Genome-wide association meta-analysis of fish and EPA+DHA consumption in 17 US and European cohorts

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Published Version</td>
<td>doi:10.1371/journal.pone.0186456</td>
</tr>
<tr>
<td>Citable link</td>
<td><a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:34651954">http://nrs.harvard.edu/urn-3:HUL.InstRepos:34651954</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA</a></td>
</tr>
</tbody>
</table>


RESEARCH ARTICLE

Genome-wide association meta-analysis of fish and EPA+DHA consumption in 17 US and European cohorts


1 Friedman School of Nutrition Science & Policy, Tufts University, Boston, MA, United States of America, 2 Nutrition and Genomics Laboratory, Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging, Tufts University, Boston, MA, United States of America, 3 Division of Statistical Genomics, Department of Genetics, Washington University School of Medicine, St. Louis, MO, United States of America, 4 Division of Preventive Medicine, Brigham and Women’s Hospital, Boston, MA, United States of America, 5 Division of Epidemiology, Human Genetics and Environmental Sciences, University of Texas Health Science Center at Houston, Houston, TX, United States of America, 6 National Institute for Health and Welfare, Helsinki, Finland, 7 Estonian Genome Center, University of Tartu, Tartu, Estonia, 8 Institute of Behavioural Sciences, University of Helsinki, Helsinki, Finland, 9 Folkhälsoan Research Centre, Helsinki, Finland, 10 Sticht Center on Aging, Wake Forest School of Medicine, Winston Salem, NC, United States of America, 11 Department of Nutrition, Harvard School of Public Health, Boston, MA, United States of America, 12 Division of Epidemiology, Erasmus MC, Rotterdam, The Netherlands, 13 Netherlands Genomics Initiative-sponsored Netherlands Consortium for Healthy Aging, Leiden, The Netherlands, 14 Department of Dietetics and Nutrition, Harokopio University, Athens, Greece, 15 William Harvey Research Institute, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, United Kingdom, 16 Department of Food and Environmental Sciences, University of Helsinki, Finland, 17 Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland, 18 Division of Epidemiology and Community Health, University of Minnesota School of Public Health, Minneapolis, MN, United States of America, 19 Department of Medicine, Epidemiology and Health Services, University of Washington, Seattle, WA, United States of America, 20 Department of Medicine, Harvard Medical School, and Division of Aging Brigham and Women’s Hospital, Boston, MA, United States of America, 21 Los Angeles Biomedical Research Institute and Department of Pediatrics, Harbor-UCLA Medical Center, Los Angeles, CA, United States of America, 22 Institute for Molecular Medicine Finland (FIMM), University of Helsinki, Helsinki, Finland, 23 University of Tartu, Estonian Genome Center, Tartu, Estonia, 24 Division of Statistical Genomics, Department of Genetics, Washington University School of Medicine, St. Louis, MO, United States of America, 25 Department of Biostatistics, Boston University School of Public Health, Boston, MA, United States of America, 26 Division of Cardiovascular Medicine, Howard University College of Medicine, Washington DC, United States of America, 27 Nutritional Epidemiology Program, USDA Human Nutrition Research Center on Aging, Tufts University, Boston, MA, United States of America, 28 Department of Chronic Disease Prevention, National Institute for Health and Welfare, Helsinki, Finland, 29 Department of Medical Genetics, University of Helsinki and University Central Hospital, Helsinki, Finland, 30 Department of Biostatistical Sciences, Wake Forest School of Medicine, Winston Salem, NC, United States of America, 31 Laboratory of Neurogenetics, National Institute of Aging, Bethesda, MD, United States of America, 32 Clinical Research Branch, National Institute on Aging, Baltimore, MD, United States of America, 33 Center for Public Health Genomics and Division of Biostatistics and Epidemiology, Department of Public Health, University of Minnesota, Minneapolis, MN, United States of America.


Received: July 9, 2015
Accepted: September 14, 2017
Published: December 13, 2017

Copyright: This is an open access article, free of all copyright, and may be freely reproduced, distributed, transmitted, modified, built upon, or otherwise used by anyone for any lawful purpose. The work is made available under the Creative Commons CC0 public domain dedication.

Data Availability Statement: The meta-analysis results from this study are available at dbGAP (accession number phs000950).
Background

Regular fish and omega-3 consumption may have several health benefits and are recommended by major dietary guidelines. Yet, their intakes remain remarkably variable both within and across populations, which could partly owe to genetic influences.

Objective

To identify common genetic variants that influence fish and dietary eicosapentaenoic acid plus docosahexaenoic acid (EPA+DHA) consumption.

Design

We conducted genome-wide association (GWA) meta-analysis of fish (n = 86,467) and EPA+DHA (n = 62,265) consumption in 17 cohorts of European descent from the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium Nutrition Working Group. Results from cohort-specific GWA analyses (additive model) for fish and EPA+DHA consumption were adjusted for age, sex, energy intake, and population stratification, and meta-analyzed separately using fixed-effect meta-analysis with inverse variance weights (METAL software). Additionally, heritability was estimated in 2 cohorts.

Results

Heritability estimates for fish and EPA+DHA consumption ranged from 0.13–0.24 and 0.12–0.22, respectively. A significant GWA for fish intake was observed for rs9502823 on chromosome 6: each copy of the minor allele (Freq = 0.015) was associated with 0.029 servings/day (~1 serving/month) lower fish consumption (P = 1.96x10^{-7}). No significant association was observed for EPA+DHA, although rs7206790 in the obesity-associated FTO gene was among top hits (P = 8.18x10^{-7}). Post-hoc calculations demonstrated 95% statistical power to detect a genetic variant associated with effect size of 0.05% for fish and 0.08% for EPA+DHA.
Introduction

Consumption of fish (including finfish and shellfish) and long-chain omega-3 fatty acids is linked to lower risk of several chronic diseases, in particular fatal coronary heart disease [1]. These beneficial associations in observational studies are supported by randomized controlled trials demonstrating favorable effects of fish or fish oil on numerous chronic disease risk factors and on cardiac mortality [1,2]. As a result, regular fish consumption is recommended by all major national and international dietary guidelines [1].

In contrast to these guidelines and in comparison to many other foods, remarkable variation exists in the amount of fish consumption within and across populations. In many Western nations, approximately one-third of individuals consume no fish at all, approximately one-third consume fish but relatively rarely (up to once per week), and approximately one-third consume fish more frequently [3]. While some of this wide variation in fish consumption is undoubtedly due to personal and environmental factors (e.g., culture, geographic residence, family habits, socioeconomic status), the potential contribution of intrinsic biologic factors, such as genetic variation, is not well established. In one analysis among Danish twins, the estimated heritability of fish consumption was 17% in men and 61% in women, based on additive genetic effects [4]. For example, potential heritability could relate to differences in genes related to taste, digestion, fatty acid metabolism, or other unknown processes related to food preferences. Yet, the potential genetic variants underlying this estimated heritability are unknown; and such heritability estimates also require further replication.

A basic concept underlying “personalized nutrition” is that a person’s genes can influence their behaviors and responses to the environment. Dietary habits, including the consumption of fish, are among the most relevant factors that influence the development of chronic diseases. Elucidating whether, and in what manner, specific genes alter fish and long-chain omega-3 fatty acid consumption would have implications for understanding influences on variation in fish intake within populations and the biology of partiality to foods. Furthermore, identification of such variants could also inform the development of personalized nutrition—dietary recommendations based on genetic preferences for consumption.

As has been seen with other characteristics such as physiologic risk factors, genome-wide association (GWA) studies may lead to discovery of novel genes and biologic pathways that influence the individual characteristic of interest. Although such studies have been performed for major macronutrients (e.g., fat, carbohydrate, protein) [5,6], few analyses have been done for specific foods [7], whose intakes may be influenced by complex characteristics of tastes, textures, aromas, and nutrient contents. The ability to undertake food-specific genetic analyses would have implications for understanding influences on variation in fish intake within populations and the biology of partiality to foods. Furthermore, identification of such variants could also inform the development of personalized nutrition—dietary recommendations based on genetic preferences for consumption.

We therefore performed a collaborative investigation to estimate heritability of and assess how common genetic variation relates to dietary consumption of both fish and long-chain omega-3 fatty acids (eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA)) as part of...
the of the CHARGE (Cohorts for Heart and Aging Research in Genomic Epidemiology) Consortium Nutrition Working Group, bringing together investigators and data from 17 US and European population-based cohort studies totaling 86,467 participants of European descent.

**Subjects and methods**

**Cohorts**

The present work was a collaboration among 17 US and European population-based cohort studies participating in the Nutrition Working Group of the CHARGE Consortium (S1 Table). These included the Atherosclerosis Risk in Communities Study (ARIC); Cardiovascular Health Study (CHS); Dietary, Lifestyle, and Genetic Determinants of Obesity and Metabolic Syndrome (DILGOM); Estonian Study; Family Heart Study (FamHS); Framingham Heart Study (FHS); Helsinki Birth Cohort Study (HBCS); Health 2000 survey (H2000); Health, Aging, and Body Composition (HealthABC) Study; Health Professionals Follow-up Study (HPFS); Invecchiare [Aging] in Chianti Area (InCHIANTI); Multi-Ethnic Study of Atherosclerosis (MESA); Nurses’ Health Study (NHS); Rotterdam Study; The Hellenic Study of Interactions between SNPs and Eating in Atherosclerosis Susceptibility (THESIAS); Women’s Genome and Health Study (WGHS); and Young Finns Study (YFS). Additional details on these cohorts have been published previously [5,6] and are provided in S1 Table. All persons studied were of European descent, consented to genetic research, and provided written informed consent. For each study, examination protocols were approved by local institutional review boards at Johns Hopkins University (ARIC, MESA), University of Washington (CHS), Epidemiology and Public Health of the Hospital District of Helsinki and Uusimaa (DILGOM), Washington University (FamHS), Boston University (FHS), University of Pittsburgh (HealthABC), Harvard University (HPFS, NHS), National Public Health Institute of Finland (HBCS), Epidemiology and Public Health of the Hospital District of Helsinki and Uusimaa (H2000), Italian National Institute of Research and Care of Aging (InCHIANTI), Erasmus Medical Center and The Netherlands Ministry of Health, Welfare and Sports (Rotterdam), Harokopio University (THESIAS), Brigham and Women’s Hospital (WGHS), University of Helsinki (YFS), and procedures were in accordance with the ethical standards of the responsible institutional or regional committee on human subject research.

**Assessment of fish and omega-3 fatty acid consumption**

Usual dietary intake was assessed in each cohort using detailed food frequency questionnaires designed to capture the dietary habits of the population under study (S2 Table). Typically, participants were asked to indicate how often, on average, they had consumed various foods and beverages over the past year according to multiple frequency categories (e.g., 9 categories ranging from <1/month to 6+/day), with usual portion sizes specified on the questionnaire or by the participant. Fish intake was generally assessed using multiple questions, such as consumption of tuna fish; dark meat fish such as salmon or sardines; other white fish; shellfish; and fried fish or fish sandwiches. For each question, the midpoint of each frequency category was used to estimate usual intake which was then multiplied by the specified portion size; these intakes were summed across all questions on fish. For this analysis, we standardized fish consumption from each cohort to 100g servings/day. In 12 cohorts, total dietary consumption of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) was estimated by linking the dietary assessment tool to a food composition table specific to the cohort (e.g., the USDA food composition database in the US). For each of the types of foods consumed, the frequency and average portion size were multiplied by the content of EPA/DHA in the food. The total was calculated by summing across all foods in the questionnaire. For cohorts that included nutrients...
from supplements, the portion of EPA+DHA from supplements was excluded from our analysis.

Heritability estimates
To evaluate potential heritability of fish and EPA+DHA consumption, we estimated heritability using family-based methods in two family-based cohorts (FamHS and FHS) using the variance components method in Sequential Oligogenic Linkage Analysis Routines (SOLAR; Texas Biomedical Research Institute; San Antonio, TX), and adjusting for age and sex. Briefly, heritability is calculated using a maximum likelihood method using the ratio of the genetic variance to total phenotypic variance.[8]

Genotyping and analysis
Genome-wide genotyping was conducted in each cohort using Affymetrix or Illumina platforms. Each study performed quality control for genotyped single nucleotide polymorphisms (SNPs) based on minor allele frequency (MAF), call rate, and departure from Hardy-Weinberg equilibrium ($S^3$ Table). Phased haplotypes from HapMap CEU were used to impute ~2.5 million autosomal SNPs using a Hidden Markov Model algorithm implemented in MACH, IMPUTE, or BimBam. Study-specific GWA analyses were conducted within each cohort using genotyped and imputed SNP dosages assuming an additive genetic model. Fish and EPA+DHA consumption were separately evaluated as the dependent variable using linear regression with robust standard error, adjusted for age, sex, energy intake (kcal/d), study-specific case ascertainment, and whether the top SNPs from the fish and EPA+DHA intake GWAS are associated with circulating EPA and DHA.

Exploratory analysis of plasma phospholipid EPA and DHA
Result from genome-wide association analyses of circulating EPA and DHA are publically available (http://faculty.washington.edu/rozenl/files/) [10]. These databases were mined to test whether the top SNPs from the fish and EPA+DHA intake GWAS are associated with circulating levels of EPA and DHA.

Results
The 17 cohorts were from the US, Estonia, Finland, Greece, Italy, and the Netherlands and included 86,467 participants with information on fish consumption and 62,265 with information on EPA+DHA consumption. Across participating cohorts, mean fish consumption ranged from...
particular Sarah Edkins and Cordelia Langford. PD is supported by the Wellcome Trust. The WGHS is supported by HL043851 and HL080467 from the National Heart, Lung, and Blood Institute and CA047988 from the National Cancer Institute, the Donald W. Reynolds Foundation and the Fondation Leducq, with collaborative scientific support and funding for genotyping provided by Amgen. The Young Finns Study has been financially supported by the Academy of Finland: grants 126925, 121584, 124282, 129378 (Salve), 117787 (Gend), and 41071 (Skidi), the Social Insurance Institution of Finland, Kuopio, Tampere and Turku University Hospital Medical Funds (grant 9M048 for TeLeht), Juho Vainio Foundation, Paavo Nurmi Foundation, Finnish Foundation of Cardiovascular Research and Finnish Cultural Foundation, Tampere Tuberculosis Foundation and Emil Aaltonen Foundation (T.L). The expert technical assistance in the statistical analyses by Irina Lisinen, Ville Aalto and Miika Helminen are gratefully acknowledged. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the other funders.

Competing interests: Luc Djousse reports receiving investigator-initiated grants (omega-3 fatty acid studies) from NIH and Amarin Pharma, Inc. Currently serving as ad hoc consultant for Amarin Pharma, Inc. Bruce Psaty reports serving on the DSMB of a clinical trial for a device funded by the manufacturer (Zoll LifeCor) and on the Steering Committee for the Yale Open Data Access Project funded by Johnson & Johnson. Paul Ridker has received research grant funds from AstraZeneca, a manufacturer of a prescription fish oil product. Oscar Franco works in ErasmusAGE, a center for aging research across the life course funded by Nestlé Nutrition (Nestec Ltd.); Metagenics Inc.; and AXA. Nestlé Nutrition (Nestec Ltd.); Metagenics Inc.; and AXA had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review or approval of the manuscript. Dr. Mozaffarian reports reports ad hoc honoraria or consulting from Bunge, Haas Avocado Board, Nutrition Impact, Amarin, Astra Zeneca, Boston Heart Diagnostics, GOED, and Life Sciences Research Organization; and scientific advisory boards, Unilever North America and Elysium Health. Harvard University holds a patent, listing Dr. Mozaffarian as one of three co-inventors, for use of trans-palmitoleic acid to prevent and treat insulin resistance, type 2 diabetes, and related conditions. All other authors report no conflicts of interest. This does not alter our adherence to PLOS ONE policies on sharing data and materials.

0.19 servings/day (FHS) to 0.75 servings/day (THISEAS) (Table 1). Mean intake of EPA+DHA consumption ranged from 89 (Rotterdam) to 563 (HBCS) mg/d and was generally consistent with findings on fish intake, except in THISEAS (Greece) which had relatively higher intakes of fish than EPA+DHA, suggesting predominant consumption of white (non-oily) fish. In general, participants in European cohorts had higher fish consumption than those in US cohorts.

The heritability estimates for fish intake were 0.13±0.03 (FamHS) and 0.24±0.02 (FHS); and for EPA+DHA intake, 0.12±0.03 (FamHS) and 0.22±0.02 (FHS). In GWA meta-analyses of fish (17 cohorts) and EPA+DHA (11 cohorts) consumption, the genomic control lambda values were 1.07 and 0.99 respectively (S1 and S2 Figs). A genome-wide significant association was observed for fish intake on chromosome 6 for rs9502823 (Table 2). The minor allele (FreqA = 0.015) was associated with 0.029 servings/day lower fish consumption (P = 1.96x10^{-5}). This SNP was mapped to LOC285768 gene of unknown function (Fig 1, top panel); and was not identified in NHGRI-EBI GWAS catalogue (http://www.ebi.ac.uk/gwas/, search on Feb 23, 2017). The second top hit was rs17396472 on chromosome 3, not achieving genome-wide statistical significance (P = 5.62x10^{-8}).

No genome-wide significant association was observed for EPA+DHA consumption (S1 and S2 Figs). The top association for EPA+DHA consumption was observed for rs11877506 (P = 1.18x10^{-7}) (Table 2). Additionally, rs7206790 in the obesity-associated FTO gene was among the top SNPs for EPA+DHA intake: the body mass index-raising G allele was associated with 7 mg/day greater EPA+DHA intake (Fig 1, bottom panel; P = 7.44x10^{-7}).

To obtain more information on the locus associated with fish consumption on chromosome 6, we investigated data from the ENCODE project. Using CEU 1000genomes data, we calculated the LD within the region 250 kb upstream and downstream from rs9502823. Mapping the SNPs found in the rs9502823 LD block to ENCODE regulatory regions, we identified rs72838923 (in complete LD with rs9502823) as a functional candidate. rs72838923 falls within a experimentally determined H3k27Ac region, identified in several cell types in ENCODE. H3k27Ac regions are thought to be markers of active enhancer activity. In addition, rs72838923 falls within DNAase Hypersensitivity Peak which were identified experimentally across 65 cell types from the ENCODE project. There is further evidence of transcription factor binding sites for FOXA1, among others in this region, from ENCODE CHIP-Seq experiments. In addition, mapping rs72838923 on the UCSC genome browser suggested that this SNP is found within a region of conservation across mammals.

Due to the varying ranges of average fish consumption in US versus European studies, we performed exploratory subgroup GWA meta-analysis stratified by geographic location. No significant associations were identified in USA nor European studies (S3 Fig) for fish or EPA +DHA consumption (S4 Fig).

A prior consortium analysis including several of these same cohorts reported on genome-wide association of SNP variants with plasma phospholipid EPA and DHA, the concentrations of which are determined by both dietary intake and endogenous metabolism regulation.[11] In exploratory analysis, we evaluated whether the top 5 hits for fish consumption and the top 4 hits for estimated dietary EPA+DHA consumption identified in the present analysis were associated with plasma phospholipid concentrations of EPA or DHA in that prior analysis [10], adjusting for multiple comparisons (9 SNPs x 2 fatty acids = Bonferroni-corrected alpha of 0.05/18 = 0.0028). No significant associations were identified (S4 Table).

Discussion

In this large GWA meta-analysis of 17 US and European cohorts totaling 86,467 participants, we found evidence that common genetic variation may be associated with consumption of
fish. We found no genome-wide significant association of common variants with EPA+DHA intake. While the sample size for the analysis of EPA+DHA was smaller than the fish intake analysis, with 62,265 individuals the analysis had 95% power to detect an effect size (heritability) of 0.08%.

We identified one locus on chromosome 6 in association with fish consumption. The SNP was mapped to LOC285768 with unknown function. The next closest gene is forkhead box Q1 (FOXQ1) which is a member of the cancer-associated forkhead-box (FOX) gene family [12].

Our evaluation of data from ENCODE, taken together, identified a functional candidate, rs72838923, that appears to lie within a transcriptionally active region of the genome. While the association was statistically significant, the magnitude of effect was small, with the minor allele being associated with a difference of 0.03 servings/day or approximately 1 serving/month of fish. This finding is more likely to be relevant for understanding the biology of food preferences than for influencing clinical outcomes, although even small differences in fish consumption, over a lifetime, could influence health. The nonsignificant top associations identified in chromosomes 1, 3, and 12 each represent intragenic regions of genes highly expressed in the brain, but these associations did not achieve genome-wide significance.

In heritability analyses, we found evidence for modest heritability of fish (0.13 to 0.24) and EPA+DHA (0.12 to 0.22). Our GWA results identified one locus in association with fish intake that cannot fully account for this observed heritability, suggesting that observed heritability might be due to remarkably small effects across a large number of SNPs, other types of genetic

### Table 1. Characteristics of the cohorts included in this analysis of the genetics of fish consumption.

<table>
<thead>
<tr>
<th>Study</th>
<th>Maximum N</th>
<th>Age</th>
<th>% Female</th>
<th>Total Fish Intake (serv/day) Median (5-95th%)</th>
<th>Dietary EPA+DHA (mg/d) Median (5-95th%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ARIC</td>
<td>9557</td>
<td>54.3±5.7</td>
<td>53</td>
<td>0.21 (0.0–0.9)</td>
<td>180 (10–730)</td>
</tr>
<tr>
<td>CHS</td>
<td>3190</td>
<td>72.3±5.4</td>
<td>61</td>
<td>0.29 (0.1–0.8)</td>
<td>191 (27–569)</td>
</tr>
<tr>
<td>DILGOM_METABO</td>
<td>3467</td>
<td>51.5±13.4</td>
<td>55</td>
<td>0.42 (0.1–1.3)</td>
<td>431 (119–1277)</td>
</tr>
<tr>
<td>DILGOM_GWA</td>
<td>604</td>
<td>52.4±13.5</td>
<td>52</td>
<td>0.44 (0.1–1.2)</td>
<td>444 (107–1233)</td>
</tr>
<tr>
<td>ESTONIAN Study</td>
<td>9920</td>
<td>48.8±20.1</td>
<td>53</td>
<td>0.21 (0.0–0.6)</td>
<td>-</td>
</tr>
<tr>
<td>FamHS</td>
<td>3640</td>
<td>52.2±13.7</td>
<td>53</td>
<td>0.14 (0.0–0.7)</td>
<td>170 (1–680)</td>
</tr>
<tr>
<td>FHS</td>
<td>7044</td>
<td>47.3±11.8</td>
<td>54</td>
<td>0.13 (0.0–0.6)</td>
<td>200 (40–640)</td>
</tr>
<tr>
<td>Health ABC</td>
<td>1494</td>
<td>74.8±2.9</td>
<td>48</td>
<td>0.20 (0.2–0.3)</td>
<td>-</td>
</tr>
<tr>
<td>Health 2000</td>
<td>1935</td>
<td>50.5±10.9</td>
<td>51</td>
<td>0.39 (0.1–1.1)</td>
<td>505 (102–1477)</td>
</tr>
<tr>
<td>HBCS</td>
<td>1701</td>
<td>61.5±2.9</td>
<td>57</td>
<td>0.44 (0.1–1.3)</td>
<td>563 (145–1895)</td>
</tr>
<tr>
<td>HPFS</td>
<td>4133</td>
<td>58.6±8.7</td>
<td>0</td>
<td>0.29 (0.1–0.9)</td>
<td>-</td>
</tr>
<tr>
<td>InCHIANTIT</td>
<td>1194</td>
<td>68.3±15.4</td>
<td>55</td>
<td>0.19 (0.0–0.5)</td>
<td>-</td>
</tr>
<tr>
<td>MESA</td>
<td>2305</td>
<td>62.7±10.2</td>
<td>52</td>
<td>0.17 (0.0–0.7)</td>
<td>100 (20–300)</td>
</tr>
<tr>
<td>NHS</td>
<td>6776</td>
<td>54.4±6.7</td>
<td>100</td>
<td>0.28 (0.1–0.8)</td>
<td>-</td>
</tr>
<tr>
<td>Rotterdam</td>
<td>4606</td>
<td>67.6±7.7</td>
<td>59</td>
<td>0.07 (0.0–0.5)</td>
<td>89 (8–443)</td>
</tr>
<tr>
<td>THISEAS</td>
<td>395</td>
<td>59.4±13.0</td>
<td>41</td>
<td>0.59 (0.0–2.0)</td>
<td>137 (4–479)</td>
</tr>
<tr>
<td>WGHS</td>
<td>22691</td>
<td>54.7±7.1</td>
<td>100</td>
<td>0.20 (0.0–0.7)</td>
<td>150 (30–470)</td>
</tr>
<tr>
<td>YFS</td>
<td>1815</td>
<td>37.8±5.0</td>
<td>56</td>
<td>0.34 (0.1–0.9)</td>
<td>357 (92–902)</td>
</tr>
</tbody>
</table>

Abbreviations: Atherosclerosis Risk in Communities Study (ARIC); Cardiovascular Health Study (CHS); Dietary, Lifestyle, and Genetic Determinants of Obesity and Metabolic Syndrome (DILGOM); Estonian Study; Family Heart Study (FamHS); Framingham Heart Study (FHS); Helsinki Birth Cohort Study (HBCS); Health 2000 survey (H2000); Health, Aging, and Body Composition (HealthABC) Study; Health Professionals Follow-up Study (HPFS); Invecchiare [Aging] in Chianti Area (InCHIANTIT); Multi-Ethnic Study of Atherosclerosis (MESA); Nurses’ Health Study (NHS); Rotterdam Study; The Hellenic Study of Interactions between SNPs and Eating in Atherosclerosis Susceptibility (THESIAS); Women’s Genome and Health study (WGHS); and Young Finns Study (YFS).

https://doi.org/10.1371/journal.pone.0186456.t001
variation such as copy number variants, epigenetic modifications, or multiple unobserved genetic interactions with unknown environmental factors. This challenge of “missing” or unaccounted for heritability is a frequent finding in GWA analyses of common diseases and traits [13]. Heritability analyses may overestimate heritability due to unmeasured shared environmental influences, for example from in utero/placental influences through childhood and adult life. In this light, our heritability findings are lower than those previously reported [4] and represent an additional important new contribution. Our findings support the need for future investigations of the possible explanations for the modest but as yet missing heritability of fish and EPA+DHA consumption.

This investigation had several strengths. Our pooling of multiple large, well-established cohorts provided a very large sample of participants for investigating our research questions. Our post-hoc power calculations demonstrate 95% statistical power to detect a genetic variant associated with an effect size of 0.05% for fish consumption and 0.08% for EPA+DHA consumption. We adjusted for total reported energy intake, which helps to address any systematic over- or under-reporting by individuals and also real differences in total food consumed (i.e., due to differences in age, sex, body size, or physical activity), facilitating evaluation of dietary composition. All the studies in the meta-analysis used comparable dietary assessment tools that were appropriate for the population under study, providing the highest quality data that can be reasonably collected across multiple large epidemiological studies.

Limitations should be considered. While dietary intakes assessed by food frequency questionnaire represent a reasonably valid method to collect data on usual dietary habits in large populations [14], such data also include measurement error, which could limit the ability to detect true associations. However, many validation studies have demonstrated that fish and EPA+DHA consumption are measured reasonably well by food frequency questionnaires, whether compared with multiple diet records or with objective circulating or tissue biomarkers [15,16,17,18]. Indeed, because biomarker levels also represent imperfect measures of “true” habitual consumption with uncorrelated errors compared to questionnaire estimates, the actual correlations of estimated fish or EPA+DHA consumption with “true” consumption are likely much higher, in the range of 0.8 or more. Compared with a candidate gene approach, GWA has lower statistical power to detect small genetic effects. Yet, candidate gene approaches for evaluating fish consumption would be strongly limited by imperfect knowledge of which genes affect known systems and biologic processes related to food preferences and, even more so, which genes may affect currently unknown systems and biologic influences on food preferences.

In summary, this large pooling project across 17 established cohorts identified modest heritability of fish and omega-3 fatty acid consumption and one genetic locus associated with fish

### Table 2. The most significant associations from genome-wide association meta-analysis of fish and EPA+DHA consumption.

<table>
<thead>
<tr>
<th>Trait</th>
<th>SNPID</th>
<th>Chr</th>
<th>Position</th>
<th>N</th>
<th>Effect/ Non-effect</th>
<th>Freq (Effect)</th>
<th>Effect</th>
<th>StdErr</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fish Intake</td>
<td>rs9502823</td>
<td>6</td>
<td>1050674</td>
<td>71910</td>
<td>A/G</td>
<td>0.02</td>
<td>-0.029</td>
<td>0.005</td>
<td>1.96E-08</td>
</tr>
<tr>
<td>(serving/day)</td>
<td>rs17396472</td>
<td>3</td>
<td>68469758</td>
<td>63102</td>
<td>A/T</td>
<td>0.98</td>
<td>0.031</td>
<td>0.005</td>
<td>5.62E-08</td>
</tr>
<tr>
<td></td>
<td>rs1860343</td>
<td>12</td>
<td>4583970</td>
<td>78153</td>
<td>T/C</td>
<td>0.52</td>
<td>-0.006</td>
<td>0.001</td>
<td>4.30E-07</td>
</tr>
<tr>
<td></td>
<td>rs1562806</td>
<td>15</td>
<td>35038800</td>
<td>61909</td>
<td>T/C</td>
<td>0.94</td>
<td>0.022</td>
<td>0.004</td>
<td>1.41E-06</td>
</tr>
<tr>
<td></td>
<td>rs16834168</td>
<td>1</td>
<td>150754770</td>
<td>77898</td>
<td>A/G</td>
<td>0.02</td>
<td>-0.020</td>
<td>0.004</td>
<td>1.58E-06</td>
</tr>
<tr>
<td>EPA+DHA</td>
<td>rs11877506</td>
<td>18</td>
<td>4917646</td>
<td>52909</td>
<td>A/G</td>
<td>0.96</td>
<td>16.313</td>
<td>3.091</td>
<td>1.18E-07</td>
</tr>
<tr>
<td>(mg/day)</td>
<td>rs2456163</td>
<td>19</td>
<td>1427432</td>
<td>36937</td>
<td>T/C</td>
<td>0.04</td>
<td>-15.560</td>
<td>3.087</td>
<td>4.20E-07</td>
</tr>
<tr>
<td></td>
<td>rs7476409</td>
<td>10</td>
<td>24809042</td>
<td>52909</td>
<td>T/C</td>
<td>0.05</td>
<td>-17.907</td>
<td>3.589</td>
<td>5.50E-07</td>
</tr>
<tr>
<td></td>
<td>rs7206790</td>
<td>16</td>
<td>52355409</td>
<td>56099</td>
<td>C/G</td>
<td>0.55</td>
<td>-7.000</td>
<td>1.420</td>
<td>7.44E-07</td>
</tr>
</tbody>
</table>

https://doi.org/10.1371/journal.pone.0186456.t002
Genome-wide association of dietary fish and EPA+DHA consumption

**Fish consumption**

![Graph showing the association between fish consumption and genetic markers](image1)

**EPA and DHA consumption**

![Graph showing the association between EPA/DHA consumption and genetic markers](image2)
consumption. These findings suggest that genetic variation may have small effects on fish consumption and, by extension, that other modifiable factors—for example, childhood diet, culture, education, income, and local availability—are the main determinants of the remarkable differences in fish consumption within and across populations, representing targets for increasing fish intake among all individuals.

Supporting information

S1 Fig. Quantile-quantile plots for (A) fish consumption and (B) EPA+DHA consumption. (TIFF)

S2 Fig. Genome-wide scans. Genome-wide association meta-analysis results of fish (A) and eicosapentanoic acid and docosahexanoic acid (B) with ~2.5 million SNPs graphed by chromosome position and–log10 p-value. (TIFF)

S3 Fig. Genome-wide scans of fish intake by geographic location. Genome-wide association meta-analysis results of fish consumption in European cohorts (A) and cohorts from the United States (B) for ~2.5 million SNPs graphed by chromosome position and–log10 p-value. (TIFF)

S4 Fig. Genome-wide scans of EPA+DHA intake by geographic location. Genome-wide association meta-analysis results of EPA+DHA consumption in European cohorts (A) and cohorts from the United States (B) for ~2.5 million SNPs graphed by chromosome position and–log10 p-value. (TIFF)

S1 Table. Description of cohorts from the CHARGE consortium. (DOCX)

S2 Table. Dietary assessment methods for CHARGE cohorts. (DOCX)

S3 Table. Genotyping methods for CHARGE cohorts. (DOCX)

S4 Table. Associations of top Fish and EPA+DHA SNPs with circulating DHA and EPA levels. (DOCX)

S1 File. Cohort Acknowledgments. (DOCX)

Acknowledgments

Disclaimer: The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or the other funders.

The Atherosclerosis Risk in Communities (ARIC) study is carried out as a collaborative study supported by National Heart, Lung, and Blood Institute contracts N01-HC-55015, N01-HC-55016, N01-HC-55018, N01-HC-55019, N01-HC-55020, N01-HC-55021, N01-HC-
55022, R01HL087641, R01HL59367 and R01HL086694; National Human Genome Research Institute contract U01HG004402; and National Institutes of Health contract HHSN2682006 25226C. The authors thank the staff and participants of the ARIC study for their important contributions. Infrastructure was partly supported by Grant Number UL1RR025005, a component of the National Institutes of Health and NIH Roadmap for Medical Research. Dr. Nettleton was supported by a K01 from the National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases (SK01DK082729-02).

The Cardiovascular Health Study (CHS) research reported in this article was supported by contracts HHSN268201200036C, HHSN268200800007C, N01HC55222, N01HC85079, N01HC85080, N01HC85081, N01HC85082, N01HC85083, N01HC85086, and grant U01HL080295 from the National Heart, Lung, and Blood Institute, with additional contribution from the National Institute of Neurological Disorders and Stroke. Additional support was provided by R01AG023629 from the National Institute on Aging. A full list of principal CHS investigators and institutions can be found at CHS-NHLBI.org. DNA handling and genotyping was supported in part by National Center for Research Resources grant M01RR00069 to the Cedars-Sinai General Clinical Research Center Genotyping core and National Institute of Diabetes and Digestive and Kidney Diseases grant DK063491 to the Southern California Diabetes Endocrinology Research Center. Dr. Mozaffarian was supported by R01 HL085710 from the National Heart, Lung, and Blood Institute.

The DILGOM study has been funded by the Academy of Finland (grant numbers 139635, 129494, 118065, 129322, 250207, 136895, 141005), the Orion-Farmos Research Foundation, the Finnish Foundation for Cardiovascular Research, and the Sigrid Jusélius Foundation. We thank the many colleagues who contributed to collection and phenotypic characterization of the clinical samples, and DNA extraction and genotyping of the data, especially Eija Hämäläinen, Minttu Jussila, Outi Törnwall, Päivi Laiho, and the staff from the Genotyping Facilities at the Wellcome Trust Sanger Institute. We would also like to acknowledge those who agreed to participate in the DILGOM study.

EGCUT received financing by FP7 grants (278913, 306031, 313010), Center of Excellence in Genomics (EXCEGEN) and University of Tartu (SP1GVARENG). We acknowledge EGCUT technical personnel, especially Mr V. Soo and S. Smit. Data analyzes were carried out in part in the High Performance Computing Center of University of Tartu.

The Family Heart Study (FamHS) work was supported in part by NIH grants 5R01 HL0 8770003, 5R01 HL08821502 (Michael A. Province) from NHLBI, and 5R01 DK07568102, 5R01 DK06833603 from NIDDK (Ingrid B. Borecki), and by the National Heart, Lung, and Blood Institute cooperative agreement grants U01 HL 67893, U01 HL67894, U01 HL67895, U01 HL67896, U01 HL67897, U01 HL67898, U01 HL67899, U01 HL67900, U01 HL67901, U01 HL67902, U01 HL65563, U01 HL6564, U01 HL6565, U01 HL6566, U01 HL6567, U01 HL6568, and U01 HL6569. The investigators thank the staff and participants of the FHS for their important contributions.

The Framingham Offspring Study and Framingham Third Generation Study (FHS) were conducted in part using data and resources from the Framingham Heart Study of the National Heart Lung and Blood Institute of the National Institutes of Health and Boston University School of Medicine. The analyses reflect intellectual input and resource development from the Framingham Heart Study investigators participating in the SNP Health Association Resource (SHARE) project. This work was partially supported by the National Heart, Lung and Blood Institute’s Framingham Heart Study (Contract No. N01-HC-25195) and its contract with Affymetrix, Inc. for genotyping services (Contract No. N02-HL-6-4278). A portion of this research utilized the Linux Cluster for Genetic Analysis (LinGA-II) funded by the Robert Dawson Evans Endowment of the Department of Medicine at Boston University School of Medicine.
and Boston Medical Center. Also supported by National Institute for Diabetes and Digestive and Kidney Diseases (NIDDK) R01 DK078616 to Drs. Meigs, Dupuis and Florez, NIDDK K24 DK080140 to Dr. Meigs, and a Massachusetts General Hospital Physician Scientist Development Award and a Doris Duke Charitable Foundation Clinical Scientist Development Award to Dr. Florez. Dr. Hivert was supported by the Centre de Recherche Medicale de l’Universite de Sherbrooke (CRMUS) and a Canadian Institute of Health Research (CHIR) Fellowships Health Professional Award. Dr. Nicola McKeown is supported by the USDA agreement No. 58-1950-7-707.

We thank all study participants as well as everybody involved in the Helsinki Birth Cohort Study. Helsinki Birth Cohort Study has been supported by grants from the Academy of Finland, the Finnish Diabetes Research Society, Folkhälsan Research Foundation, Novo Nordisk Foundation, Finska Läkaresällskapet, Signe and Ane Gyllenberg Foundation, University of Helsinki, European Science Foundation (EUROSTRESS), Ministry of Education, Ahokas Foundation, Emil Aaltonen Foundation.

The Health, Aging and Body Composition (Health ABC) study was supported in part by the Intramural Research Program of the NIH, National Institute on Aging contracts N01AG62101, N01AG62103, and N01AG62106. The genome-wide association study was funded by NIA grant R01 AG032098 to Wake Forest University Health Sciences and genotyping services were provided by the Center for Inherited Disease Research (CIDR). CIDR is fully funded through a federal contract from the National Institutes of Health to The Johns Hopkins University, contract number HHSN268200782096C.

The use of Health 2000 data in this study has been financially supported by the Academy of Finland (grant 250207) and Orion-Farmos Research Foundation. The authors would like to thank the many colleagues who contributed to collection and phenotypic characterization of the clinical samples, and DNA extraction and genotyping of the data, especially Eija Hämäläinen, Minttu Jussila, Outi Törnwall, Päivi Laiho, and the staff from the Genotyping Facilities at the Wellcome Trust Sanger Institute. They would also like to acknowledge those who agreed to participate in the H2000 study.

Invecchiare in Chianti (aging in the Chianti area, InCHIANTI) study investigators thank the Intramural Research Program of the NIH, National Institute on Aging who are responsible for the InCHIANTI samples. Investigators also thank the InCHIANTI participants. The InCHIANTI study baseline (1998-2000) was supported as a “targeted project” (ICS110.1/RF97.71) by the Italian Ministry of Health and in part by the U.S. National Institute on Aging (Contracts: 263 MD 9164 and 263 MD 821336).

The Multi-Ethnic Study of Atherosclerosis (MESA) and MESA SHARe project are conducted and supported by contracts N01-HC-95159 through N01-HC-95169 and RR-024156 from the National Heart, Lung, and Blood Institute (NHLBI). Funding for MESA SHARe genotyping was provided by NHLBI Contract N02-HL-6-4278. The authors thank the participants of the MESA study, the Coordinating Center, MESA investigators, and study staff for their valuable contributions. A full list of participating MESA investigators and institutions can be found at http://www.mesa-nhlbi.org.

The NHS and HPFS are supported by the National Cancer Institute (NHS: UM1 CA186 107, HPFS:UM1 CA167552) with additional support for genotyping. The NHS Breast Cancer GW scan was performed as part of the Cancer Genetic Markers of Susceptibility initiative of the NCI (R01CA40356, U01-CA98233). The NHS/HPFS type 2 diabetes GWAS (U01HG004399) is a component of a collaborative project that includes 13 other GWAS funded as part of the Gene Environment-Association Studies (GENEVA) under the NIH Genes, Environment and Health Initiative (GEI) (U01HG004738, U01HG004422, U01HG004402, U01HG004729, U01HG004726, 01HG004735, U01HG004415, U01HG004436, U01HG004423, U01HG004
with additional support from individual NIH Institutes (NIDCR: U01DE018993, U01DE018903; NIAAA: U10AA008401, NIDA: P01DA013423; NCI: CA63464, CA54281, CA136792, Z01CP010200). Assistance with phenotype harmonization and genotype cleaning, as well as with general study coordination, was provided by the GENEVA Coordinating Center (U01HG004446). Genotyping was performed at the Broad Institute of MIT and Harvard, with funding support from the NIH GEI (U01HG04424), and Johns Hopkins University Center for Inherited Disease Research, with support from the NIH GEI (U01HG004438) and the NIH contract "High throughput genotyping for studying the genetic contributions to human disease" (HHSN268200782096C). The NHS/HPFS CHD GWAS was supported by Merck/Rosetta Research Laboratories, North Wales, PA. The NHS/HPFS Kidney GWAS was supported by NIDDK: 5P01DK070756.

The generation and management of GWAS genotype data for the Rotterdam Study is supported by the Netherlands Organization of Scientific Research NWO Investments (nr. 175.010.2005.011, 911-03-012), the Research Institute for Diseases in the Elderly (014-93-015; RIDE2), EUROSPAN (European Special Populations Research Network:LSHG-CT-2006-01947), the Netherlands Organization for Scientific Research (Pionier, 047.016.009, 047.017.043(050-060-810)), Erasmus Medical Center and the Centre for Medical Systems Biology (CMSB I and II and Grand; National Genomics Initiative) of the Netherlands Genomics Initiative (NGI); The Rotterdam Study is further funded by Erasmus Medical Center and Erasmus University, Rotterdam, Netherlands Organization for the Health Research and Development (ZonMw), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry for Health, Welfare and Sports, the European Commission (DG XII), and the Municipality of Rotterdam. We thank Pascal Arp, Mila Jhamai, Dr Michael Moorhouse, Marijn Verkerk, and Sander Bervoets for their help in creating the GWAS database. The authors are grateful to the study participants, the staff from the Rotterdam Study and the participating general practitioners and pharmacists.

The Hellenic study of Interactions between SNPs and Eating in Atherosclerosis Susceptibility (THISEAS) study thanks the Genotyping Facility at the Wellcome Trust Sanger Institute for typing the THISEAS samples and in particular Sarah Edkins and Cordelia Langford. PD is supported by the Wellcome Trust.

The WGHS is supported by HL043851 and HL080467 from the National Heart, Lung, and Blood Institute and CA047988 from the National Cancer Institute, the Donald W. Reynolds Foundation and the Fondation Leduq, with collaborative scientific support and funding for genotyping provided by Amgen.

The Young Finns Study has been financially supported by the Academy of Finland: grants 126925, 121584, 124282, 129378 (Salve), 117787 (Gendi), and 41071 (Skidi), the Social Insurance Institution of Finland, Kuopio, Tampere and Turku University Hospital Medical Funds (grant 9M048 for TeLeht), Juho Vainio Foundation, Paavo Nurmi Foundation, Finnish Foundation of Cardiovascular Research and Finnish Cultural Foundation, Tampere Tuberculosis Foundation and Emil Aaltonen Foundation (T.L). The expert technical assistance in the statistical analyses by Irina Lisinen, Ville Aalto and Mika Helminen are gratefully acknowledged.

**Author Contributions**

**Conceptualization:** Dariush Mozaffarian, Toshiko Tanaka.

**Data curation:** Dariush Mozaffarian, Toshiko Tanaka.

**Formal analysis:** Mary K Wojczynski, Jennifer A Nettleton, Kati Kristiansson, Jari Lahti, Marilyn C Cornelis, Frank J. A van Rooij, Stavroula Kanoni, Mary F Feitosa, Julius S Ngwa, Ani Manichaikul, Terho Lehtimäki, Rozenn Lemaitre, Toshiko Tanaka.

Project administration: Dariush Mozaffarian, Toshiko Tanaka.


Writing – original draft: Dariush Mozaffarian, Toshiko Tanaka.


References


