overall other CVD were derived from random-effect meta-analysis.

CI, confidence interval; CVD, cardiovascular disease; OR, odds ratio; VTE, venous thromboembolism.

sensitivity analyses excluding rs1741 showed similar results. We also used sensitivity analyses to assess the possibility of violation of the exclusion-restriction assumption by genetic pleiotropy. However, six of the eight SNPs were associated with EPA at nominal significance ($P < 0.05$), thus it cannot be ruled out that the association of AA with ASCVD may be partly driven by EPA. Second, the role of endogenous AA might not correspond to that of exogenous AA intake. However, plasma AA composition relates to dietary or supplemental AA intake dose-dependently over a wide range of AA intake (82–3600 mg/day) [11]. Third, the findings might not apply beyond Europeans or to populations that do not consume animal foods, although causes usually act consistently but are not always relevant [51]. Fourth, the genetic variants predicting AA in the sex-specific analysis were from both sexes. Accordingly, the sex-specific estimates might be conservative but the directions should be unchanged. Fifth, influential genetic variants might be attenuated by compensatory or feedback mechanisms, likely biasing estimates toward the null [52]. Sixth, selection bias is possible for stroke and other CVD typically occurring later than IHD, which may bias the associations towards the null. Seventh, the CVD cases in the overall and sex-specific samples of the UK Biobank were ascertained at different timepoints (two years later for the overall sample) and based on slightly different ICD codes, which may lead to

some discrepancies in the estimates, particularly for PAD. Finally, some participants may have multiple correlated CVDs, so meta-analyses of the sex-specific estimates for overall ASCVD or other CVD may overestimate the precision of the estimates. However, co-existing CVD could also be consequences of AA.

5. Conclusions

Our study suggests positive associations of genetically predicted plasma phospholipid AA with ASCVD and VTE, independent of the Flores haplotype rs174547, with potentially stronger associations in men than women. Clarifying the roles of AA in different types of CVD may provide insight into the underlying mechanisms and corresponding interventions, as well as potentially elucidating actionable reasons for men being more vulnerable to CVD than women.

Data Sharing Statement

The datasets generated and/or analysed during the current study are publicly available.

Funding Sources

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Author Contributions

CMS designed the study. TZ analysed the data and drafted the paper. CMS and JVZ critically revised the paper. All authors read and approved the final manuscript.

Declaration of Competing Interests

All the authors have nothing to disclose.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ebiom.2020.103189.

References

- [1] Yudkin J. Patterns and trends in carbohydrate consumption and their relation to disease. *Proc Nutr Soc* 1964;23:149–62.
- [2] Oppenheimer GM, Benrubi ID. McGovern's Senate Select Committee on Nutrition and Human Needs versus the meat industry on the diet-heart question (1976–1977). *Am J Public Health* 2014;104(1):59–69.
- [3] Keys A. Diet and the epidemiology of coronary heart disease. *J Am Med Assoc* 1957;164(17):1912–9.
- [4] Keys A. Seven countries. A multivariate analysis of death and coronary heart disease. Harvard University Press; 1980.
- [5] US Senate Select Committee on Nutrition and Human Needs. 1st ed. Washington DC: US Government Printing Office; 1977.
- [6] US Department of Health and Human Services and US Department of Agriculture. 2015–2020 Dietary Guidelines for Americans. 8th Edition. Available from: <https://health.gov/dietaryguidelines/2015/guidelines/>.
- [7] Ramsden CE, Zamora D, Majchrzak-Hong S, Faurot KR, Broste SK, Frantz RP, et al. Re-evaluation of the traditional diet-heart hypothesis: analysis of recovered data from Minnesota Coronary Experiment (1968–73). *BMJ (Clinical research ed)* 2016;353:i1246.
- [8] Ramsden CE, Zamora D, Leelarthaeapin B, Majchrzak-Hong SF, Faurot KR, Suchindran CM, et al. Use of dietary linoleic acid for secondary prevention of coronary heart disease and death: evaluation of recovered data from the Sydney Diet Heart Study and updated meta-analysis. *BMJ (Clinical research ed)* 2013;346:e8707.
- [9] Bhatt DL, Steg PG, Miller M, Brinton EA, Jacobson TA, Ketchum SB, et al. Cardiovascular risk reduction with icosapent ethyl for hypertriglyceridemia. *N Engl J Med* 2019;380(1):11–22.
- [10] Aung T, Halsey J, Kromhout D, Gerstein HC, Marchioli R, Tavazzi L, et al. Associations of Omega-3 Fatty Acid supplement use with cardiovascular disease risks: meta-analysis of 10 trials involving 77917 individuals. *JAMA Cardiol* 2018;3(3):225–34.
- [11] Kawashima H. Intake of arachidonic acid-containing lipids in adult humans: dietary surveys and clinical trials. *Lipids Health Dis* 2019;18(1):101.
- [12] DiNicolantonio JJ, OK J. Importance of maintaining a low omega-6/omega-3 ratio for reducing platelet aggregation, coagulation and thrombosis. *Open heart* 2019;6(1):e001011.
- [13] Sonnweber T, Pizzini A, Nairz M, Weiss G, Tancevski I. Arachidonic acid metabolites in cardiovascular and metabolic diseases. *Int J Mol Sci* 2018;19(11).
- [14] Piper K, Garelnabi M. Eicosanoids: atherosclerosis and cardiometabolic health. *J Clin Transl Endocrinol* 2020;19:100216.
- [15] Marklund M, Wu JHY, Imamura F, Del Gobbo LC, Fretts A, de Goede J, et al. Biomarkers of Dietary Omega-6 fatty acids and incident cardiovascular disease and mortality. *Circulation* 2019;139(21):2422–36.
- [16] Harris WS, Tintle NL, Ramachandran VS. Erythrocyte n-6 fatty acids and risk for cardiovascular outcomes and total mortality in the framingham heart study. *Nutrients* 2018;10(12).
- [17] Sakai M, Kakutani S, Tokuda H, Suzuki T, Kominami M, Egawa K, et al. Arachidonic acid and cerebral ischemia risk: a systematic review of observational studies. *Cerebrovasc Dis Extra* 2014;4(3):198–211.
- [18] Nielsen MS, Schmidt EB, Stegger J, Gorst-Rasmussen A, Tjonneland A, Overvad K. Adipose tissue arachidonic acid content is associated with the risk of myocardial infarction: a Danish case-cohort study. *Atherosclerosis* 2013;227(2):386–90.
- [19] Chowdhury R, Warnakula S, Kunutsor S, Crowe F, Ward HA, Johnson L, et al. Association of dietary, circulating, and supplement fatty acids with coronary risk: a systematic review and meta-analysis. *Ann Intern Med* 2014;160(6):398–406.
- [20] Hooper L, Al-Khudairy L, Abdelhamid AS, Rees K, Brainard JS, Brown TJ, et al. Omega-6 fats for the primary and secondary prevention of cardiovascular disease. *Cochrane Database Syst Rev* 2018;11:Cd011094.
- [21] Calder PC, Campoy C, Eilander A, Fleith M, Forsyth S, Larsson PO, et al. A systematic review of the effects of increasing arachidonic acid intake on PUFA status, metabolism and health-related outcomes in humans. *Br J Nutr* 2019;121(11):1201–14.
- [22] Oe H, Hozumi T, Murata E, Matsuura H, Negishi K, Matsumura Y, et al. Arachidonic acid and docosahexaenoic acid supplementation increases coronary flow velocity reserve in Japanese elderly individuals. *Heart* 2008;94(3):316–21.
- [23] Dwyer JH, Allayee H, Dwyer KM, Fan J, Wu H, Mar R, et al. Arachidonate 5-lipoxygenase promoter genotype, dietary arachidonic acid, and atherosclerosis. *N Engl J Med* 2004;350(1):29–37.
- [24] Zhao JV, Schooling CM. Effect of linoleic acid on ischemic heart disease and its risk factors: a Mendelian randomization study. *BMC Med* 2019;17(1):61.
- [25] Yuan S, Back M, Bruzelius M, Mason AM, Burgess S, Larsson S. Plasma Phospholipid Fatty Acids, FADS1 and risk of 15 cardiovascular diseases: a mendelian randomisation study. *Nutrients* 2019;11(12).
- [26] Yuan T, Si S, Li Y, Li W, Chen X, Liu C, et al. Roles for circulating polyunsaturated fatty acids in ischemic stroke and modifiable factors: a Mendelian randomization study. *Nutr J* 2020;19(1):70.
- [27] Tucci S, Vohr SH, McCoy RC, Vernot B, Robinson MR, Barbieri C, et al. Evolutionary history and adaptation of a human pygmy population of Flores Island. Indonesia. *Sci*. 2018;361(6401):511–6.
- [28] Guan W, Steffen BT, Lemaitre RN, Wu JHY, Tanaka T, Manichaikul A, et al. Genome-wide association study of plasma N6 polyunsaturated fatty acids within the cohorts for heart and aging research in genomic epidemiology consortium. *Circ Cardiovasc Genet* 2014;7(3):321–31.
- [29] Nikpay M, Goel A, Won HH, Hall LM, Willenborg C, Kanoni S, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. *Nat Genet* 2015;47(10):1121–30.
- [30] Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, et al. The UK Biobank resource with deep phenotyping and genomic data. *Nature* 2018;562(7726):203–9.
- [31] Malik R, Chauhan G, Traylor M, Sargurupremraj M, Okada Y, Mishra A, et al. Multi-ancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet* 2018;50(4):524–37.
- [32] Shah S, Henry A, Roselli C, Lin H, Sveinbjörnsson G, Fatemifar G, et al. Genome-wide association and Mendelian randomisation analysis provide insights into the pathogenesis of heart failure. *Nat Commun* 2020;11(1):163.
- [33] Roselli C, Chaffin MD, Weng LC, Aeschbacher S, Ahlberg G, Albert CM, et al. Multi-ethnic genome-wide association study for atrial fibrillation. *Nat Genet* 2018;50(9):1225–33.
- [34] Romanelli F, Valenca M, Conte D, Isidori A, Negro-Vilar A. Arachidonic acid and its metabolites effects on testosterone production by rat Leydig cells. *J Endocrinol Invest* 1995;18(3):186–93.
- [35] Erkan LG, Altinbas B, Guvenc G, Alcay S, Toker MB, Ustuner B, et al. Brain thromboxane A2 via arachidonic acid cascade induces the hypothalamic-pituitary-gonadal axis activation in rats. *Auton Neurosci* 2015;189:50–5.
- [36] Luo S, Au Yeung SL, Zhao JV, Burgess S, Schooling CM. Association of genetically predicted testosterone with thromboembolism, heart failure, and myocardial infarction: mendelian randomisation study in UK Biobank. *BMJ (Clinical research ed)* 2019;364:l476.
- [37] Schooling CM, Luo S, Au Yeung SL, Thompson DJ, Karthikeyan S, Bolton TR, et al. Genetic predictors of testosterone and their associations with cardiovascular disease and risk factors: a mendelian randomization investigation. *Int J Cardiol* 2018;267:171–6.
- [38] Koletzko B, Reischl E, Tanjung C, Gonzalez-Casanova I, Ramakrishnan U, Meldrum S, et al. FADS1 and FADS2 polymorphisms modulate fatty acid metabolism and dietary impact on health. *Annu Rev Nutr* 2019;39:21–44.
- [39] Bowden J, Del Greco MF, Minelli C, Davey Smith G, Sheehan NA, Thompson JR. Assessing the suitability of summary data for two-sample Mendelian randomization analyses using MR-Egger regression: the role of the I2 statistic. *Int J Epidemiol* 2016;45(6):1961–74.
- [40] Falconer D, Mackay T. Introduction to Quantitative Genetics. 4th ed. Essex, England Longman: Harlow, England: Prentice Hall; 1996.
- [41] Lemaitre RN, Tanaka T, Tang W, Manichaikul A, Foy M, Kabagambe EK, et al. Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis

- of genome-wide association studies from the CHARGE Consortium. *PLoS Genet* 2011;7(7):e1002193.
- [42] Lloyd-Jones LR, Robinson MR, Yang J, Visscher PM. Transformation of summary statistics from linear mixed model association on all-or-none traits to odds ratio. *Genetics* 2018;208(4):1397–408.
- [43] Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* 2013;37(7):658–65.
- [44] Paternoster R, Brame R, Mazerolle P, Piquero A. Using the correct statistical test for equality of regression coefficients. *Criminology* 1998;36:859–66.
- [45] Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol* 2016;40(4):304–14.
- [46] Kark JD, Kaufmann NA, Binka F, Goldberger N, Berry EM. Adipose tissue n-6 fatty acids and acute myocardial infarction in a population consuming a diet high in polyunsaturated fatty acids. *Am J Clin Nutr* 2003;77(4):796–802.
- [47] Fava C, Ricci M, Melander O, Minuz P. Hypertension, cardiovascular risk and polymorphisms in genes controlling the cytochrome P450 pathway of arachidonic acid: a sex-specific relation? *Prostaglandins Other Lipid Mediat* 2012;98(3–4):75–85.
- [48] Dessì M, Noce A, Bertucci P, Manca di Villahermosa S, Zenobi R, Castagnola V, et al. Atherosclerosis, dyslipidemia, and inflammation: the significant role of polyunsaturated Fatty acids. *ISRN Inflamm* 2013;2013:191823.
- [49] Gupta C, Goldman AS. The arachidonic acid cascade is involved in the masculinizing action of testosterone on embryonic external genitalia in mice. *Proc Natl Acad Sci U S A* 1986;83(12):4346–9.
- [50] Gelbenegger G, Postula M, Pecun L, Halvorsen S, Lesiak M, Schoergenhofer C, et al. Aspirin for primary prevention of cardiovascular disease: a meta-analysis with a particular focus on subgroups. *BMC medicine* 2019;17(1):198.
- [51] Lopez PM, Subramanian SV, Schooling CM. Effect measure modification conceptualized using selection diagrams as mediation by mechanisms of varying population-level relevance. *J Clin Epidemiol* 2019;113:123–8.
- [52] Haycock PC, Burgess S, Wade KH, Bowden J, Relton C, Davey Smith G. Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. *Am J Clin Nutr* 2016;103(4):965–78.