Omega-3 Fatty Acids and Acute Coronary Syndrome

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ABSTRACT

Omega-3 fatty acids have been promoted as cardioprotective nutrients but there is some controversy regarding their role in the prevention of cardiovascular diseases. Additionally, knowledge of their effects on acute coronary syndrome (ACS) is limited.

We conducted a systematic review and meta-analysis to investigate the association between the consumption of fish, which are main source of omega-3 fatty acids, and the risk for ACS in the general population. We found that fish consumption was inversely associated with ACS risk. A dose-response relationship was observed and each 100 g serving of fish per week was associated with a 5% risk reduction in ACS.

To provide more insight on the mechanisms through which omega-3 fatty acids may act to influence ACS risk, we investigated whether omega-3 fatty acid intake modulates the genetic predisposition to ACS. We undertook a case-only gene-diet interaction study of individuals with early onset ACS, using data from the GENESIS-PRAXY (GENdEr and Sex determInantS of Cardiovascular Disease from bench to beyond in PRemature Acute Coronary Syndrome) study. Our results indicate that potential interactions may exist between omega-3 fatty acids and ACS-associated polymorphisms, but replication is necessary for definite evidence.

In conclusion, our findings suggest that omega-3 fatty acids are beneficial for ACS risk reduction and may interact with polymorphisms that confer ACS risk. Additional research is required to fully understand the mechanisms of omega-3 fatty acids in modifying the genetic risk of ACS.

RÉSUMÉ

Les acides gras oméga-3 ont été promus en tant que nutriments cardioprotecteurs mais il y a une certaine controverse quant à leur rôle dans la prévention des maladies cardiovasculaires. Par ailleurs, la connaissance de leurs effets sur le syndrome coronarien aigu (SCA) est limitée.

Nous avons effectué une revue systématique avec méta-analyse pour étudier l'association entre la consommation de poisson et le risque de SCA dans la population générale. Nos résultats ont démontré que la consommation de poisson était inversement associée au risque de SCA. Une relation dose-réponse a été observée et chaque portion de 100 g de poisson par semaine était associée à une diminution de 5 % du risque de SCA.

Afin de mieux comprendre les mécanismes par lesquels les acides gras oméga-3 pourraient agir pour influencer le risque de SCA, nous avons étudié si l'apport en acides gras oméga-3 modulerait la prédisposition génétique au SCA. Nous avons entrepris une étude d'interaction gène-alimentation de personnes ayant présenté un SCA de façon prématurée, en utilisant les données de l'étude GENESIS-PRAXY (GENdEr and Sex determInantS of Cardiovascular Disease from bench to beyond in PRemature Acute Coronary Syndrome). Nos résultats suggèrent que des interactions pourraient exister entre les acides gras oméga-3 et les polymorphismes associés avec le SCA, mais des réplications futures sont nécessaires pour des réponses définitives.

En conclusion, les acides gras oméga-3 seraient bénéfiques pour la réduction du risque de SCA et pourraient interagir avec les polymorphismes qui confèrent un risque d'avoir le SCA. Des recherches supplémentaires sont nécessaires afin d'élucider les mécanismes des acides gras oméga-3 dans la modification du risque génétique de SCA.

PREFACE

This manuscript-based thesis incorporates two manuscripts:

- Manuscript #1 “Fish Consumption and Acute Coronary Syndrome: a Meta-Analysis”
- Manuscript #2 “Omega-3 Fatty Acids and the Genetic Risk of Early Onset Acute Coronary Syndrome”

The thesis conforms to the requirements of McGill University for manuscript-based theses. The manuscripts were integrated with the mandatory thesis components, and connecting texts were included to provide a logical progression. Since manuscripts are concisely written research reports intended for scientific journals, additional sections were also incorporated to provide a more detailed description of some aspects of the manuscripts. Some redundancy is inevitable in a manuscript-based thesis, particular regarding the general introduction and discussion of the thesis and those of the individual manuscripts, but all effort was made to present a coherent scholarly work. References were combined in a single chapter after the final conclusion.

CONTRIBUTION OF AUTHORS

As the first author of both manuscripts, I was involved in all stages of the manuscript, including study conception and design, statistical analysis, interpretation of results, and manuscript writing and revision.

Manuscript #1: Dr. Pilote, as thesis supervisor and senior author supervised all aspects of the work executed. Drs. Pilote, Stark and Thanassoulis contributed to the critical review of the paper.

Manuscript #2: Dr. Pilote obtained funding as a principal investigator of GENESIS-PRAXY and helped conceptualize the study question and design. Drs. Pilote, Thanassoulis, Engert, Stark and Avgil Tsadok provided guidance for the analyses and data interpretation, and critically reviewed the paper.

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LIST OF ABBREVIATIONS

ACS	acute coronary syndrome
AHA	American Heart Association
ALA	α-linolenic acid
BMI	body mass index
CI	confidence interval
CVD	cardiovascular diseases
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FFQ	food frequency questionnaire
GWAS	genome-wide association studies
HWE	Hardy-Weinberg equilibrium
MI	myocardial infarction
MUFA	monounsaturated fatty acids
SES	socioeconomic status
SFA	saturated fatty acids
SNP	single nucleotide polymorphism
OR	odds ratio
IQR	interquartile range
RR	risk ratio
PUFA	polyunsaturated fatty acids
VLDL	very low-density lipoprotein

1 INTRODUCTION

1.1 Overview of Cardiovascular Diseases and Acute Coronary Syndrome

Cardiovascular diseases (CVD) are a leading cause of morbidity and mortality and are projected to remain the principal cause of death worldwide[1]. About one-third of all global deaths are attributable to CVD and it is estimated that the number of CVD-related deaths will reach 23.3 million by 2030[1, 2]. Acute coronary syndrome (ACS), which consists of myocardial infarction (MI) with or without ST-segment elevation and unstable angina, accounts for the majority of deaths due to CVD and portends a substantial disease and economic burden[3, 4]. According to the Heart Disease and Stroke Statistics 2013 Update by the American Heart Association (AHA), ACS resulted in more than 1.1 million hospitalizations in the United States in 2010[5]. Yet, this disease burden is occurring increasingly frequently at unnecessarily young ages and for the most part would be preventable by adopting a healthy lifestyle, including a cardioprotective diet[6].

1.2 Omega-3 Fatty Acids and Cardiovascular Diseases

A high intake of omega-3 fatty acids from fatty or oily fish has been suggested to be cardioprotective[6]. The omega-3 fatty acids found in fish are the polyunsaturated fatty acids (PUFA) eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), in contrast to α-linolenic acid (ALA; 18:3n-3) that is plant-derived and found primarily in nuts, flax seeds and canola oil. EPA and DHA can be biosynthesized from ALA, but this bioconversion is limited in humans, and therefore blood and tissue levels of EPA and DHA are largely dependent on dietary intake of preformed EPA and DHA[7].

The interest in omega-3 fatty acids and CVD started about half a century ago, when low rates of acute MI were observed among Greenland Inuit populations, despite their high fat diets[8]. This "Inuit Paradox" led to a multiple epidemiological studies in the 1970's, revealing an association between the large amounts of fish consumed by this population and a decreased cardiovascular risk[9]. Following these initial investigations, a vast number of animal model studies, observational studies and clinical trials were conducted, and omega-3 fatty acids were found to be associated with many cardiovascular benefits[7]. The beneficial effects of omega-3 fatty acids in CVD have been ascribed mainly to the marine-derived EPA and DHA, and hence, the use of the term omega-3 fatty acids in the scientific literature usually refers to these two main constituents[10].

Today, national and international nutritional guidelines from recognized health agencies and professional organizations advocate the importance of omega-3 fatty acids for both the primary and secondary prevention of CVD[7]. Both fish consumption and omega-3 fatty acid supplements are promoted as a means to increase omega-3 fatty acid intake. Recommendations for the general population include the consumption of two servings of fish per week, preferably fatty fish, for an average of 250 mg of EPA+DHA per day. Among those with documented CVD, 1 g of EPA+DHA per day is advised through fish consumption or supplements as needed. Among patients who need to lower triglycerides levels, the recommendations suggest taking fish oil supplements providing 2 to 4 g of EPA+DHA per day, as advised under physician care[11]. Yet, controversy has been recently raised when it was demonstrated from meta-analyses that omega-3 fatty acid supplements did not have an effect on cardiovascular risk[12, 13].

The effect of omega-3 fatty acids in CVD is complex and involves multiple mechanisms[14]. One of the ways through which omega-3 fatty acids may influence disease status is through the interaction with genetic factors[15]. Drastic changes from earlier diets to the current Western diets have unfavorably changed the omega-6 to omega-3 fatty acid ratio[10, 16]. It is presumed that inadequate evolutionary adaptation to those dramatic transformations in the typical diet has brought about interactions between diet and certain genetic polymorphisms that could increase CVD risk[17]. However, there is a paucity of information regarding such interactions.

To date, the majority of studies investigating the association between omega-3 fatty acids and CVD have examined their role in secondary prevention and/or have looked at combined outcomes or cardiovascular mortality as an outcome. The exact role of omega-3 fatty acids in ACS remains to be determined. To fully understand the biological mode of action of omega-3 fatty acids, exploration of the potential modulatory effect of omega-3 fatty acids in the genetic risk for ACS is also needed.

1.3 Aim and Objectives

This thesis aims to investigate the effect of omega-3 fatty acids in ACS. Specifically, the objectives were:

1) To systematically evaluate the association between fish consumption and ACS among healthy adults; and
2) To examine whether there are interactions between omega-3 fatty acids and genetic polymorphisms that predispose to ACS among patients with early onset ACS.

2 LITERATURE REVIEW

Despite clinical observations of the cardiometabolic properties of omega-3 fatty acids, many of the specific molecular mechanisms have not been fully elucidated. In this chapter, a review of the mechanisms of actions of omega-3 fatty acids and a meta-analysis on the association between fish consumption and ACS are presented.

2.1 Overview of the Mechanisms of Actions of Omega-3 Fatty Acids

Evidence suggests that omega-3 fatty acids have an array of effects on physiological cardiovascular risk factors as well as on cardiovascular outcomes. Animal experiments and human studies have demonstrated that omega-3 fatty acids regulate genes in various tissues, by acting as cell signaling molecules, secondary messengers or modulator molecules, or by directly altering the transcription of specific genes[15].

2.1.1 Triglyceride-Lowering Effects

Omega-3 fatty acids lower plasma triglycerides via the regulation of genes that are important in lipid homeostasis[18]. Intakes of 3 to 4 g of omega-3 fatty acids have been shown to reduce plasma triglycerides by 20 to 50%[19]. The mode of action includes the inhibition of the assembly and secretion of hepatic very low-density lipoprotein (VLDL), which results in a reduction in fatty acid substrates for triglyceride synthesis due to decreased de novo lipogenesis, increased β-oxidation of fatty acids and reduced transportation of nonesterified fatty acids to the liver[7, 18, 19].

2.1.2 Improvement of Endothelial Function

Various trials have demonstrated that supplementation with omega-3 fatty acids improves endothelial function[20]. This may be mediated through the incorporation of omega-3 fatty acids into cellular phospholipids and the simultaneous reduction of omega-6 fatty acids[20]. It has been postulated that omega-3 fatty acids may have a role in the synthesis of endothelial nitric oxide and may promote nitric oxide-induced endothelial relaxation[7, 10]. Additionally, DHA may decrease the expression of vascular cell adhesion molecule 1, intercellular adhesion molecule 1, E-selectin, interleukin-6 and interleukin-8 in endothelial cells, thereby reducing endothelial dysfunction[18, 21].

2.1.3 Anti-Inflammatory Effects

Atherosclerosis, the main cause of ACS, arises and develops as a consequence of inflammation[22, 23]. Omega-3 fatty acids are key PUFA that may hinder inflammatory processes. Omega-3 fatty acids in the diet are incorporated in the phospholipid membranes of platelets, endothelial cells and inflammatory cells, thereby displacing the omega-6 fatty acid arachidonic acid (20:6n-6)[24]. The replacement of arachidonic acid by omega-3 fatty acids results in the decreased production of arachidonic acid-derived pro-inflammatory eicosanoids, including prostaglandins, thromboxanes, leukotrienes, and hydroxyeicosatetraenoic acids[25, 26]. In addition, EPA can act as a competitive substrate for cyclo-oxygenase and lipoxygenase enzymes to produce alternative eicosanoids that are less inflammatory[27]. Moreover, omega-3 fatty acids may be involved in the regulation of inflammatory gene expression by modulating transcription factors such as nuclear factor kappa B and peroxisome proliferator-activated receptors[24].

2.1.4 Inhibition of Platelet Aggregation and Thrombosis

Omega-3 fatty acids may lower CVD risk by inhibiting platelet aggregation, thus decreasing the risk for thrombosis[7, 18]. In vitro studies indicate that omega-3 fatty acids act as antagonists of pro-aggregatory thromboxane A2/prostaglandin H2 receptor in human platelets[28, 29]. As well, omega-3 fatty acids may down-regulate the expression of platelet-derived growth factors A and B in mononuclear blood cells[30].

2.1.5 Reduction of Blood Pressure and Heart Rate

Evidence suggests that omega-3 fatty acids have a dose-dependent hypotensive effect, reducing both systolic and diastolic blood pressure, and also decrease resting heart rate[31-33]. Potential mechanisms include direct effects on electrophysiological pathways, as well as indirect effects such as the improvement of ventricular diastolic filling and augmentation of vagal tone[7].

2.1.6 Anti-Arrhythmic Effects

The anti-arrhythmic properties of omega-3 fatty acids have been indicated by various studies[7, 10, 18, 34]. Direct influences on atrial and ventricular myocyte electrophysiology have been suggested. Omega-3 fatty acids are potent inhibitors of membrane ion channels in cardiac myocytes, including voltage-gated sodium channels, and have an effect on cell-cell connexins, which contribute to reduced arrhythmia[7, 34].

2.1.7 Inhibition of Plaque Formation

Omega-3 fatty acids have been indicated as being anti-atherogenenic and inhibiting plaque formation. Atherosclerotic plaques have been shown to be dynamic and responsive to dietary modification[35]. Omega-3 fatty acids, particularly EPA, can readily incorporate into atherosclerotic plaques and decrease the infiltration of inflammatory and immune cells as well as decrease their activity in the plaque[36]. In turn, this results in structural changes and improved plaque stability. Furthermore, it has been demonstrated that supplementation with omega-3 fatty acid ethyl esters lowers the expression of inflammatory genes in plaques[37].

In summary, omega-3 fatty acids exert a range of actions which include the improvement of triglyceride levels, endothelial function, inflammation, platelet aggregation, thrombosis, blood pressure, arrhythmia and plaque stability. The cardioprotective effects of omega-3 acids appear to be due to the integration of these various intricate signaling mechanisms, rather than through a single pathway.

2.2 Preface to Manuscript #1

Recently, the cardioprotective role of omega-3 fatty acids was challenged, when meta-analyses failed to show evidence for an association between omega-3 fatty acid supplements and cardiovascular outcome risk reduction[12, 13]. The contradictory findings on omega-3 fatty acids and clinical outcomes raise an important question in that omega-3 fatty acids may have a potentially different effect depending on whether they come from supplements or food sources such as fish. The effects may also differ in primary vs. secondary prevention. Given that there is no comprehensive synthesis on the effects of fish consumption in the primary prevention of ACS, we evaluated the association between fish consumption and ACS in healthy adults by systematically reviewing the literature and conducting a dose-response meta-analysis.

The abstract of this meta-analysis was presented as a moderated poster at the AHA EPI/NPAM (Epidemiology and Prevention/Nutrition, Physical Activity and Metabolism) 2014 Scientific Sessions and published in Circulation (Circulation 2014;129:AMP65). The manuscript was accepted at The American Journal of Medicine and is in press (Am J Med 2014 pii:S0002-9343(14)00355-6), and has been formatted according to the journal's guidelines.

2.3 Manuscript #1: Fish Consumption and Acute Coronary Syndrome: a Meta-Analysis

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Abstract

Background: Findings on the association between fish consumption and acute coronary syndrome are inconsistent. We assessed the role of fish consumption in acute coronary syndrome by conducting a dose-response meta-analysis.

Methods: We conducted a literature search of Medline and Embase databases from 1966 to June 2013 for prospective cohort and case-control studies that evaluated the association between fish consumption and acute coronary syndrome among general populations without cardiovascular disease history. Additional studies were identified via hand search of references of relevant articles. Estimates of relative risk (RR) were pooled using random effects model. Sex and age effects were also evaluated.

Results: Our search retrieved 11 prospective cohort and 8 case-control studies, totaling 408,305 participants. Among prospective cohort studies, the highest category of fish consumption, i.e. ≥4 times per week, was associated with the greatest risk reduction in acute coronary syndrome (RR 0.79, 95% CI 0.70-0.89). In dose-response analysis, each additional 100 g serving of fish per week was associated with a 5% reduced risk (RR per serving 0.95, 95% CI 0.92-0.97). Subgroup analysis and meta-regression suggested that the risk reduction did not differ across sex or age groups. No heterogeneity was observed among prospective cohort (p=0.73) and case-control (p=0.29) studies. There was no evidence of publication bias.

Conclusion: Our meta-analysis demonstrated that there is an inverse association between fish consumption and the risk of acute coronary syndrome. Fish consumption appears beneficial in the primary prevention of acute coronary syndrome and higher consumption is associated with greater protection.

Introduction

Fish, especially fatty fish, are a rich source of omega-3 fatty acids. Omega-3 fatty acids are polyunsaturated fatty acids, consisting of eicosapentaenoic acid (EPA; 20:5) and docosahexaenoic acid (DHA; 22:6), which have been shown to have anti-inflammatory, anti-thrombotic and anti-arrhythmic effects, improve blood lipid profile, and help in vascular relaxation and plaque stability[38]. Yet, controversy exists as to the efficacy of omega-3 fatty acids in preventing cardiovascular diseases and recent meta-analyses have indicated that omega-3 fatty acid supplements are not associated with cardiovascular disease risk reduction[13, 39]. Conversely, it appears that fish, as opposed to omega-3 fatty acid supplements, may be beneficial to cardiovascular health, and the distinction between fish versus omega-3 fatty acid intake requires further exploration.

Evidence from meta-analyses indicate the cardioprotective effects of fish consumption in relation to different endpoints such as cerebrovascular diseases, heart failure and overall cardiovascular mortality[40-44], but none of these reviews have assessed the role of fish consumption in the primary prevention of acute coronary syndrome. Findings from observational studies, including long-term prospective cohort and case-control studies, have not been consistent, with some studies, but not all, reporting an association between fish consumption and acute coronary syndrome. A systematic review and quantitative analysis of these studies is therefore needed to clarify the association between fish consumption and acute coronary syndrome.

The aim of this study was to investigate the association between fish consumption and acute coronary syndrome by conducting a dose-response meta-analysis. As a

secondary objective, we evaluated whether this association varied according to sex and age.

Methods

The Meta-analysis Of Observational Studies in Epidemiology (MOOSE) protocol[45] was followed throughout the design, implementation, analysis and reporting of this systematic review and meta-analysis study.

Study Selection

We conducted a literature search of Medline and Embase databases from 1966 to June 2013 for studies that evaluated the association between fish consumption and acute coronary syndrome, using search terms for fish ("fish" OR "fish meat" OR seafood") in combination with those for acute coronary syndrome ("acute coronary syndrome" OR "myocardial infarction" OR "heart infarction" OR "heart infarct") (Supplement S1). References of relevant articles were hand searched to identify additional studies. Studies were included if they met the following criteria: 1) prospective cohort or case-control study design; 2) fish consumption as exposure of interest; 3) acute coronary syndrome as outcome of interest; 4) the most adjusted relative risk (RR) and 95% confidence interval (CI) were reported; 5) the study population consisted of general adult populations without pre-existing disease or without a previous acute coronary syndrome event; 6) for dose-response analysis, the number of cases and participants or person-years for each category of fish consumption were reported (or data were available to calculate them). We restricted studies to those published in English or French. If a study reported a measure of

association and 95% CI for men and women, the results were treated as two separate studies in the meta-analysis. For studies that reported results only by different types of acute coronary syndrome (e.g. fatal and nonfatal myocardial infarction) or by different types of fish (e.g. low- and high-fat fish), the RRs were pooled. Finally, if data were shared or duplicated in more than one study, the first published or most detailed one was included in the analysis.

Data Extraction

Relevant data were independently extracted by two reviewers using a predesigned data collection form. Disagreements were resolved by consensus through discussion, or upon consultation of a third reviewer. The collected data included first author's last name, year of publication, country where the study was conducted, duration and person-years of follow-up, sample size and proportion of men and women, mean and range of age, type and number of acute coronary syndrome events, method used to assess fish consumption, categories of fish consumption, most adjusted RR and corresponding 95% CI for each category of fish consumption, and the variables included in the multivariable model.

Quality Assessment

Quality assessment was performed using the Newcastle Ottawa Scale, which is one of the most comprehensive and valid tools available for assessing the quality of non-randomized studies (cohort and case-control studies) in meta-analyses[46]. Scores range

from 0 to 9, with a higher score indicating better methodological quality. Studies with a score ≥7 were considered as being of higher quality.

Statistical Analysis

As some studies reported category of consumption in frequency, we first standardized these intake levels by converting frequency into grams per day, using 100 g as a standard portion size for fish as per dietary guidelines. The mean or median fish intake per category of each study was then used to categorize the levels of intake into five standardized intervals, namely "less than once per month" (the reference category), "1 to <4 times per month", "1 to <2 times per week", "2 to <4 times per week", and "≥4 times per week". We assumed that the reported reference exposure category from all studies represented a level of intake that was similar to the standardized reference category. When a range of intake was reported rather than the mean or median, the midpoint value of the upper and lower boundaries of the fish intake category was used as the average intake. If the highest level of intake was open ended (e.g. ≥5 times per week), we assumed that that boundary had the same magnitude as the closest category. If a study did not report the lower boundary of the lowest category of fish consumption, it was considered to be zero. Additionally, if the mean or median or average amount of fish consumption from 2 or more categories of a single study fell into the same standardized category of fish intake, the estimates were pooled. Dose-response meta-analysis was conducted using generalized least squares method for trend estimation (GLST) of summarized dose-response data[47]. Restricted cubic splines with 3 knots at fixed percentiles were used to examine potential nonlinear relationship between fish

consumption and acute coronary syndrome. Potential departure from a linear relationship was assessed by testing the null hypothesis that the coefficient of the second spline is equal to zero. Furthermore, we conducted subgroup analysis and meta-regression to investigate the effect of sex and age on the potential association between fish consumption and acute coronary syndrome.

The DerSimonian and Laird random effects model[48], which considers both within- and between-study variation, was used to calculate summary estimates and 95% CIs for each category of fish consumption. Heterogeneity among studies was assessed by using the I^2 statistic test[49]. Potential publication bias was assessed by using funnel plots, Egger's regression asymmetry test[50] and Begg's rank correlation test[51]. All statistical analyses were conducted using STATA version 12.1 (StataCorp, College Station, TX, USA).

Results

Literature Search

The initial search identified 1,185 potentially relevant articles (276 from MEDLINE and 909 from Embase). Among them, 246 were duplicates. Two additional articles were identified via hand search. After initial screening, based on titles and abstracts, 37 articles remained. After full-text assessment, 18 articles were excluded for various reasons. Thus, the final set of studies consisted of 19 studies (Figure 1).

Study Characteristics

The combined studies included 11 prospective cohort[52-62] and 8 case-control[63-70] studies, totaling 408,305 participants (398,177 for prospective cohort and 10,128 for case-control studies), with 47.1% being male. The mean age was 55.8 years (age range 20 to 84 years). There were 8,517 cases of acute coronary syndrome. The majority of the studies were from US (5 studies) and Japan (3 studies). The remaining were from various European countries and one study was from China. Among prospective cohort studies, the average follow-up time was 11.2 years, ranging from 4 to 30 years (Table 1; Table 2).

Fish Consumption and Acute Coronary Syndrome

Among prospective cohort studies, a significant association was observed between fish consumption and a reduced risk of acute coronary syndrome (Table 3; Figure 2). The highest category of fish consumption, i.e. ≥4 times per week, was associated with the greatest risk reduction in acute coronary syndrome (RR 0.79, 95% CI 0.70-0.89). No evidence of heterogeneity was found (I^2=0.0%, p=0.73). Although most of the studies (9 out of 11) were of high quality, we conducted an analysis to exclude the studies of low quality, and obtained similar results. Since all except 2 studies reported hazard ratios (HRs) as measures of association, we performed a separate analysis for these studies. The results were similar, with the highest category of fish consumption being associated with a 21% reduced risk of acute coronary syndrome (HR 0.79, 95% CI 0.69-0.90). In sensitivity analysis excluding the largest study, we also found a significant reduction in the risk of acute coronary syndrome (RR 0.76, 95% CI 0.66-0.88). Furthermore, the results did not differ according to country.

Among case-control studies, fish consumption also appeared to reduce the risk of acute coronary syndrome (RR 0.76, 95% CI 0.67-0.87 for 1 to <2 times per week), but the association was non-significant for the highest versus lowest category. Similar to prospective cohort studies, little heterogeneity was observed (I^2=20.2%, p=0.29). Generally, the case-control studies were of poorer quality, and only 2 studies were considered as being of high quality, with their score being 7 each. In sensitivity analysis, no substantial change was seen after excluding the largest study.

Dose-Response Analysis

Eight prospective cohort studies provided sufficient data to conduct a dose-response analysis. It was found that each additional 100 g serving of fish per week was associated with a risk reduction in acute coronary syndrome by 5% (RR 0.95, 95% CI 0.92-0.97). We did not find evidence for a nonlinear relationship (p=0.92).

Effect of Sex and Age

There were 6 studies (5 prospective cohort and 1 case-control studies) that were conducted among men only and 4 studies (2 prospective cohort and 2 case-control studies) that were among women only. The subgroup analysis of prospective cohort studies of men versus women did not support a sex difference in the association between fish intake and acute coronary syndrome (RR 0.84, 95% CI 0.70-1.01 and RR 0.80, 95% CI 0.61-1.06 for men and women respectively). There was also no evidence that the association differed by sex according to meta-regression (RR 0.95, 95% CI 0.56-1.64).

In additional meta-regression analyses where we included age as a continuous variable, there was no significant effect of age on the association between fish consumption and acute coronary syndrome. However, there was only modest variation in age across the included studies, with most studies having a mean age in the range of 47 to 62 years.

Publication Bias

No evidence of publication bias was found among the prospective cohort and case-control studies. This was supported by funnel plots which did not show presence of asymmetry (Supplement S2), as well as the Egger's test (p=0.60) and the Begg's test (p=0.44).

Discussion

Our meta-analysis demonstrated that there is an inverse association between fish consumption and the risk of acute coronary syndrome. We observed a greater risk reduction with increasing fish intake, and with each additional 100 g serving per week, the risk of acute coronary syndrome was further reduced by 5%.

This study represents an updated investigation of the role of fish consumption in acute coronary syndrome. While several meta-analyses of observational studies have evaluated the effect of fish consumption on different cardiovascular outcomes[71-75], none of them have specifically examined acute coronary syndrome as an outcome. The landmark DART trial demonstrated that fish consumption was beneficial for the secondary prevention of myocardial infarction, with ≥2 servings of fish per week being

associated with reduced cardiovascular mortality risk[76]. Yet, the benefits of omega-3 fatty acid supplements in the secondary prevention of cardiovascular diseases remain controversial in light of recent meta-analyses that have combined the evidence from randomized controlled trials and found no association[13, 39]. Additionally, there is also a need to distinguish between primary versus secondary prevention. We explored the relationship between fish and acute coronary syndrome among generally healthy populations, as opposed to clinical trials. Our findings from prospective cohort studies confirm that fish consumption is beneficial in the primary prevention of acute coronary syndrome.

The potential differential effect of omega-3 fatty acids from fish versus supplements has previously been highlighted. It has been suggested that the bioavailability and functioning of nutrients obtained from foods compared to supplements may differ[77]. A 6-week experimental study comparing salmon to fish oil capsules found that EPA and DHA from dietary fish were more effectively incorporated into plasma lipids, leading to higher plasma concentrations of omega-3 fatty acids[78]. In another study where participants were randomly assigned to the consumption of oily fish or omega-3 fatty acid capsules providing the same amount of EPA and DHA, EPA content in erythrocytes was found to rise more rapidly in the fish group[79]. Thus, while omega-3 fatty acids have been shown to favorably impact markers of cardiovascular diseases, whether they come from a complex food matrix such as fish, or are in an isolated form, could possibly explain why differences are observed between fish versus omega-3 fatty acid supplements.

It has also been indicated that adherence to high omega-3 fatty acid diets in nutritional interventions may be poor[80]. It is plausible that one of the reasons why fish have a cardioprotective effect relative to supplements is that adherence to fish consumption may be higher. Fish can be included in the diet as a high quality protein food and contain a wide array of other valuable nutrients including vitamins (A, D, B_3, B_6, B_{12}) and minerals (calcium, phosphorus, selenium, iron, magnesium, potassium, iodine). These nutrients have diverse properties that are beneficial to the overall health. Higher vitamin D levels have also been linked to reduced acute coronary syndrome mortality and morbidity[81]. Hence, when considering omega-3 fatty acid intake for cardiovascular benefits, a diet-based approach including fish, rather than supplements, may be warranted.

Most of the studies included in this meta-analysis used food frequency questionnaires to assess fish consumption. Although fish consumption is a recognized proxy for omega-3 fatty acid intake, blood concentrations of omega-3 fatty acids may reflect dietary intake more strongly[82]. Some studies have suggested that low blood levels of omega-3 fatty acids may be associated with an increased risk of acute coronary syndrome[83-85]. The omega-3 index, which is the sum of EPA and DHA in erythrocyte membranes, expressed as a percentage of total erythrocyte fatty acids, is a novel biomarker of blood concentrations of omega-3 fatty acids[86]. The omega-3 index has been indicated as a reliable biomarker for assessing long-term omega-3 fatty acid intake, and fish consumption has been shown to strongly correlate with higher omega-3 index[87]. It has also been shown that men are better able to increase blood levels of omega-3 fatty acids with dietary advice on fish intake, likely because men consume larger portion sizes

than women, which may not be captured by dietary assessment with food frequency questionnaires[80].

This study summarizes the evidence from observational studies but presents with some limitations. Our meta-analysis consisted of studies that adjusted for many important confounders including socioeconomic status and various lifestyle behaviors such as different dietary factors, physical activity, and smoking. However, residual confounding is still possible, given that fish consumption may be associated with healthier lifestyle behaviors that are difficult to measure. Another limitation is that we had a relatively small sample of studies which cannot eliminate the possibility of publication bias. Whether the association between fish consumption and acute coronary syndrome differed by sex was inconclusive, possibly due to our small number of studies. We also did not find an age effect, but this could be due to the narrow age range. It would have been equally interesting to evaluate whether the association differed by type of fish or acute coronary syndrome, but we did not have enough studies to conduct these analyses.

In conclusion, fish consumption appears to be beneficial in the primary prevention of acute coronary syndrome. An inverse association between fish consumption and the risk of acute coronary syndrome was observed. A dose-response relationship was found between fish consumption and the reduced risk of acute coronary syndrome. Future studies are needed to further investigate whether sex differences and age effects exist. Moreover, more research is required to elucidate whether this association varies according to the type of fish.

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Disclosures

None.

Table 1. Characteristics of Included Cohort Studies on Fish Consumption and Acute Coronary Syndrome

Author, year of publication (country)	Years & duration of follow-up	Number of participants	Mean age (range), years	Number of cases (outcome)	Dietary assessment tool	Categories of fish consumption	Covariates
Ascherio, 1995 (US)[52]	1986-1992 6	44,895 men	57.5 (40-75)	547 (nonfatal MI)	self-administered FFQ	<1/mo, 1-3/mo, 1/wk, 2-3/wk, 4-5/wk, ≥6/wk	age, BMI, smoking, alcohol consumption, history of hypertension, history of diabetes, history of hypercholesterolemia, family history of MI before 60 years, profession, intake of n-3 fatty acids
Bjerregaard, 2010 (Denmark)[53]	1993-2003 7.6	25,573 men 28,653 women	56 (50-64)	854 (nonfatal MI)	self-administered FFQ	0-24 g/d, 25-35 g/d, 36-47 g/d, 48-64 g/d, >64 g/d (men); 0-22 g/d, 23-31 g/d,32-41 g/d, 42-54 g/d, >55 g/d (women)	education, smoking, alcohol intake, BMI, history of diabetes, systolic blood pressure, serum cholesterol, physical activity, dietary intake of fruits and vegetables, total energy intake, dietary intake of SFA, MUFA and PUFA, menopausal status
Daviglus, 1997 (US)[54]	1957-1959 30	1,822 men	47.6 (40-55)	293 (fatal MI)	questionnaire-based interview by nutritionists	0 g/d, 1-17 g/d, 18-34 g/d, ≥35 g/d	age, education, religion, systolic pressure, serum cholesterol, number of cigarettes smoked, BMI, diabetes, electrocardiographic abnormalities, intake of energy, cholesterol, SFA, MUFA, PUFA, total protein, carbohydrate, alcohol, iron, thiamine, riboflavin, niacin, vitamin C, beta carotene and retinol
de Goede, 2010 (Netherlands)[55]	1993-2007 11.3	9,604 men 11,738 women	42.1 (20-65)	64 (fatal MI) 252 (nonfatal MI)	self-administered FFQ	<3.3 g/d, 3.3-7.3 g/d, 7.4-14 g/d, >14 g/d	age, sex, BMI, total energy intake, ethanol intake, smoking, SES, vitamin or mineral supplement use, use of drugs for hypertension or hypercholesterolemia, family history of CVD, SFA intake, fruit and vegetable intake
Hu, 2002 (US)[56]	1980-1994 16	84,688 women	46.5 (34-59)	1,029 (nonfatal MI)	self-administered FFQ	<1/mo, 1-3/mo, 1/wk, 2-4/wk, ≥5/wk	age, time periods, smoking, BMI, alcohol intake, menopausal status, post-menopausal hormone use, vigorous to moderate activity, use of aspirin, multivitamin use, vitamin E supplement use, history of hypertension, hypercholesterolemia, diabetes, intake of trans fat, ratio PUFA:SFA, dietary fiber
Iso, 2006 (Japan)[57]	1990-2001 10	19,985 men 21,593 women	49.5 (40-59)	198 (total MI)	self-administered FFQ	median 23 g/d, 51 g/d, 78 g/d, 114 g/d,	age, sex, smoking, alcohol intake, BMI, history of hypertension and diabetes, medication use for

						180 g/d	hypercholesterolemia, education, sports at leisure time, dietary intake of fruits, vegetables, SFA, MUFA, n-6 PUFA, cholesterol and total energy, public health centres
Kuhn, 2013 (Germany)[58]	1994-1998 8.1	20,292 men 28,023 women	50.5 (35-65)	605 (total MI)	self-administered FFQ	<7.5 g/d, 7.5-14.5 g/d, 14.5-21.5 g/d, 21.5-31.1 g/d, >31.1 g/d	age, sex, study centres, energy intake, alcohol intake, BMI, waist circumference, physical activity, education, smoking, diabetes
Morris, 1995 (US)[59]	1982-1988 4	21,185 men	62 (40-84)	281 (total MI)	self-administered FFQ	<1/wk, 1/wk, 2-4/wk, ≥5/wk	age, level of fish consumption, aspirin and beta-carotene assignment, smoking, alcohol consumption, obesity, diabetes, vigorous exercise, parental history of MI before 60, history of hypertension, history of hypercholesterolemia, vitamin supplement use, SFA intake
Mozaffarian, 2003 (US)[60]	1989-2000 9.3	1,526 men 2,384 women	72.7 (≥65)	363 (nonfatal MI)	self-administered FFQ picture-sort version	1/mo, 1-3/mo, 1/wk, 2/wk, ≥3/wk	age, sex, education, diabetes, smoking, pack-years of smoking, tuna/other fish and fried fish/fish sandwich consumption, BMI, systolic blood pressure, LDL cholesterol, HDL cholesterol, triglycerides, C-reactive protein, intake of SFA, alcohol, beef/pork, fruits and vegetables
Yamagishi, 2008 (Japan)[61]	1988-2003 12.7	22,881 men 35,091 women	56.1 (40-79)	329 (fatal MI)	self-administered FFQ	median 20 g/d, 33 g/d, 45g/d, 62 g/d, 86 g/d (men); 21 g/d, 33 g/day, 46g/d, 62 g/d, 85 g/d (women)	age, sex, history of hypertension and diabetes, smoking, alcohol consumption, BMI, mental stress, walking, sports, education, total energy, dietary intake of cholesterol, SFA, n-6 PUFA, vegetables and fruits
Yuan, 2001 (China)[62]	1986-1989 12	18,244 men	55.8 (45-64)	113 (fatal MI)	questionnaire-based interview	<30 g/wk, 30-<60 g/wk, 60-<100 g/wk, 100-<150 g/wk, ≥150 g/wk	age, total energy intake, level of education, BMI, smoking, number of cigarettes smoked, number of alcoholic drinks consumed, history of diabetes, history of hypertension

ACS: acute coronary syndrome; BMI: body mass index FFQ: food frequency questionnaire; MI: myocardial infarction; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SES: socioeconomic status; SFA: saturated fatty acids.

Table 2. Characteristics of Included Case-Control Studies on Fish Consumption and Acute Coronary Syndrome

Author, year of publication (country)	Years	Number of participants	Mean age (range), years	Number of cases (outcome)	Dietary assessment tool	Categories of fish consumption	Covariates
Gramenzi, 1990 (Italy)[63]	1983-1989	936 women	49 (21-69)	287 (total MI)	questionnaire-based interview	<1/wk, 1/wk, >1/wk	age, area of residence, education, smoking, hyperlipidaemia, diabetes, hypertension, BMI, intake of carrots, green vegetables, fresh fruit, meat, ham and salami, butter, total fat score, coffee consumption, alcohol consumption
Lockheart, 2007 (Norway)[64]	1995-1997	211 men and women	62.35 (45-75)	106 (total MI)	interview using FFQ	median 32 g/d, 99 g/d (low-fat); 12 g/d, 52 g/d (high-fat)	age, marital status, education, family history of heart disease, smoking, energy intake
Martinez-Gonzalez, 2002 (Spain)[65]	1999-2001	277 men 65 women	61.6 (<80)	171 (total MI)	self-administered FFQ	<60 g/d, >77 g/d	age, sex, hospital, smoking, BMI, high blood pressure, high blood cholesterol, diabetes, leisure-time physical activity, SES, intake of olive oil, fiber, fruits, vegetables, alcohol, meat/meat products and white bread/rice/pasta
Oliveira, 2010 (Portugal)[66]	1999-2003	1,460 men 1,556 women	52 (33-69)	820 (nonfatal MI)	interview using FFQ	median <35.5 g/d, ≥35.5 g/d (excluding cod); <13.5 g/d, ≥13.5 g/d (cod)	sex, age, education, total energy intake, intake of fruit, refined cereals and white meat, smoking, regular physical activity, family history of MI, BMI, menopause, hormone replacement therapy
Panagiotakos, 2005 (Greece)[67]	2000-2001	1,562 men 364 women	60.1 (49-75)	848 (nonfatal ACS)	questionnaire-based interview	never, <150 g/wk, 150-300 g/wk, >300 g/wk	age, sex, smoking, hypertension, hypercholesterolemia, HDL cholesterol, LDL cholesterol, diabetes, physical inactivity, BMI, food items consumed
Sasazuki, 2001 (Japan)[68]	1996-1998	1,340 men 506 women	59.5 (40-79)	458 (nonfatal MI)	questionnaire-based interview	<2/wk, 2-3/wk, ≥4/wk	smoking, alcohol use, sedentary job, leisure-time physical activity, hyperlipidemia, hypertension, diabetes, angina pectoris, obesity, tofu consumption, fruit consumption
Tavani, 2001 (Italy)[69]	1995-1999	675 men 310 women	60 (25-79)	507 (nonfatal MI)	interview using FFQ	<1/wk, 1-<2/wk, ≥2/wk	age, sex, education, BMI, cholesterol, smoking, coffee, alcohol, meat, vegetables, fruit, calorie intakes, physical activity, hyperlipidemia, diabetes, hypertension, family history of MI in first-degree relatives

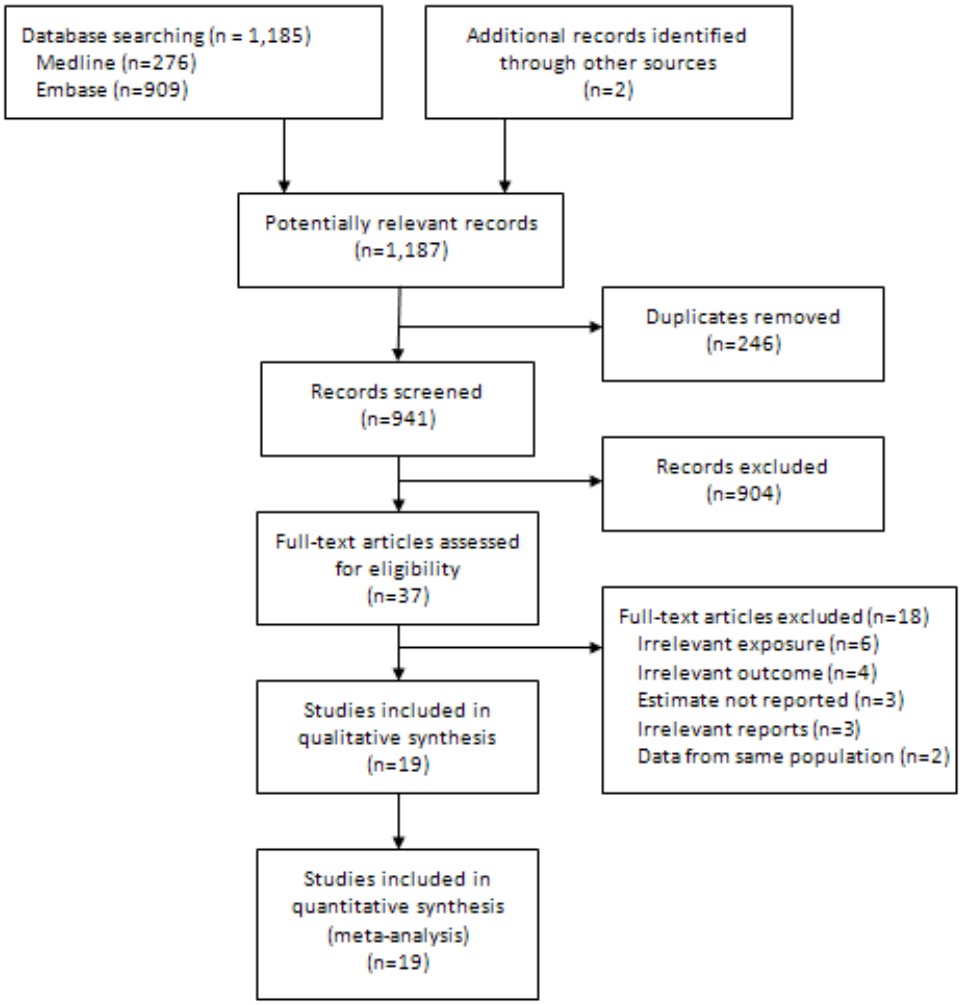
Wennberg, 2011 (Sweden)[70]	1987-1999	648 men 218 women	58.7 (34-77)	392 (total MI)	self-administered FFQ	<1/mo, 1/mo-<1/wk, 1-2/wk, >2/wk	apolipoprotein B/apolipoprotein A-I, smoking, systolic blood pressure, diabetes, education, consumption of fruit and vegetables, consumption of wine, consumption of strong beer, level of physical activity

ACS: acute coronary syndrome; BMI: body mass index; FFQ: food frequency questionnaire; MI: myocardial infarction; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SES: socioeconomic status; SFA: saturated fatty acids.

Table 3. Pooled Measures of Acute Coronary Syndrome According to Category of Fish Consumption

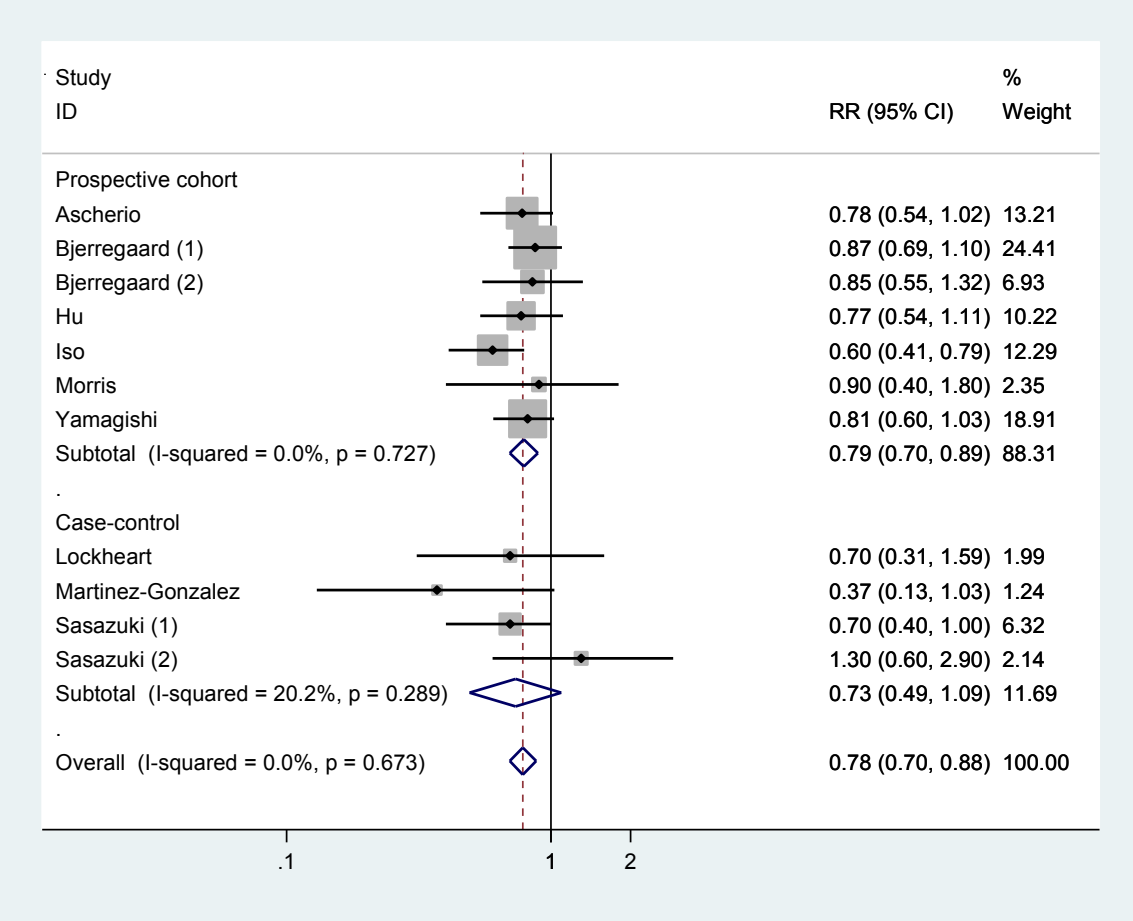
	RR (95% CI) per category of fish consumption				
	<once per month	1 to <4 times per month	1 to <2 times per week	2 to <4 times per week	≥4 times per week
Prospective cohort	1.00	**0.82 (0.72-0.92)**	0.85 (0.72-1.01)	**0.83 (0.71-0.96)**	**0.79 (0.70-0.89)**
Highest quality studies	1.00	**0.84 (0.74-0.95)**	**0.81 (0.73-0.91)**	**0.84 (0.73-0.95)**	**0.79 (0.70-0.89)**
Hazard ratio only	1.00	**0.83 (0.73-0.95)**	**0.81 (0.72-0.90)**	**0.81 (0.70-0.95)**	**0.79 (0.69-0.90)**
Largest study excluded	1.00	**0.82 (0.72-0.92)**	0.85 (0.72-1.01)	**0.80 (0.67-0.96)**	**0.76 (0.66-0.88)**
Case-control	1.00	--	**0.76 (0.67-0.87)**	0.84 (0.64-1.10)	0.73 (0.49-1.09)

Figure 1. Flow Diagram of Selection of Studies on Fish consumption and Acute Coronary Syndrome*



*Adapted from PRISMA 2009 flow diagram

Figure 2. Meta-Analysis of Prospective and Case-control Studies on Fish Consumption and Acute Coronary Syndrome Comparing the Highest (≥4 times per week) to Lowest (<once per month) Category of Intake



3 Gene-Environment and Gene-Diet Interactions in Cardiovascular Diseases

In our literature review, fish consumption was associated with a reduced risk of ACS. To explore the underlying mechanisms, we considered gene-environment interactions. Several studies have suggested potential gene-environment and gene-diet interactions in CVD, but omega-3 fatty acids and genetic interactions in ACS have not been previously studied. In view of this lack of information, we investigated whether the beneficial effect of omega-3 fatty acids in ACS could be due to the modulation of genetic risk. We used a case-only design to examine whether omega-3 fatty acids interact with ACS-associated single nucleotide polymorphisms (SNPs).

In the next sections, gene-environment and gene-diet interactions and their link to CVD are described. An overview of the case-only methodology is given in chapter 4. Manuscript #2 is subsequently presented in chapter 5.

3.1 Gene-Environment Interactions in Cardiovascular Diseases

CVD are complex multifactorial diseases. Current knowledge on multifactorial diseases stipulates that such complex chronic conditions arise due to an interplay between genetic factors and environmental exposures. Gene-environment interactions refer to the joint effect of one or more genes with one or more environmental factors that cannot be readily explained by their separate effects on disease risk[88]. They occur when certain genetic factors interact with certain environmental factors to modify the risk of a particular disease. These interactions can happen between genetic polymorphisms and various environmental or lifestyle factors, e.g. diet, physical activity, smoking, stress[89, 90].

3.2 Gene-Diet Interactions in Cardiovascular Diseases

Gene-diet interaction refers to effect modification that arises as a result of the interaction between genetic factors and diet. Nutrients, foods and dietary patterns have an undeniable role in cardiometabolic health. Nutrigenetics and nutrigenomics have emerged as two fields of research which explore interactions between genes and diet. Whereas nutrigenetics refers to how an individual's genetic makeup influences dietary response and nutritional requirements, nutrigenomics refers to how dietary factors modify the genetic effect associated with polymorphisms[91]. The latter is of particular relevance in disease prevention and reduction of disease burden at the population level.

3.3 Gene-Diet Interactions of Omega-3 Fatty Acids in Cardiovascular Diseases

A number of studies have attempted to investigate omega-3 fatty acids in relation to clinical manifestations of the atherosclerotic process, including lipid metabolism, inflammation, hemostasis, vascular function and insulin sensitivity, with several studies indicating interactions between omega-3 fatty acids and genetic factors[92]. There is some noteworthy evidence to suggest that omega-3 fatty acids could modulate the genetic predisposition to cardiovascular risk. For instance, Dwyer et al. were one of the first to suggest that omega-3 fatty acids could modulate the effect of genes in the inflammatory pathway to influence the onset of atherosclerosis[93]. A more recent study by Lu et al. suggested that dietary intake of omega-3 fatty acids modulates the effect of FADS1 polymorphism on plasma cholesterol concentrations[94]. However, despite considerable progress in identifying gene-environment interactions that may influence CVD, our understanding of gene-diet interactions remains limited.

4 The Case-Only Design

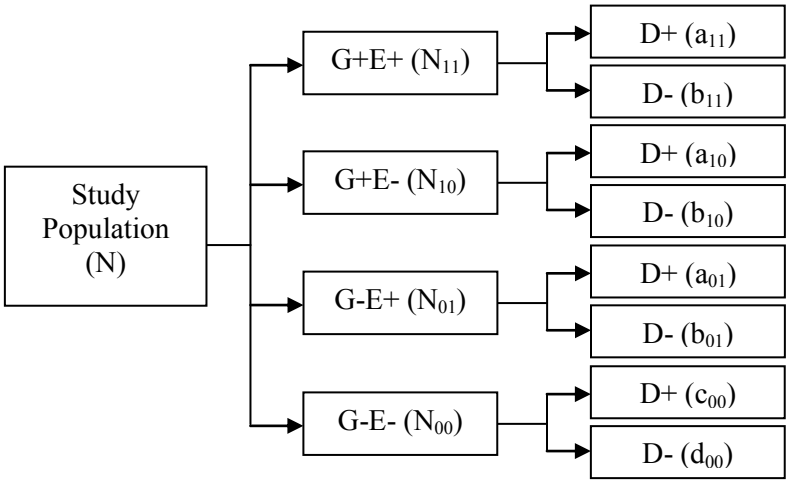
4.1 Overview of the Case-Only Design

The case-only design is an epidemiologic approach that is useful for analyzing gene-gene and gene-environment interactions[95]. This methodology was introduced in the 1980's as an attempt to avoid some of the limitations of cohort and case-control studies. For instance, cohort studies are often time-consuming, expensive and require a large sample size. Case-control studies represent a feasible way of investigating common interacting factors, but they are often underpowered to detect interactions between less frequent interacting factors[96]. As well, case-control studies are subject to numerous biases, such as selection bias and recall bias. Accordingly, the choice of an appropriate control group is crucial to avoid spurious findings due to confounding. However, finding the appropriate control group is fraught with difficulty.

The fundamental aspect of case-only studies is that they allow the testing of gene-environment interactions without the need for control subjects. The case-only analysis is an efficient method that has been demonstrated to have greater statistical power than cohort and case-control studies with similar sample size and produce more precise interaction estimates (lower variance)[97-99]. The case-only interaction estimates are valid only under the assumption that the interacting factors are uncorrelated in the source population[100]. Under this independence assumption, the estimate reflects interaction under the multiplicative scale and is equivalent to the true interaction risk ratio (RR)[96, 101].

The calculation of gene-environment interaction estimate (GxE_{RR}), i.e. the joint effect of the gene (G) and environment (E) on disease (D), is illustrated in Figure 1 (see next page).

Figure 1. Calculation of Gene-Environment Interaction Risk Ratio (GxE_{RR})



Joint effect of G and E

	D+	D-	Total
G+E+	a_{11}	b_{11}	N_{11}
G-E-	c_{00}	d_{00}	N_{00}

$$RR_{GE} = \frac{a_{11}/N_{11}}{c_{00}/N_{00}}$$

Independent effect of G (among E-)

	D+	D-	Total
G+	a_{10}	b_{10}	N_{10}
G-	c_{00}	d_{00}	N_{00}

$$RR_{G} = \frac{a_{10}/N_{10}}{c_{00}/N_{00}}$$

Independent effect of E (among G-)

	D+	D-	Total
E+	a_{01}	b_{01}	N_{01}
E-	c_{00}	d_{00}	N_{00}

$$RR_{E} = \frac{a_{01}/N_{01}}{c_{00}/N_{00}}$$

Case-Only Study (D+)

	G+	G-
E+	a_{11}	a_{01}
E-	a_{10}	c_{00}

$$\text{Case-only OR} = \frac{a_{11}c_{00}}{a_{10}a_{01}} = \text{Term I}$$

Total Cohort (D+ and D-)

	G+	G-
E+	N_{11}	N_{01}
E-	N_{10}	N_{00}

$$\text{G-E OR} = \frac{N_{11}N_{00}}{N_{10}N_{01}} = \text{Term II}$$

$$GxE_{RR} = \frac{RR_{GE}}{RR_G * RR_E} = \frac{\frac{a_{11}/N_{11}}{c_{00}/N_{00}}}{\frac{a_{10}/N_{10}}{c_{00}/N_{00}} * \frac{a_{01}/N_{01}}{c_{00}/N_{00}}} = \frac{\left(\frac{a_{11}c_{00}}{a_{10}a_{01}}\right)}{\left(\frac{N_{11}N_{00}}{N_{10}N_{01}}\right)} = \frac{\text{Term I}}{\text{Term II}}$$

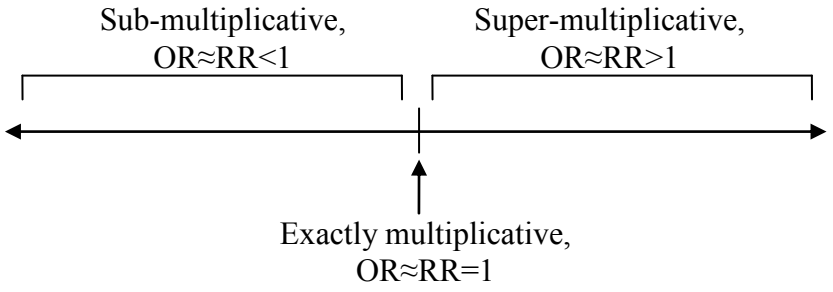
(Adapted from Gatto NM et al. [101])

From the equation given in Figure 1, we note that when Term II = 1, i.e. when there is no association between G and E in the population, Term I (the case-only OR) will be equal to the true gene-environment interaction RR (GxE_{RR}), hence the need for the independence assumption.

4.2 Interpreting the Case-Only Odds Ratio

Effects of exposure to joint effects, e.g. the joint effect of a genetic and an environmental factor, exist on a continuum. As well, effect modification is scale dependent. Under the independence assumption, the case-only OR will approximate the interaction RR and will provide an assessment of interaction on the multiplicative scale[102]. Figure 2 below illustrates the continuum and scale dependence of effect modification in case-only studies.

Figure 2. Continuum and Scale Dependence of Effect Modification in Case-Only Studies



Accordingly, an OR>1 or OR<1 indicates that there is a multiplicative effect (i.e. interaction on the multiplicative scale), and an OR=1 indicates an exactly multiplicative effect (i.e. no interaction on the multiplicative scale).

4.3 Sources of Bias in Case-Only Studies

The violation of the independence assumption will yield a biased estimate and an elevated type I error[88]. For instance, a causal association between genes and environment may arise for genes involved in behavioral traits such as addiction, or in cases of symptomatic gene status which may ultimately lead to behavior modification[88]. However, in the real-world setting, the independence assumption is rarely violated given that individuals are usually unaware of their genetic status and thus, alterations in behavior based on genetic status are unlikely to occur[101]. Additionally, according to Mendelian principles, alleles segregate randomly in the population regardless of environmental exposures, such that genetic and environmental factors should be independent[101, 103, 104]. Another potential source of error in case-only studies of gene-environment interactions, as in all genetic studies, is population stratification bias (i.e. confounding by ethnicity), and this can be controlled through stratification[101, 105]. In a systematic review and meta-analysis of case-only gene-environment interaction studies, bias was not found to be common[103]. Therefore, when the independence assumption holds, the case-only design represents a powerful and precise method to analyze gene-environment interactions.

5 RESULTS

5.1 Preface to Manuscript #2

The role of omega-3 fatty acids in modulating the ACS risk conferred by genetic polymorphisms has not been previously studied. To better understand how omega-3 fatty acids may influence ACS risk, we conducted a case-only gene-diet interaction study to evaluate potential interactions between omega-3 fatty acids and ACS-associated SNPs. These SNPs are well-known and none of them are known to be associated with omega-3 fatty acids. We used data from the GENESIS-PRAXY (GENdEr and Sex determInantS of Cardiovascular Disease from bench to beyond in PRemature Acute Coronary Syndrome) study, which consists of a patient population of adults aged 18 to 55 years with early onset ACS[106].

The abstract was presented as poster at the AHA EPI/NPAM 2014 Scientific Sessions and published in Circulation (Circulation 2014;129:AP414). The manuscript was accepted for publication in Nutrition, Metabolism and Cardiovascular Diseases and is in press (Nutr Metab Cardiovasc Dis 2014 pii: S0939-4753(14)00198-7), and has been formatted according to the journal's guidelines.

5.2 Manuscript #2: Omega-3 Fatty Acids and the Genetic Risk of Early Onset Acute Coronary Syndrome

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*See Appendix 1 and 2 for GENESIS-PRAXY co-investigators and participating centres

Abstract

Background and Aims: Recent gene-environment interaction studies suggest that diet may influence an individual's genetic predisposition to cardiovascular risk. We evaluated whether omega-3 fatty acid intake may influence the risk for acute coronary syndrome (ACS) conferred by genetic polymorphisms among patients with early onset ACS.

Methods and Results: Our population consisted of 705 patients of white European descent enrolled in GENESIS-PRAXY, a multicentre cohort study of patients aged 18 to 55 years and hospitalized with ACS. We used a case-only design to investigate interactions between the omega-3 index (a validated biomarker of omega-3 fatty acid intake) and 30 single nucleotide polymorphisms (SNPs) robustly associated with ACS. We used logistic regression to assess the interaction between each SNP and the omega-3 index. Interaction was also assessed between the omega-3 index and a genetic risk score generated from the 30 SNPs. All models were adjusted for age and sex. An interaction for increased ACS risk was found between carriers of the chromosome 9p21 variant rs4977574 and low omega-3 index (OR 1.57, 95% CI 1.07-2.32, p=0.02), but this was not significant after correction for multiple testing. Similar results were obtained in the adjusted model (OR 1.55, 95% CI 1.05-2.29, p=0.03). We did not observe any interaction between the genetic risk score or any of the other SNPs and the omega-3 index.

Conclusion: Our results suggest that omega-3 fatty acid intake may modify the genetic risk conferred by chromosome 9p21 variation in the development of early onset ACS and requires independent replication.

Introduction

It is well-recognized that cardiovascular diseases (CVD) are complex multifactorial conditions involving genetic and environmental risk factors[89]. Although gene-environment interactions could be part of additional mechanisms that contribute to cardiovascular outcomes, little is known about how environmental factors, such as diet, interact with an individual's genetic predisposition to early onset acute coronary syndrome (ACS).

Recent gene-environment interaction studies of CVD, including gene-diet interaction studies[94, 107], suggest that diet may influence an individual's genetic predisposition to CVD. Evidence also suggests a plausible interplay between an individual's genetic variation and omega-3 fatty acid intake[92, 93]. An increased intake of omega-3 fatty acids, consisting of eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3), has been indicated to be cardioprotective[6]. The associations between omega-3 fatty acids and CVD risk have been demonstrated in many studies[7, 10, 18]. However, although omega-3 fatty acid intake has been suggested to increase survival following cardiovascular events, not all trials have shown an association between omega-3 fatty acid intake and cardiovascular risk[108-110].

The omega-3 index (i.e. the sum of the percentage of EPA and DHA in total erythrocyte fatty acids) is a well-validated biomarker that has been shown to be highly reliable for assessing usual omega-3 fatty acid intake over the long-term[111]. It is based on the fatty acid composition of the erythrocyte that has a lipid bilayer that reflects the fatty acids in cell membranes of tissues and organs such as the heart[112]. Additionally, the slower fatty acid remodelling of the erythrocyte better reflects long-term dietary habits as

compared with plasma fatty acids[112]. The fatty acid composition of erythrocytes has also been demonstrated to be stable after myocardial infarction (MI) and after clinical intervention post-infarction[113]. In clinical trials, the omega-3 index has been inversely associated with CVD[111, 114] and studies have shown that a low level of erythrocyte EPA+DHA is an independent predictor of increased risk for ACS[83, 84].

In this study, we aimed to evaluate whether omega-3 fatty acid levels, as measured by the omega-3 index, may influence the genetic risk conferred by genetic variants that predispose to ACS. We hypothesized that low omega-3 fatty acid levels will interact with the genetic predisposition to ACS to increase the risk for early onset ACS.

Methods

Study Population

The study population consisted of patients enrolled in GENESIS-PRAXY (GENdEr and Sex determInantS of cardiovascular disease: from bench to beyond Premature Acute Coronary SYndrome), a multicentre prospective cohort study of adults hospitalized with early onset ACS. The protocol and methods of the GENESIS-PRAXY study have been previously described[106]. Briefly, eligible participants included patients aged 18 to 55 years, admitted with a diagnosis of ACS to a participating hospital, fluent in English and/or French, and able to provide informed consent. The study began in January 2009 and includes 24 sites across Canada, one in the US and one in Switzerland. In Quebec, a multicentre ethics review allowed for the McGill University Health Centre to act as the central review board and coordinate ethics approval for all centres. All other centres received ethics approval from their respective hospital ethics review boards.

For the present study, our population was restricted to patients of white European ethnicity, which made up more than 90% of enrolled participants. This was done as a means to avoid population stratification bias (i.e. confounding by ethnicity), an issue which can arise in genetic studies, including case-only studies.

Data collection

Study participants were approached by a trained research nurse within 48 hours of hospital admission. After consent, personal and medical data were collected via self-administered questionnaires and medical chart reviews. Patients also provided a blood sample which was immediately centrifuged. Serum and plasma were distributed into aliquots, and then stored locally at -80°C until being transported in dry ice to the McGill University Health Centre in Montreal, Canada.

Omega-3 Index

The fatty acid composition of erythrocytes was determined using fast gas chromatography methods at the University of Waterloo[115]. Briefly, erythrocyte fatty acids were determined by isolating the lipids with a double extraction protocol, followed by transesterification with boron trifluoride in methanol with hexane to generate fatty acid methyl esters[116]. Thirty-two fatty acid methyl esters were consistently identified and quantitated, and data were expressed as absolute concentrations (μg fatty acid/mL erythrocytes). Individual fatty acids were also expressed as the relative weight percentage (relative %) of total fatty acids. The omega-3 index was calculated by adding the relative % of EPA and the relative % of DHA.

DNA Extraction and Genotyping

ACS includes MI and unstable angina. These conditions of coronary heart disease (CAD) share common etiology and pathology and represent a continuum of severity, with MI being the most severe. We considered the top 30 single nucleotide polymorphisms (SNPs) robustly associated with MI/CAD from recent genome-wide association studies (GWAS). These SNPs have strong evidence for a true association with MI (minimum p-value for association $<5x10^{-8}$) and have all been replicated in at least one additional independent sample[117-125]. A 30 SNP genetic risk score (GRS) was generated from an unweighted count of the risk alleles at each SNP, with a theoretical range from 0 to 60. The SNPs used in the GRS were not in linkage disequilibrium (i.e. uncorrelated, $r^2 <0.3$). For some patients (13%), data on certain SNPs were unavailable such that GRS could not be directly calculated. In these cases, imputation was done by taking the average score of the sample population for each particular SNP.

DNA extraction, quantification, quality control tests and plating were carried out by the McGill University and Genome Quebec Innovation Centre. Approximately 34-45 SNPs were combined into a single Sequenom panel. This technology provided cost-effective genotyping, with an error rate below 0.05%. For the SNPs that failed genotyping on the Sequenom platform (<10%), genotyping was performed using the TaqMan platform.

Statistical Analysis

We used a case-only design to investigate the potential interaction between the omega-3 index and the genetic predisposition to ACS. Case-only designs allow for

efficient testing of gene-environment interactions, provide a more precise estimate of a gene-environment interaction and are more powerful than case-control designs with the same sample size[95]. This method assesses departure from the multiplicative joint effect and the case-only interaction odds ratio (OR) is interpreted as the multiplicative interaction between gene and environment, under the assumption that the gene and environmental exposure are independent of each other in the source population of the cases[101]. According to Mendelian principles, alleles segregate randomly in the population regardless of environmental exposures, such that genetic and environmental factors should be independent[101, 103, 104]. Additionally, the independence assumption is unlikely to be violated given that individuals are usually unaware of their genetic status and thus, alterations in behavior based on genetic status are improbable[101]. Moreover, to our knowledge, none of the ACS-associated SNPs have been associated with omega-3 fatty acids in prior studies and thus, it is unlikely that an individual's genotype for any of these 30 SNPs has a direct effect on omega-3 fatty acid intake and/or plasma levels.

We estimated allele frequencies by the gene-count method, and Hardy-Weinberg equilibrium (HWE) was assessed for each SNP using a chi-square test. The interaction between the omega-3 index and each ACS-associated SNP was assessed using logistic regression to estimate the case-only interaction OR and 95% confidence interval (CI). The omega-3 index was dichotomized using the median, and SNPs were dichotomized using those homozygous for the non-risk allele as the reference group. In few cases where the frequency of the homozygote for the non-risk allele was too low (rs10953541, rs11206510, rs1122608, rs12413409, rs17114036, rs1746048, rs17465637, rs17609940, rs646776), the heterozygous variant genotype was combined with the reference group to

maximize statistical power. Thus, in the analyses, an OR greater than 1 would indicate synergistic interaction for increased ACS risk between having the risk allele and low omega-3 index. Interaction was also assessed for the relative % of EPA and DHA separately. In multivariable logistic regression, adjustments for age and sex were made. Adjustments for multiple testing were performed using the false discovery rate (FDR) method. We evaluated the interaction between the omega-3 index and the GRS, with GRS dichotomized at the median score. Given that those who had a previous MI may have altered their dietary behavior and could consequently have different omega-3 indices than those who never had a MI, as a sensitivity test, we performed subgroup analyses by separating those who had a previous MI before inclusion into this cohort from those who did not.

All statistical analyses were conducted using STATA version 12.1 (StataCorp, College Station, TX, USA).

Results

Characteristics of Study Population

Our study sample consisted of 705 patients of white European descent with complete genotyping and fatty acid data. The median age was 49 years and 72.1% were male. The omega-3 index was generally low with a median of 3.35% (interquartile range (IQR) 2.81% to 4.07%) (Figure 1; Supplement S3 for the distribution of EPA and DHA separately). Patients with a previous MI had significantly higher omega-3 index compared to those without a history of MI (3.74% vs. 3.48%, p=0.03). The genotype frequencies of each ACS-associated SNP are illustrated in Table 1. The allele frequencies

were similar across study regions (data not shown). None of the SNPs deviated from HWE ($p>0.05$). The median GRS value was 31.0 (IQR 29.0 to 34.0).

Gene-Environment Interaction

We did not observe any interaction between the GRS and either the omega-3 index, EPA or DHA, but a significant interaction was observed between the chromosome 9p21 variant rs4977574 and the omega-3 index (Table 2). In univariable logistic regression, an interaction for increased ACS risk was found between carriers of the chromosome 9p21 variant rs4977574 and low omega-3 index (OR 1.57, 95% CI 1.07-2.32, p=0.02). Similar results were obtained after adjusting for age and sex (adjusted OR 1.55, 95% CI 1.05-2.29, p=0.03). However, the results did not retain statistical significance after correction for multiple testing. Interestingly, the interaction involving the chromosome 9p21 variant rs4977574 was significant with only one component of the omega-3 index, DHA (adjusted OR 1.57, 95% CI 1.06-2.31, p=0.02), but not EPA (adjusted OR 1.36, 95% CI 0.93-2.01, p=0.12). We did not find evidence for interactions between the other SNPs and the omega-3 index. Additional exploratory interactions were performed with EPA and DHA for all SNPs and a few nominally significant interactions were found (Supplement S4). In subgroup analysis, the interaction for rs4977574 among those without a history of MI (adjusted OR 1.70, 95% CI 1.11-2.59, p=0.01) remained statistically significant.

Discussion

Our study investigated potential a gene-diet interaction that may influence early onset ACS. We identified a possible interaction between omega-3 index and the 9p21 rs4977574 risk allele, indicating that carriers of this risk allele who have a poor intake of omega-3 fatty acids may be at higher risk for early onset ACS.

The 9p21 region is one of the strongest genetic predictors of CVD or MI, and has been shown to regulate the expression of the nearby CDKN2A and CDKN2B genes[126]. The 9p21 locus has also been associated with CAD severity[127]. Thus, it is possible that the interaction detected between the 9p21 variant rs4977574 and the omega-3 index applies to more severe cases of ACS (i.e. MI's). In addition, our findings suggest that the interaction may be mediated by low DHA (one component of the omega-3 index), as the interaction with the 9p21 rs4977574 risk allele was seen with low DHA but not with low EPA. This may be due to independent effects of EPA versus DHA on cardiovascular health[128], but it could also be related to the differences in EPA and DHA as blood biomarkers of dietary intake of omega-3 fatty acids[129]. Even in the erythrocyte, EPA can change rapidly in response to recent short-term dietary intake of omega-3 fatty acids, while changes in DHA tend to be gradual and require longer dietary interventions[130]. Other potential genetic interactions were suggested for EPA and DHA separately, as well as for those with or without a history of MI.

While gene-diet interaction studies investigating ACS-associated SNPs are limited, a recent study examined interactions between polymorphisms in the 9p21 chromosomal region and diet[107]. Using data from the INTERHEART and FINRISK studies, Do et al. investigated whether dietary patterns modulate the effect of genetic

variation in chromosome 9p21[107]. They found a reduction in the MI risk associated with the 9p21 risk allele among participants consuming a "prudent" diet high in raw fruits and vegetables. Although our results were not found to be statistically significant after correction for multiple testing, they should stimulate further investigation and replication studies in light of prior study findings which suggest that the risk conferred by the 9p21 variant can be modified by a "prudent" diet[107].

Despite the progress made in identifying gene-environment and gene-diet interactions, our understanding of the underlying biological pathways through which these interactions occur is limited. Omega-3 fatty acids may modify genetic risk via their role in gene expression and transcription. They are involved in the regulation of the expression of key proteins involved in inflammation, lipid metabolism and energy utilization in various tissues including the heart potentially through the regulation of transcription factors and other intermediates.

The consideration of epigenetics is also essential in gene-environment or gene-diet interactions. Epigenetic research has provided innovative insights into the mechanisms of CVD, especially by exploring interactions between heritability and environmental factors. It has been postulated that the rapid transformations in diet, such as the change in the ratio of omega-6 to omega-3 fatty acids, over the past centuries have disturbed the interplay between nutrients and the human genome, thereby resulting in increased CVD risk[17]. This represents a novel perspective in gene-diet interactions that could explain the dynamics of omega-3 fatty acids in modulating the cardiovascular risk conferred by susceptibility alleles.

Our study has several strengths and limitations. The case-only methodology is a key strength, which provides an efficient study design for assessing gene-environment multiplicative interactions. In addition, we used the omega-3 index, an extremely reliable measure, as a biomarker for long-term omega-3 fatty acid intake, which allowed for an accurate measure of this dietary factor. A limitation of our investigation is that it is not possible to establish causality from our observational approach. The study also had a relatively small sample size, which reduces statistical power, and the results need to be confirmed in another independent study. As well, the study was performed among patients with early onset ACS, who may be expected to have a higher genetic predisposition to ACS. We obtained different results when separate analyses were conducted with EPA and DHA, possibly due to the narrow range of their relative % and the associated lack of power. Furthermore, ACS consists of a heterogeneous spectrum of conditions, with varying symptoms from unstable angina to MI, and this heterogeneity may have also reduced the power of this study. Our subgroup analyses separating those with and without a history of MI could also have been underpowered and these results should be interpreted with even more caution. As our study was a multicentre one, there is the possibility of confounding by study region. Nonetheless, the allele frequencies were verified and found to be similar in each region. Moreover, our study population was of European descent, and although this restriction was helpful to avoid population stratification bias, further exploration is needed to determine whether the results are generalizable to other ethnic groups.

To conclude, our results suggest that omega-3 fatty acid intake may modify the genetic risk conferred by chromosome 9p21 variation in the development of early onset

ACS, but require independent replication in other cohorts. Further validation research is also warranted to examine whether this interaction occurs in other ethnic groups. Identifying interactions between diet, nutrients and genetic variation in CVD will not only help to elucidate the underlying mechanistic pathways, but also further clarify the fundamental disease pathology. Ultimately, the progress of this area of research will provide evidence for targeting certain dietary interventions to individuals at high genetic cardiovascular risk with wide-reaching public health implications.

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Disclosures

None.

Figure 1. Distribution of Omega-3 Index

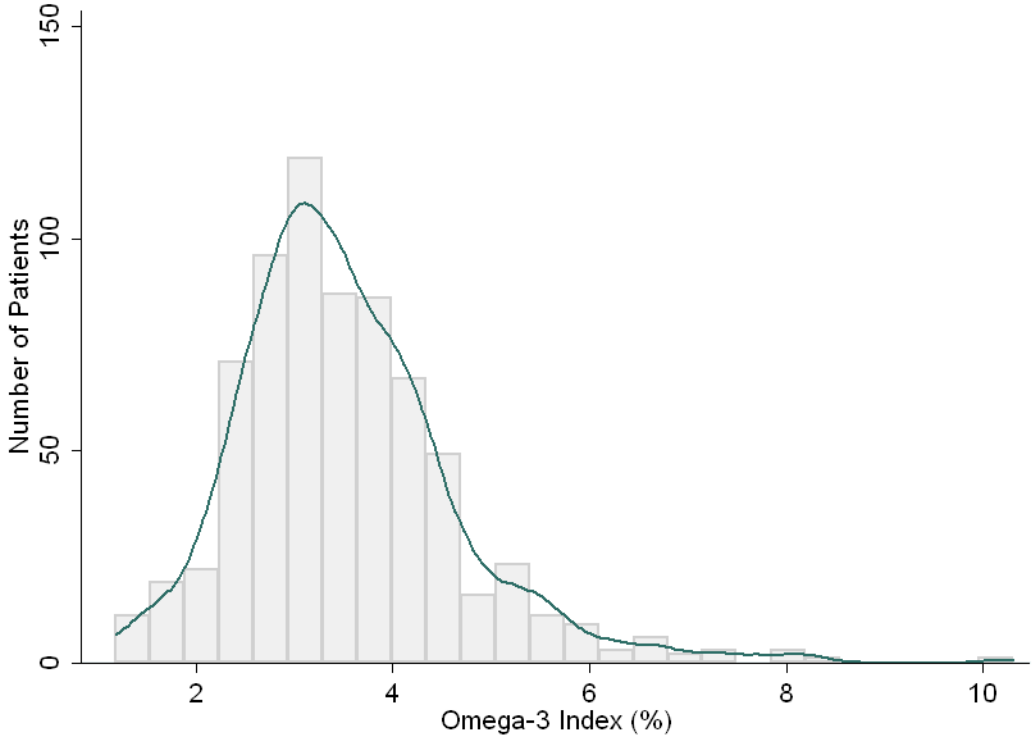

Table 1. Genotype Frequencies of Acute Coronary Syndrome-Associated Single Nucleotide Polymorphisms

SNP	Gene	Non-risk allele	Risk allele	Non-risk allele homozygote (%)	Heterozygote (%)	Risk allele homozygote (%)
rs10953541	7q22/BCAP29	T	C	6.7	39.2	54.1
rs11206510	PCSK9/BSND	C	T	3.2	31.2	63.8
rs1122608	LDLR/SMARCA4	T	G	6.6	37.1	56.4
rs11556924	ZC3HC1	A	G	12.0	47.3	40.7
rs12190287	TCF21	G	C	11.9	48.0	40.0
rs12413409	CYP17A1/CNNM2/NT5C2	T	C	0.7	13.3	85.7
rs12526453	PHACTR1	G	C	48.8	40.0	10.8
rs12936587	PEMT	A	G	18.0	50.4	31.2
rs1412444	LIPA	C	T	42.0	41.7	11.1
rs17114036	PPAP2B	C	T	0.8	15.4	82.7
rs1746048	CXCL12/HNRNPA3P1	T	C	1.7	18.5	79.9
rs17465637	MIA3	T	G	7.8	38.9	52.9
rs17609940	ANKS1A	C	G	4.1	31.0	64.6
rs216172	SMG6-SRR	G	C	38.5	44.5	16.8
rs2259816	HNF1A	C	A	39.2	47.7	13.0
rs2505083	KIAA1462	A	G	31.6	45.9	22.0
rs2895811	HHIPL1	T	C	33.3	47.8	17.9
rs3184504	SH2B3	C	T	25.3	46.4	28.3
rs3798220	LPA	T	C	93.9	5.7	0.3
rs3825807	ADAMTS7	C	T	18.5	49.1	29.4
rs46522	UBE2Z/GIP	C	T	21.1	50.9	27.7
rs4773144	COL4A1-A2	T	C	32.0	47.3	20.3
rs4977574	CDKN2A, CDKN2B	A	G	18.3	47.8	31.2
rs579459	ABO	T	C	57.1	36.1	6.6
rs646776	CELSR2/PSRC1-SORT1	C	T	4.6	31.9	62.9
rs6725887	WDR12	T	C	74.6	23.4	1.7
rs964184	APOA5/APOC3/ZNF259	G	C	73.2	24.6	2.2
rs974819	PDGFD	G	A	46.6	38.9	11.3
rs9818870	MRAS	C	T	67.1	30.2	2.4
rs9982601	SLC5A3/MRPS6/KCNE2 /C21orf82	G	A	71.2	25.6	3.2

SNP: single nucleotide polymorphism.

Table 2. Interaction between Acute Coronary Syndrome-Associated Single Nucleotide Polymorphisms and Omega-3 Index

	Unadjusted		Adjusted[a]	
SNP	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
rs10953541	0.96 (0.71,1.29)	0.79	0.95 (0.71,1.28)	0.75
rs11206510	1.07 (0.79,1.47)	0.66	1.07 (0.78,1.47)	0.66
rs1122608	1.18 (0.88,1.59)	0.27	1.17 (0.87,1.58)	0.30
rs11556924	1.14 (0.72,1.79)	0.57	1.16 (0.73,1.83)	0.53
rs12190287	0.87 (0.55,1.37)	0.55	0.85 (0.54,1.34)	0.47
rs12413409	1.02 (0.67,1.56)	0.93	0.98 (0.64,1.50)	0.92
rs12526453	1.17 (0.87,1.58)	0.29	1.18 (0.88,1.59)	0.28
rs12936587	1.08 (0.74,1.59)	0.68	1.05 (0.71,1.54)	0.82
rs1412444	0.85 (0.63,1.16)	0.31	0.86 (0.63,1.17)	0.34
rs17114036	1.14 (0.76,1.70)	0.54	1.09 (0.73,1.64)	0.66
rs1746048	0.98 (0.68,1.42)	0.91	0.98 (0.68,1.43)	0.94
rs17465637	1.15 (0.85,1.54)	0.36	1.14 (0.84,1.53)	0.40
rs17609940	1.00 (0.73,1.36)	0.98	0.99 (0.73,1.36)	0.97
rs216172	0.94 (0.69,1.27)	0.69	0.95 (0.70,1.29)	0.75
rs2259816	0.99 (0.73,1.34)	0.94	0.97 (0.72,1.32)	0.85
rs2505083	1.17 (0.85,1.61)	0.34	1.15 (0.83,1.59)	0.39
rs2895811	0.80 (0.58,1.09)	0.16	0.81 (0.59,1.11)	0.18
rs3184504	1.09 (0.78,1.53)	0.62	1.08 (0.77,1.53)	0.64
rs3798220	1.05 (0.57,1.95)	0.88	1.08 (0.58,2.01)	0.81
rs3825807	0.95 (0.65,1.40)	0.81	0.94 (0.64,1.39)	0.77
rs46522	0.84 (0.58,1.21)	0.35	0.83 (0.58,1.20)	0.33
rs4773144	1.12 (0.81,1.53)	0.50	1.13 (0.82,1.55)	0.47
rs4977574	**1.57 (1.07,2.32)**	**0.02**	**1.55 (1.05,2.29)**	**0.03**
rs579459	1.22 (0.91,1.65)	0.19	1.20 (0.89,1.62)	0.24
rs646776	0.81 (0.59,1.10)	0.17	0.82 (0.60,1.12)	0.22
rs6725887	0.92 (0.65,1.29)	0.63	0.93 (0.66,1.31)	0.68
rs964184	1.08 (0.77,1.50)	0.66	1.10 (0.79,1.54)	0.58
rs974819	0.77 (0.57,1.04)	0.09	0.75 (0.56,1.02)	0.07
rs9818870	0.77 (0.56,1.05)	0.10	0.76 (0.55,1.05)	0.09
rs9982601	0.80 (0.58,1.12)	0.19	0.81 (0.59,1.13)	0.22

[a]Adjusted for age and sex.

CI: confidence interval; OR: odds ratio; SNP: single nucleotide polymorphism.

6 DISCUSSION

The goal of the work undertaken in this thesis was to investigate the role of omega-3 fatty acids in ACS.

In manuscript #1, we performed a systematic review and meta-analysis of the association between fish consumption and ACS. The rationale for this study was that the source of omega-3 fatty acids in the diet may potentially have a different effect on disease risk and there is no evidence synthesis on the effect of fish consumption on ACS risk. We combined the evidence from observational studies and found that fish consumption is beneficial for the primary prevention of ACS. A dose-response relationship was observed such that there was a greater risk reduction with increasing fish consumption. While there are certain inherent limitations to observational studies, the real-world setting is advantageous for such dietary exposures[131]. In addition, the studies included in our meta-analysis adjusted for numerous dietary and health behavioral factors. We also recognize the possibility of self-reported bias and inaccurate estimations of fish consumption, but the effect would unlikely differ across categories of fish consumption.

Contrary to recent findings which indicated that omega-3 fatty acid supplements did not have a secondary preventive effect for cardiovascular outcomes[12, 13], our literature review supports fish consumption as being beneficial for ACS risk reduction. The issue could lie in the adherence to fish oil capsules in intervention trials, whereas observational studies capture people who consistently have omega-3 fatty acids in their diet. Intervention studies require behavioral changes while observational studies do not, and adherence to fish oil supplements in intervention studies has been found to be poor[80].

Furthermore, fish can be a relatively inexpensive food commodity and provide a range of other healthful nutrients, which are not available through omega-3 fatty acid supplements. Research has increasingly emphasized the importance of consuming specific foods and adopting distinct dietary patterns as compared to individual nutrient intake[132, 133]. It has been argued that isolated forms of nutrients, such as those from supplements, may not have the same impact as sources of nutrients in a food matrix[77, 133]. Thus, from a public health perspective, it may be more appropriate to highlight the cardiovascular benefits of fish as a source of omega-3 fatty acids rather than omega-3 fatty acid supplements per se.

In manuscript #2, we investigated gene-diet interactions in ACS and examined whether omega-3 fatty acid intake, as assessed by blood biomarkers, could modify the risk conferred by ACS-associated polymorphisms in the GENESIS-PRAXY cohort. The study had a case-only design, which is an efficient method to assess gene-environment interactions[95]. Our findings were suggestive of interactions between omega-3 fatty acids in erythrocytes and ACS-associated SNPs, particularly the 9p21 variant rs4977574, indicating that carriers of this polymorphism with low levels of omega-3 fatty acids have an increased risk of early onset ACS beyond the sum of the risks associated with these two factors.

An aspect of our gene-diet interaction study was that we used the omega-3 index as a measure of omega-3 fatty acid intake. Blood levels reflect strongly dietary intake and the advantage of using erythrocytes to calculate the omega-3 index is that it reflects long-term exposure to omega-3 fatty acids[111, 112]. In addition, dietary biomarkers are useful to objectively assess dietary exposures and to avoid the bias of self-reported dietary

intakes[134]. However, one concern regarding the use of the omega-3 index is that both fish consumption and omega-3 fatty acid supplements have been shown to be independent predictors of the omega-3 index[87]. Our case-only study did not permit to differentiate between patients who took omega-3 fatty acid supplements and those who did not. This requires further investigation and should be considered in future studies on the omega-3 index. Nonetheless, considering the findings from our literature search, it is conceivable that fish consumption would be more favorable than omega-3 fatty acid supplements in the potential modification of the predisposition to early onset ACS. Therefore, those who are genetically predisposed to ACS may derive an increased benefit from a higher consumption of fish, compared to the general population.

While the case-only design is an efficient way of examining gene-environment interactions, one of its limitations is that it only provides an assessment of the multiplicative interaction, and does not allow the investigation of interactions on the additive scale, as compared to cohort and case-control studies. It may also be prone to biases when the independence assumption is violated. Notably, non-independence can arise in cases of symptomatic gene status which lead to behavior modifications or when there is confounding by ethnicity. None of the 30 ACS-associated SNPs included in our study are known to be associated with omega-3 fatty acids and it is also unlikely that the PRAXY patients were aware of their gene status. As well, our population was restricted to those of European descent to reduce population stratification bias. Thus, these biases were improbable in our study.

To our knowledge, this is the first investigation of interactions between omega-3 fatty acids and ACS-associated SNPs, and we hope that it can serve as a foundation for

future explorations of the role of omega-3 fatty acids in gene-diet interactions. We nonetheless acknowledge that the results need to be replicated and validated in other ethnicities.

The purpose of studying gene-environment and gene-diet interactions is not only to understand the complete etiology of complex multifactorial diseases like CVD, but also to inform targeted personalized prevention strategies so as to maximize health and minimize disease. Currently, the biological mechanisms of gene-environment interactions are not well understood, but taken together, our findings provide evidence of the cardioprotective effect of fish and omega-3 fatty acids in ACS.

7 CONCLUSION

This thesis helped in better understanding important facets of the protective role of omega-3 fatty acids in ACS. The findings of our studies highlight the value of modifiable dietary behaviors in improving cardiovascular health. We found that fish consumption was associated with a lowered risk of ACS in a dose-response manner and could be particularly beneficial in a personalized approach for those who have a genetic predisposition to ACS. Future research, including interventional trials comparing fish and omega-3 fatty acid supplements, as well as gene-diet interaction and replication studies, are warranted to further explore the potential of omega-3 fatty acids in reducing the burden of ACS at the population level.

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9 APPENDIX

Supplement S1. Search Strategies for Studies on Fish Consumption and Acute Coronary Syndrome

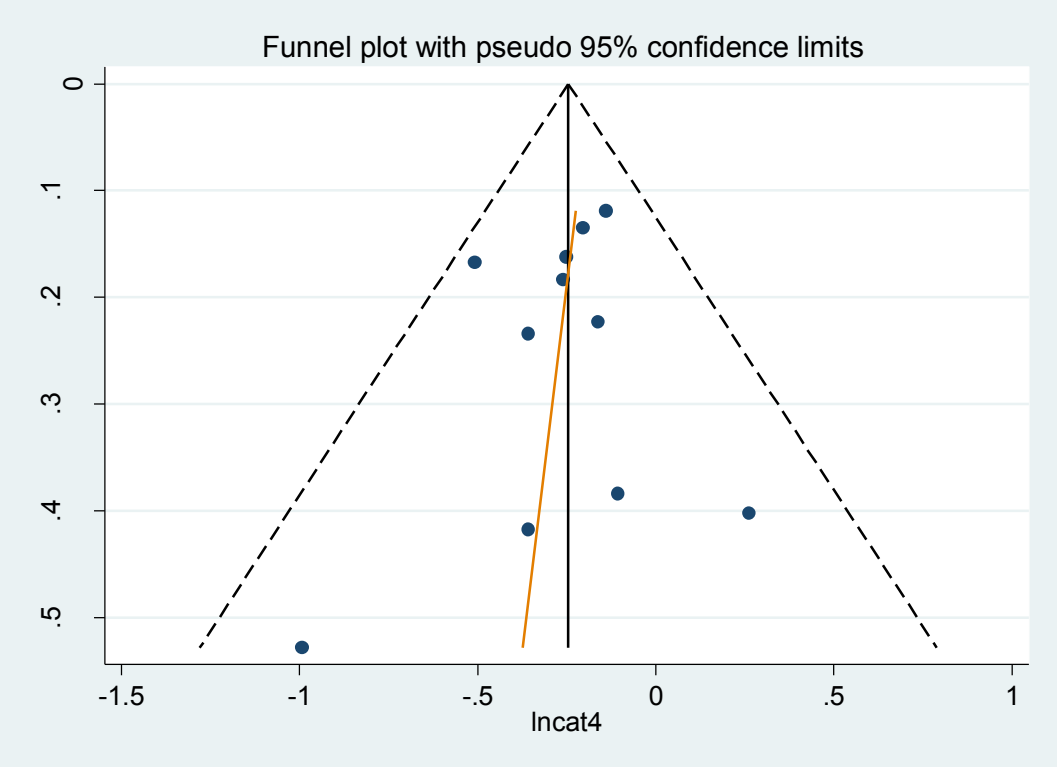
Medline

1. "Fishes"[Mesh]
2. "Seafood"[Mesh]
3. “fish”[tiab]
4. 1 or 2 or 3
5. "Acute Coronary Syndrome"[Mesh]
6. "Myocardial Infarction"[Mesh]
7. “acute coronary syndrome”[tiab]
8. “myocardial infarction”[tiab]
9. “heart infarction”[tiab]
10. “heart infarct”[tiab]
11. 5 or 6 or 7 or 8 or 9 or 10
12. 4 and 11
13. limit 12 to (English or French)
14. limit 13 to human

Embase

1. exp fish/ or exp fish meat/
2. exp sea food/
3. fish*.tw.
4. seafood*.tw.
5. 1 or 2 or 3 or 4
6. exp acute coronary syndrome/ or exp heart infarction/
7. myocardial infarction.tw.
8. acute coronary syndrome.tw.
9. 6 or 7 or 8
10. 5 and 9
11. limit 10 to (English or French)
12. limit 11 to yr="1966 - 2013"
13. limit 12 to human

Supplement S2. Funnel Plot of Studies on Fish Consumption and Acute Coronary Syndrome



Supplement S3. Concentration and Relative % of Eicosapentaenoic Acid and Docosahexaenoic Acid

Figure 1A. Concentration of EPA in Erythrocytes

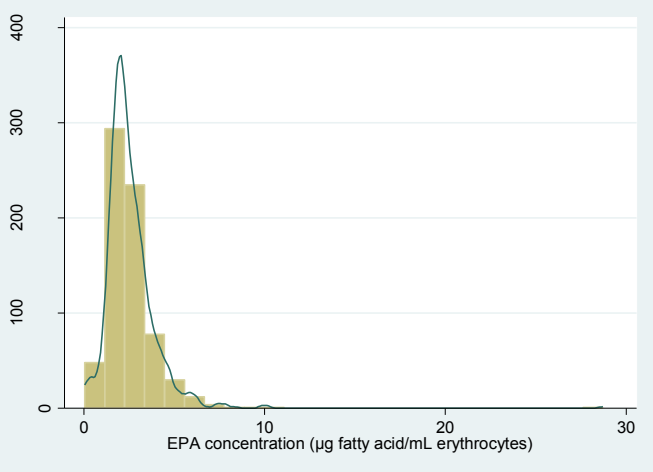


Figure 1B. Distribution of Relative % of EPA

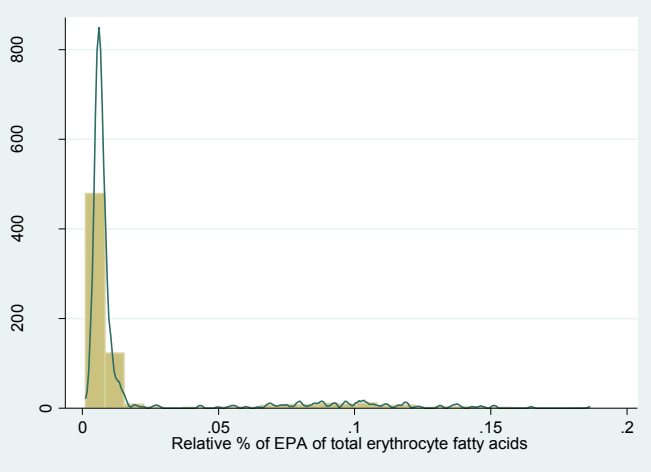


Figure 2A. Concentration of DHA in Erythrocytes

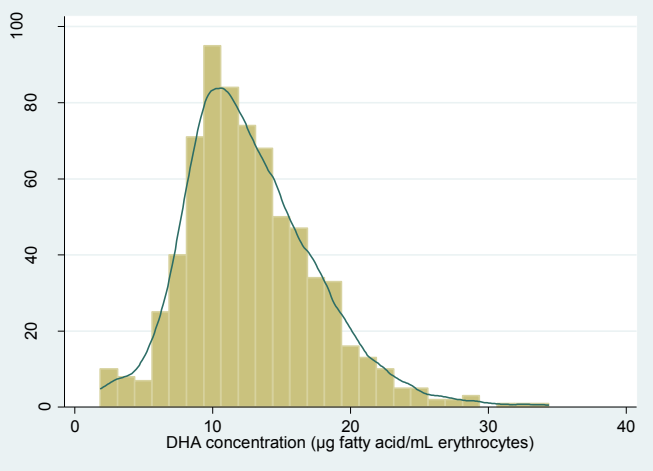
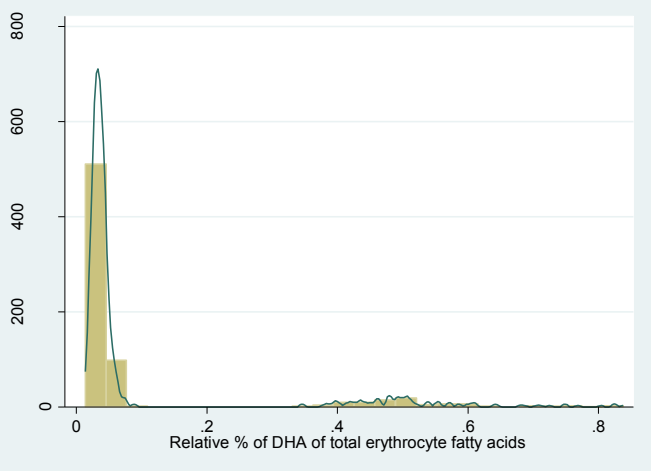


Figure 2B. Distribution of Relative % of DHA



Supplement S4. Interaction between Acute Coronary Syndrome-Associated Single Nucleotide Polymorphisms and Eicosapentaenoic Acid and Docosahexaenoic Acid

	EPA		DHA	
SNP	**OR (95% CI)[a]**	**p-value**	**OR (95% CI)[a]**	**p-value**
rs10953541	1.09 (0.59,0.81)	1.46	1.02 (0.76,1.38)	0.88
rs11206510	1.20 (0.88,1.64)	0.26	0.99 (0.73,1.36)	0.97
rs1122608	1.10 (0.82,1.49)	0.52	1.12 (0.83,1.51)	0.45
rs11556924	0.48 (0.30,0.78)	**0.00**	1.36 (0.86,2.14)	0.19
rs12190287	1.11 (0.70,1.75)	0.66	0.95 (0.60,1.50)	0.83
rs12413409	1.03 (0.67,1.58)	0.89	1.04 (0.68,1.59)	0.87
rs12526453	1.04 (0.77,1.40)	0.79	1.12 (0.84,1.51)	0.44
rs12936587	1.01 (0.68,1.48)	0.98	0.87 (0.59,1.28)	0.48
rs1412444	0.88 (0.65,1.20)	0.42	0.83 (0.61,1.12)	0.22
rs17114036	0.99 (0.66,1.49)	0.97	1.15 (0.77,1.73)	0.50
rs1746048	1.14 (0.79,1.65)	0.49	1.06 (0.73,1.53)	0.78
rs17465637	**1.37 (1.02,1.85)**	**0.04**	0.98 (0.73,1.32)	0.90
rs17609940	1.15 (0.84,1.57)	0.37	0.95 (0.70,1.30)	0.76
rs216172	0.86 (0.64,1.17)	0.35	1.02 (0.75,1.39)	0.89
rs2259816	0.90 (0.66,1.22)	0.49	1.00 (0.73,1.35)	0.98
rs2505083	0.95 (0.69,1.31)	0.75	1.04 (0.75,1.43)	0.83
rs2895811	0.99 (0.72,1.36)	0.95	**0.70 (0.51,0.97)**	**0.03**
rs3184504	1.01 (0.72,1.42)	0.96	1.02 (0.73,1.44)	0.89
rs3798220	1.63 (0.87,3.08)	0.13	0.97 (0.52,1.80)	0.93
rs3825807	0.99 (0.67,1.45)	0.95	0.96 (0.65,1.41)	0.84
rs46522	0.88 (0.61,1.26)	0.48	0.81 (0.56,1.16)	0.25
rs4773144	1.20 (0.87,1.64)	0.27	1.10 (0.80,1.52)	0.54
rs4977574	1.36 (0.93,2.01)	0.12	**1.57 (1.06,2.31)**	**0.02**
rs579459	1.27 (0.94,1.72)	0.12	1.23 (0.91,1.66)	0.18
rs646776	0.81 (0.59,1.10)	0.17	0.96 (0.71,1.31)	0.81
rs6725887	1.02 (0.73,1.44)	0.90	0.96 (0.68,1.35)	0.80
rs964184	**1.47 (1.05,2.06)**	**0.02**	1.13 (0.81,1.57)	0.48
rs974819	0.95 (0.71,1.29)	0.76	0.74 (0.55,1.00)	0.05
rs9818870	0.95 (0.69,1.31)	0.75	0.83 (0.60,1.13)	0.24
rs9982601	1.15 (0.83,1.60)	0.39	0.79 (0.57,1.10)	0.16

[a]Adjusted for age and sex.

CI: confidence interval; DHA: docosahexaenoic acid; EPA: eicosapentaenoic acid; OR: odds ratio; SNP: single nucleotide polymorphism.

Appendix 1. Co-Investigators for GENESIS-PRAXY

Co-Principal Investigators
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McMaster University/Hamilton Health Sciences (Juravinski Site), Hamilton, Ontario, Canada	Mike Rokoss
Ottawa Hospital, Ottawa, Ontario, Canada	Michele Turek
St Michael's Hospital, Toronto, Ontario, Canada	Asim Cheema
London Health Sciences Centre, London, Ontario, Canada	Shahar Lavi
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Hôpital Général de Montréal, Montréal, Québec, Canada	Thao Huynh
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Hôpital Général Juif-Sir Mortimer B. Davis, Montréal, Québec, Canada	Mark Eisenberg
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Inselspital, University of Bern, Switzerland and Lausanne University Hospital, Lausanne, Switzerland	Nicolas Rodondi