Interactions between dietary \( n \)-3 fatty acids and genetic variants and risk of disease

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Abstract
Nutritional genomics has undergone rapid development and the concept is now very popular with the general public. Therefore, there is increasing demand for knowledge on adapting dietary composition to the genome. Our aim has been to undertake a systematic review so as to find out the level of evidence existing on whether the effects of \( n \)-3 fatty acids on health can be modulated by genetic variation.

A systematic literature search was conducted on studies that jointly analyse the effect of one or more genetic variants in candidate genes and \( n \)-3 fatty acids. Both observational and experimental studies were included. Results are classified in accordance with whether the study was undertaken on intermediate phenotypes (plasma lipid concentrations, glucose, inflammation markers, anthropometric measurements) or disease phenotypes (cancer, cardiovascular diseases, metabolic syndrome, etc) and whether it was experimental or observational. A wide diversity of genetic variants and little consistency in the publication of replication studies was found. Greater consistency was observed in studies that involved the \( FADS1 \) and \( FADS2 \) locus in the determination of \( n \)-3 fatty acid concentrations in biological samples. Most of the studies were designed to measure gene-diet interactions and not diet-gene interactions. Despite the fact that multiple studies have shown statistically significant interactions between \( n \)-3 fatty acids and certain genetic variants on intermediate and disease phenotypes, the individual level of evidence is very low and recommendations cannot be made on increasing or reducing the intake of \( n \)-3 fatty acids based on each individual's genotype.

Key words: Nutrigenetics: \( n \)-3 fatty acids: genes: cardiovascular: cancer

Background and objectives
The effect of \( n \)-3 fatty acids on health has been analysed in thousands of studies throughout the last 50 years. However, there are still great discrepancies over their effects on the different intermediate and final disease phenotypes, as well as on the optimum amount of the same, or on their relationship with \( n \)-6 fatty acids or other components of the diet. Contributing to this divergence of results obtained in the different studies may not only be the methodological differences between studies, but also, of great importance, the influence of genetic variability among study participants on the effects analysed. There are multiple candidate genes that may have an influence on modulating the effects of \( n \)-3 fatty acids on different disease phenotypes. The most relevant gene candidates will depend on the phenotype studied and, possibly, will be different for each of them (cardiovascular disease, cancer, diabetes, neurodegenerative diseases, etc). Thus, in the era of Nutritional Genomics, it is essential to have a good knowledge of the possible relevance of genetic variability on the effects of \( n \)-3 fatty acid intake in order to make more individualised dietary recommendations and to obtain several, optimal effects for each individual and for each phenotype. Likewise, the study of the influence of genetic variability on certain candidate genes will contribute to a better understanding of how the \( n \)-3 fatty acid mechanism acts on the different disease phenotypes and how to make progress on the different pathways involved.

One of the first studies to show that the effect of a genetic variant on an intermediate phenotype of cardiovascular disease could be modulated by the amount of polyunsaturated

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fatty acids (PUFA) consumed in the diet was undertaken among participants of the Framingham Study by our research group. In this study, we observed that the effect of the -75G > A polymorphism in the APOA1 gene promoter on plasma HDL-C concentrations was modulated by total PUFA intake. Thus, when PUFA intake was low (<4% of energy), GG subjects had statistically higher HDL-C concentrations than did carriers of the A allele. Conversely, when PUFA intake was high (>8% of energy), HDL-C concentrations in carriers of the A allele were higher than those of GG subjects. This interaction was found mainly in women and was non-significant in men. Although, in that study, we did not analyse the separate effects of n-3 fatty acids, it did provide a setting-off point for the analysis of gene-diet interactions and for the establishment of a basic methodology for carrying out subsequent studies by our group and other research groups. Likewise, Luan et al. showed a gene-diet interaction between PUFA intake and intermediate phenotypes associated with obesity and insulin resistance. Specifically, they showed that the Pro12Ala polymorphism in the PPARG2 interacted with the PUFA/saturated fatty acids (SFA) relationship of the diet in determining BMI and fasting insulin. Likewise, these investigators did not separate the different types of PUFA in their analyses. When we investigated the possible replication of this gene-diet interaction in a multiethnic Asian population, we did not find statistically significant results, underscoring the need of replicating gene-diet interactions in different populations as a first step to increasing the scientific level of evidence of those findings.

In general, very few interactions have survived the replication test. Thus, limiting the clinical applicability of current findings to the design of personalized diets based on genetic information. Various factors have contributed to this, including the difficulty of accurately measuring dietary intake in observational population studies, and the lack of compliance with long-term diets in large-scale nutritional interventions. Nevertheless, the methodological quality of nutrigenomic studies has been steadily improving. Today, there is an increasing number of publications on the different fields of nutritional epidemiology in general, as well as on the study of the effects of n-3 fatty acids on health. In addition, ‘in vitro’ studies and experimental animal models have shown that n-3 fatty acids influence regulation of gene expression in a number of pathways, supporting the relevance of this dietary components and their genetic interactions.

**Objectives**

The aim of this study was to perform a systematic review to assess the level of evidence supporting the effect of genetic variability in modulating the effects of n-3 fatty acids on intermediate and disease phenotypes.

**Methods**

**Criteria for considering studies for this review**

**Types of studies.** This review was not restricted to randomized controlled trials, but included any epidemiological study independently of its design, in which the interaction of n-3 fatty acids and any number of genetic variants has been studied in relation to intermediate and disease phenotypes. On presenting results, studies were divided into two different categories depending on the type of study: (1) Observational studies, which included both, cross-sectional studies as well as case-control and cohort studies and (2) Intervention studies, which included studies in which a dietary intervention was undertaken and where the contribution of n-3 fatty acids was studied. Both controlled and uncontrolled studies, with or without randomization were analysed. There was no restriction on language, publication type and sample size.

**Types of participants.** There was no restriction on the basis of gender, age, ethnicity, study setting or other characteristics of participants. Studies undertaken on a general healthy population as well as studies that involve different types of patients with certain intermediate or final disease phenotypes were included. On analysing each study, the sample type is indicated in order to ensure the homogeneity of comparisons.

**Types of n-3 fatty acid measurements.** All observational or intervention studies in which a measurement of the n-3 fatty acid intake contributed by diet (total or separated as LC n-3 fatty acids (LC n-3 fatty acids), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), alpha-linolenic acid (ALA) or others was undertaken by means of the different types of diet questionnaires, including semiquantitative food frequency questionnaires (FFQ), 24-h recalls or dietary records, were included. Also included were those studies that measured total or specific n-3 fatty acids on different kinds of biological samples (subcutaneous fat, erythrocytes, plasma, breast milk or serum). There is an excellent review on the validity of each of these methods for correctly measuring n-3 fatty acids in different situations. This review concludes that the summarised quality of n-3 fatty acid estimates derived from FFQ is good or acceptable according to the (EU)ropean micronutrient RECommendations Aligned) EUR-RECA scoring system and that no dietary intake method is superior to any other. With regard to measurements of n-3 fatty acids in different biological samples, the literature describes subcutaneous fat as the best reference method. We also included all intervention studies in which n-3 fatty acids were administered under various preparations (enriched foods, oils, pills, etc).

**Types of genetic variants.** Any study that reported one or more genetic variants of any kind [single nucleotide polymorphisms (SNP), insertions, deletions, copy number variations (CNV)] were included. Haplotypes as well as combined analysis of genetic variant were also included.

**Types of outcome measures.** All types of diseases were included, both disease phenotypes (cancer, cardiovascular diseases, neurodegenerative diseases, metabolic syndrome, diabetes, etc), and intermediate disease phenotypes (plasma lipids, plasma glucose and insulin, anthropometric measures, inflammation markers, etc).
Search methods for identification of studies

A systematic literature search was performed in April 2011. The literature search was conducted in the following databases: Medlars Online International Literature (MEDLINE), via PubMed; EMBASE; and Latin American and Caribbean Health Sciences Literature (LILACS). We first searched in MEDLINE (from 1966 to 2011) using common medical subject heading (MESH) terms and adapted for use in other databases. The following terms were used: n-3 or n-3 fatty acid or n-3 fatty acids; or n-3 or n-3 fatty acids; or polyunsaturated fatty acid or polyunsaturated fatty acids; or unsaturated fatty acids or essential fatty acids; or alpha-linolenic acid or ALA; or eicosapentaenoic acid or EPA; or docosahexaenoic acid or DHA; or docosapentaenoic acid or DPA; or alpha-linolenic acid or ALA or long chain fatty acids. These terms were combined in the search with the genetic terms in order to find studies that analyse the genetic influence on the effects of n-3 fatty acids. The genetic terms used were: genes; or genetic variants; or gene variants; or gene variant; or polymorphisms. Furthermore, as several of the studies found using those key words were carried out on animals, plants or in vitro, another term was added to the search to further limit the studies. The term added was ‘human/s’.

Processing of articles

Initial screening of articles was undertaken on the basis of abstracts. If it was clear from the abstract that the article did not meet the inclusion criteria, it was rejected. Titles and abstracts were screened by both authors for inclusion based on study design, n-3 fatty acids assessment, genetic analysis and outcome measures. The full text of the selected article was retrieved so as to analyse the complete information. Full text articles were also searched for any further relevant publications. Both authors extracted data from the studies and study details were summarised in tables or in text. Although there are various study quality indicators that could be applied, we have opted, in this review, not to apply them for including or excluding a study, as this is a time consuming process and what we wanted at the onset was to have a wide vision of the studies undertaken that had analysed the genetic influence on the multiple effects of n-3 fatty acids on health. Nevertheless, in the review and information gathering process, the authors have taken into account whether the study fulfils the minimum quality indicators, making a comment on the study when some limitation is detected (reduced sample size, lack of adjustment for potential confounders, poor quality of genetic analysis, etc).

Results

From the various search terms introduced, the widest number of articles was found with the ‘PUFA and genes’ combination. This search resulted in 5674 papers. However, the screening of the abstracts showed that it was too general a search as it were included all types of PUFA and not only n-3. Restricting the search to the terms ‘n-3 fatty acids and genes’ resulted in 670 papers. The screening of those abstracts revealed that the search was still too wide, as it included studies of all kinds on animals, plants, cultivated cells, etc., so the term ‘humans’ was added, resulting in 150 papers. As the term ‘genes’ is also non-specific, as it includes studies of all kinds in which analyses of expression, regulation, etc were carried out, the search was narrowed by adding the term ‘gene variants’ or ‘genetic variants’. This selection turned out to be too restrictive, as only twenty-five papers were found. Having tested various strategies of search term combinations, it was observed that the term ‘polymorphism’, is more suitable than ‘genetic variant’, as it results in a greater number of papers that fulfil the inclusion criteria of focusing on epidemiological studies that simultaneously analyse the effect of genetic variants and n-3 fatty acids on intermediate or final disease phenotypes. Sixty papers were obtained in this way. Of which fifteen were discarded for being reviews or for not studying the theme even though they had been selected by the aforementioned key words indicated. In addition, the reference lists of the selected articles were scanned, which resulted in another four original publications for inclusion.

Influence of genetic variants in n-3 fatty acids concentration in different biological samples

On analysing the literature on the influence of the different genetic variants on the effects of n-3 fatty acids in intermediate and disease phenotypes, we noted that in recent years various articles have been published showing several highly consistent effects of certain genetic variants, namely at desaturases loci, on PUFA concentrations in different tissues. We believe that these genetic variants may be very important in further studies, so we began first of all to analyse the effects of those variants. The delta-6 desaturase (D6D) and delta-5 desaturase (D5D) are membrane-bound enzymes that catalyze the rate-limiting formation of LC-PUFA. D6D catalyzes the conversion of ALA (18 : 3n-3) and linoleic acid (LA, 18 : 2n-6) into stearidonic acid (STD, 18 : 4n-3) and γ-linolenic acid (GLA, 18 : 3n-6), respectively. D5D catalyzes the conversion of eicosatetraenoic acid (ETA, 20 : 4n-3) and dihomoy-γ-linolenic acid (DGLA, 20 : 3n-6) into EPA (20 : 5n-3) and arachidonic acid (AA, 20 : 4n-6), respectively. The desaturase-encoding genes (FADS1 for D5D and FADS2 for D6D) form a gene cluster on chromosome 11 together with a third desaturase gene, FADS3, of lesser known function. The first epidemiological study in which a significant association was shown between variation in that cluster and PUFA concentrations was carried out by Schaeffer et al. on 727 Germans of the European Community Respiratory Health Survey I. After analysing eighteen SNP, they found that eleven of them presented highly significant associations with the concentrations of various PUFA in serum phospholipids. Thus, carriers of the minor alleles at several SNP (rs174544, rs174553, rs174556, rs174561, rs8384458, rs968567, rs99780, rs174570, rs2072114, rs174583, or rs174589) had enhanced levels of the precursor PUFA with two or three double bonds and reduced levels of LC-PUFA with ≥4 double bonds and major product-to-substrate interactions between dietary n-3 fatty acids and genetic variants
ration of the n-6 pathway (AA-to-LA ratio) and the n-3 pathway (EPA-to-ALA ratio).

Interestingly, fatty acids belonging to other pathways like oleic acid and DHA, whose source is mainly nutritional, did not show significant associations with those genetic variants. Subsequent studies have consistently replicated the associations between polymorphisms in the FADS1 and FADS2 genes and PUFA concentration measurements in different biological samples including breast milk\(^9\)–\(^10\). In the excellent review on this subject undertaken by Latka et al.\(^19\), those studies indicating the SNP analysed and the fatty acids for which an association has been found in different tissues are presented in detail. These associations between the FADS1/FADS2 cluster and PUFA concentrations have also been confirmed in genome wide association studies (GWAS)\(^20\). Other GWAS have also reported associations between the FADS1/FADS2 cluster and plasma lipid concentrations\(^21\),\(^22\).

Therefore, it will be informative to accumulate additional evidence related to the most relevant polymorphisms in FASD1 and FASD2 in future studies seeking to analyse the influence of n-3 fatty acids on disease, as the overall concentrations of n-3 fatty acids will not depend only on intake, but will also be modulated by these genetic variants. However, bearing in mind that the discovery of the importance of these polymorphisms is recent, most of the studies undertaken that have focused on genetic variants have not analysed them, so we do not have the full data. There are, however, several recent studies that have studied the influence of genetic variants in FASD1 and FASD2 as modulators of the effects of dietary n-3 fatty acids on various phenotypes that is summarised in the corresponding tables depending on the study design.

To organise the presentation of results and bearing in mind that we have not selected a specific disease but all phenotypes that have been researched, we will first consider the study type, whether it is an observational or experimental study. As most studies are observational, within them we will consider whether they have studied intermediate or disease phenotypes so as to present them in different tables. Intervention studies will be analysed jointly.

Interaction between n-3 fatty acids and genetic variants in determining intermediate disease phenotypes in observational studies

One of the first studies to separately study the effects of n-3 and n-6 fatty acids in modulating the effects of a genetic variant on an intermediate cardiovascular risk phenotype was carried out by our research group on participants in the Framingham Study\(^23\). Regulation of gene expression by PUFA can occur through interaction with specific or non-specific ligands. Specifically, it has been shown that PUFA can interact directly with transcription factors such as peroxisome proliferator-activated receptor α (PPARα), a nuclear transcription factor that regulates multiple genes involved in lipid homeostasis. One genetic variant in the PPARα gene, consisting of a G484C transversion at the codon 162, creates a missense mutation that alters leucine to valine (L162V) and has functional consequences on receptor activity depending on the concentration of the ligand. PUFA are natural ligands of PPARα and, therefore, their concentration can increase or reduce the gene expression of the PPARα gene, which in turn modulates multiple genes. In the Framingham Study we found a statistically significant interaction between the L162V polymorphism and dietary PUFA intake in determining plasma triglycerides and apoC-III concentrations (Table 1)\(^24\)–\(^34\). For both parameters, those with the 162V allele had lower concentrations of TG and apoC-III with higher intake of PUFA. In contrast, among homozygotes for the 162L allele, PUFA intake did not decrease either TG or apoC-III concentrations. We also explored the effect of n-3 and n-6 fatty acids and found an effect similar for both. Later, Chan et al.\(^24\) attempted to study this interaction in Chinese, Malays and Asian Indians participating in the 1998 Singapore National Health Survey. However, they found that the L162V variant was not polymorphic in the Asian population. Therefore, their genetic analysis focused on the V227A variant which is common among Asians, but not in Caucasian populations. Although they did not analyse the effects of n-3 and n-6 fatty acids separately, a statistically significant interaction was found between the V227A polymorphism and dietary PUFA in HDL-C concentrations in Chinese women. Even though this interaction may have been observed in a different phenotype, it contributes to supporting the hypothesis that the effects of the variation in the PPAR4 gene are modulated by PUFA. Some years later, Volcik et al.\(^25\) evaluated the influence of PPAR4 genetic variation on the association between PUFA intake (specifically n-6 and LC n-3 fatty acids) and plasma lipid concentration in the biethnic Atherosclerosis Risk in Communities (ARIC) Study. Although they found no significant interactions between the PPAR4 L162V polymorphism and plasma lipids, they did find an interaction with the 3′UTR C→T SNP polymorphism in the same gene. Being a biethnic population, factors related with the different prevalence or relevance of certain genetic variants between populations may have had an influence on the results.

In addition to these observational studies, an intervention study in which the PUFA/SFA ratio was modified in the diet, concluded that the L162V in the PPARα gene significantly contributes to modulating the different response observed among individuals\(^35\). In a more detailed way, Rudkowska et al.\(^36\) showed that n-3 fatty acids regulate gene expression levels differently in subjects carrying the PPARα L162V polymorphism. These authors also examined the effects of n-3 fatty acids on LPL activity taking into consideration the presence of the PPARα L162V polymorphism, and observing that n-3 fatty acids increase the transcription rate of LPL to a greater extent in L162-PPARα than V162-PPARα alleles\(^37\). All of this contributes to increasing the evidence that the effects of n-3 fatty acids on plasma triglyceride concentrations and related measures are modulated by the PPARα gene variation. However, the level of evidence is still insufficient to be able to make dietary recommendations on n-3 fatty acid intake based on the determination of this polymorphism.

Whereas most of the previous gene-PuFA interactions related to plasma lipids focused on PPARα, there has been a current shift towards other genes, including the FADS genes.
Table 1. Observational studies analyzing \(n\)-3 fatty acids and genetic variants in determining intermediate phenotypes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study population</th>
<th>Phenotype</th>
<th>Genetic variant (s)</th>
<th>Interaction</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tai et al.(^{(23)})</td>
<td>Cross-sectional study in participants in the Framingham Offspring Study (1003 men and 1103 women)</td>
<td>Plasma triglycerides and apoC-III concentrations</td>
<td>PPARA (L162V polymorphism)</td>
<td>Yes</td>
<td>Carriers of the 162V allele had significantly lower plasma triglycerides and apoC-III concentrations when consuming a high PUFA diet, in which (n)-3 and (n)-6 fatty acids seem to have a similar role.</td>
</tr>
<tr>
<td>Chan et al.(^{(24)})</td>
<td>Cross-sectional study among Participants in the 1998 Singapore National Health Survey: 1964 men (1318 Chinese, 364 Malays and 282 Asian Indians) and 2284 women (1581 Chinese, 397 Malays and 306 Indian Indians)</td>
<td>Plasma lipids</td>
<td>PPARA (V227A polymorphism)</td>
<td>Yes</td>
<td>In carriers of the A227 allele, increasing PUFA intake was associated with lower HDL-C concentrations. In those who were homozygous for the common allele, this association was much weaker</td>
</tr>
<tr>
<td>Volcik et al.(^{(25)})</td>
<td>Cross-sectional study among participants in the biethnic Atherosclerosis Risk in Communities (ARIC) Study (10134 whites and 3480 African Americans)</td>
<td>Plasma lipids</td>
<td>PPARA (L162V and 3’UTR C → T polymorphisms)</td>
<td>Yes</td>
<td>A significant interaction between the PPARA 3’UTR C → T SNP and long-chain (n)-3 fatty acids on LDL-C concentrations in African American participants. No significant interaction with the PPARA L162V SNP.</td>
</tr>
<tr>
<td>Lai et al.(^{(26)})</td>
<td>Cross-sectional study among participants in the Framingham Offspring cohort (1001 men and 1147 women)</td>
<td>Plasma lipids, remnant-like particle concentrations, and lipoprotein particle size</td>
<td>APOA5 SNP: −1131T &gt; C, −3A &gt; G, IVS3 + 476G &gt; A, and 1258T &gt; G and 56C &gt; G</td>
<td>No with (n)-3 fatty acids</td>
<td>Significant gene-diet interactions between the −1131T &gt; C polymorphism and total PUFA intake were found in determining fasting triglycerides, remnants and particle size. However, the PUFA-APOA5 interactions were specific for dietary (n)-6 fatty acids. No significant interaction with (n)-3 fatty acids were found</td>
</tr>
<tr>
<td>Verduci et al.(^{(27)})</td>
<td>Cross-sectional study in obese children (53 girls and 68 boys)</td>
<td>Plasma adiponectin and HOMA-IR</td>
<td>ADIPOQ gene (SNP 276G &gt; T)</td>
<td>Yes</td>
<td>In obese children, carriers of the SNP 276G &gt; T may be at increased risk of metabolic complications compared with noncarriers, possibly due to the n-6/n-3 LC-PUFA ratio in phospholipids</td>
</tr>
<tr>
<td>Ylönen et al.(^{(28)})</td>
<td>Cross-sectional etudy among 571 non-diabetic relatives of subjects with type II diabetes.</td>
<td>Plasma glucose and IR</td>
<td>PPARG2 (Pro12Ala SNP)</td>
<td>Yes</td>
<td>The PPARG polymorphism modulate the associations marine (n)-3 fatty acids with glycemic metabolism and fasting free fatty acids</td>
</tr>
<tr>
<td>Lu et al.(^{(29)})</td>
<td>Cross-sectional investigation in the Doetinchem Cohort Study (3575 subjects)</td>
<td>Plasma lipids</td>
<td>FADS cluster (rs174546, rs482548, and rs174570 polymorphisms)</td>
<td>Yes</td>
<td>Significant associations between rs174546 genotypes and total and non-HDL-cholesterol concentrations in the group with a high intake of (n)-3 PUFA but not in the low intake group</td>
</tr>
<tr>
<td>Kim et al.(^{(30)})</td>
<td>Cross-sectional study in Koreans (580 men, 614 women).</td>
<td>Serum phospholipids, adiponectin, HOMA-IR</td>
<td>ADIPOQ: (−11391G &gt; A; −11377C &gt; G; H241P; Y111H; G90S; R221S; 45T &gt; G; 276G &gt; T polymorphisms)</td>
<td>No with n-3</td>
<td>The 276G carriers with a higher proportion of 18:2n6 exhibited more pronounced IR characteristics. No interaction with n-3 was observed</td>
</tr>
<tr>
<td>Zhou et al.(^{(31)})</td>
<td>Cross-sectional study in Chinese (195 men and 386 women). Erythrocyte membrane fatty acids were measured</td>
<td>Inflammatory markers</td>
<td>IL-6 (-572 C &gt; G polymorphism)</td>
<td>Yes</td>
<td>Erythrocyte (n)-3 PUFA intake modulated the effects of IL-6 -572 genotype on HDL-C concentrations in males but not in females</td>
</tr>
</tbody>
</table>
Table 1 also provides a summary of the other studies found during this review that analyse the interaction between n-3 fatty acid levels and intermediate disease phenotypes including plasma lipids, anthropometric measurements, insulin resistance and inflammatory markers. Of all of them, we would like to mention in particular and because of its latest findings involving microRNAs (short post-transcriptional regulators that bind to complementary sequences in the 3′UTR of multiple target mRNAs, usually resulting in their silencing), the study recently published by Richardson et al. \(^{34}\). The authors found significant interactions between the rs8887 minor allele in the perilipin 4 \((PLIN4)\) gene with the n-3 fatty acid intake modulating anthropometrics. Moreover, in \textit{silico} analysis of the \textit{PLIN4} 3′UTR sequence surrounding the rs8887 minor allele predicted a seed site for the human microRNA-522 (miR-522). In vitro results suggested that the interaction with PUFA acts in part through creation of a miR-522 regulatory site.

### Interaction between n-3 fatty acids and genetic variants in determining disease phenotypes in observational studies

In this section, we have reviewed the studies that analyse the influence of the n-3 fatty acids, either originating from the diet or through their measurement in biological samples and different disease phenotypes (Table 2) \(^{38-47}\). We have considered disease phenotypes to be the different types of cardiovascular disease (coronary heart disease, stroke), cancer (breast, lung, colo-rectal, prostate), metabolic syndrome, diabetes, etc.

Various studies have been carried out that have investigated the interaction between intake of n-3 fatty acids and the risk of different types of cancer. The primary working hypotheses was that marine n-3 fatty acids may provide protection against breast cancer, as shown in previous experimental studies. It is, moreover, hypothesized that the inter-individual differences in the ability to protect cells from cytotoxic lipid peroxidation products may determine the protective effect of marine n-3 fatty acids on breast cancer. The glutathione S-transferases (GST) are potential major catalysts in the elimination of these beneficial by-products. These enzymes are polymorphic and many of them have been studied in relation with different types of cancer, hence the great importance currently placed on them as modulators of cancer risk faced with different environmental exposures \(^{48}\). There are three well-characterized isozymes, \textit{GSTM1}, \textit{T1}, and \textit{P1}, in which polymorphisms have been extensively studied with respect to DNA adducts and cancer. Gago-Dominguez et al. \(^{38}\) hypothesized that women possessing low activity GST genotypes \((GSTM1\) null, \textit{GSTT1} null and \textit{GSTP1} AB/BB) might exhibit a stronger marine n-3 fatty acid—breast cancer inverse association than those possessing the high activity genotypes. To check this hypothesis, they undertook a nested case-control study among the participants in the Singapore Chinese Health Study, a population-based, prospective investigation of diet and cancer risk. At recruitment (from April 1993 to December 1998), information on usual diet over the last year was obtained by a semi-quantitative food frequency questionnaire. In 2002,
Observational studies analyzing N-3 fatty acids and genetic variants in determining disease phenotypes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study population</th>
<th>Phenotype</th>
<th>Genetic variant(s)</th>
<th>Interaction</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gago-Dominguez et al.</td>
<td>Nested case-control study in women in the Singapore Chinese Health Study, a population-based, prospective investigation of diet and cancer risk</td>
<td>Breast cancer incidence</td>
<td>Glutathione S-transferases (GST) genotypes (GSTM1 null, GSTT1 null and GSTP1 AB/BB)</td>
<td>Yes (GSTP1 and quartiles of marine n-3)</td>
<td>Women with genetic polymorphisms encoding lower or no enzymatic activity of GSTM1, GSTT1 and/or GSTP1 experienced more breast cancer protection from marine n-3 fatty acids than those with high activity genotypes.</td>
</tr>
<tr>
<td>Hedelin et al.</td>
<td>Population-based case-control study of prostate cancer (n = 1,499 cases and 1,130 controls) in Sweden</td>
<td>Prostate cancer</td>
<td>COX-2 gene: rs2745557, rs20432, rs4648276, rs5275 and rs689470</td>
<td>Yes COX-2 gene (rs5275 SNP) and salmon-type fish intake</td>
<td>The association between marine fatty acids and the risk of prostate cancer was modified by genetic variation in the COX-2 gene. Strong inverse associations with increasing intake of salmon-type fish only observed among carriers of the variant allele of the rs5275.</td>
</tr>
<tr>
<td>Fradet et al.</td>
<td>Case-control study (n = 506 aggressive incident prostate cancer cases and 506 controls) in Cleveland, USA</td>
<td>Prostate cancer</td>
<td>COX-2 gene: rs689466, rs20417, rs2745557, rs5277, rs2066826, rs5275, rs2206593, rs689470, and rs4648310</td>
<td>Yes COX-2 gene (rs 4648310 SNP) and LC n-3 fatty acids intake</td>
<td>The inverse association between LC n-3 fatty acids and the risk of aggressive prostate cancer was modified by genetic variation in the COX-2 gene. Strong protective association among carriers of the rs4648310 variant allele.</td>
</tr>
<tr>
<td>Stern et al.</td>
<td>Case-control study (n = 753 cases and 799 controls) in a study of risk factors in USA</td>
<td>Colo-rectal cancer</td>
<td>XRCC1-194, XRCC1-399 and XRCC3-241</td>
<td>Yes XRCC1 gene and n-6/n-3 ratio</td>
<td>The effect of the XRCC1 polymorphisms on colorectal cancer risk is modulated by the n-6/n-3 ratio. A high n-6/n-3 ratio increased the risks in subjects carrying the XRCC1 Trp194 allele or the Arg399 allele.</td>
</tr>
<tr>
<td>Stern et al.</td>
<td>Nested case-control study in the Singapore Chinese Health Study (310 cases and 1,176 controls)</td>
<td>Colo-rectal cancer</td>
<td>XRCC1: rs1799782 and rs25487, PARP: rs1136410, and rs3219145. OGG1: rs1052133; XPD: rs1799793 and rs13181</td>
<td>Yes PARP gene (rs1136410) and marine n-3 PUFA</td>
<td>The PARP Val762Ala SNP modified the association between marine n-3 PUFA and colorectal cancer risk, with no evidence of interaction among colon cancer. Carriers of the 762Ala allele have higher colorectal cancer risk with a high intake of marine n-3 PUFA.</td>
</tr>
<tr>
<td>Guerreiro et al.</td>
<td>Case-control study in 116 controls and 99 patients with Crohn’s disease</td>
<td>Crohn’s disease and pro- and anti-inflammatory cytokines</td>
<td>Seven SNP in the IL1, TNFA, LTA, and ILE genes</td>
<td>Yes TNFA and n-3 PUFA</td>
<td>Low intake of n-3 PUFA and high n-6/n-3 PUFA ratio in patients with the TNFA 857 polymorphism were associated with higher disease risk.</td>
</tr>
<tr>
<td>Martinelli et al.</td>
<td>Case-control study in the Verona Heart Study (610 cases of angiographically documented CAD and 266 controls)</td>
<td>Angiographically proven coronary artery disease, inflammatory markers</td>
<td>Thirteen SNP tagging the greatest variability of the FADS gene region</td>
<td>Yes FADS genotypes and erythrocyte PUFA</td>
<td>Haplotypes of the FADS gene cluster, including variants associated with an elevated AA/LA, were also associated to both a higher hs-CRP concentration and greater risk of coronary artery disease. This was modulated by the the ratio of AA to LA on erythrocytes.</td>
</tr>
<tr>
<td>Dwyer JH et al.</td>
<td>Cohort of 470 healthy, middle-aged women and men from the Los Angeles Atherosclerosis Study</td>
<td>Carotid-artery intima-media thickness, and markers of inflammation</td>
<td>5-LO genotypes</td>
<td>Yes 5-LO and n-3</td>
<td>Increased dietary arachidonic acid significantly enhanced the apparent atherogenic effect of the 5-LO genotype, whereas increased intake of n-3 fatty acids blunted the effect.</td>
</tr>
</tbody>
</table>
they identified 399 cases of incident breast cancer among female cohort subjects. Of those cases, they were only able to carry out a genetic analysis on 258 cases. For marine n-3 fatty acid intake, subjects were categorized based on quartile distribution values among females. They examined the association between n-3 fatty acids and breast cancer stratified by GSTM1, GSTT1 and GSTP1 genotypes and found a stronger protection of the n-3 fatty acids in the low than high activity genotype subgroups. Thus, the risk of breast cancer in high (quartiles 2–4) v. low (quartile 1) consumers of marine n-3 fatty acids, was reduced by half in women with the genotype GSTP1 AB/BB genotype (P<0.05), achieving similar results on the limit of statistical significance for the other genotypes. The authors concluded that the Chinese women carriers of the genetic polymorphisms encoding lower or no enzymatic activity of GSTM1, GSTT1 and/or GSTP1 experienced more breast cancer protection from marine n-3 fatty acids than those with high activity genotypes, this being the first study to report that association. It has not been possible to replicate these results in subsequent studies, so the level of evidence of the interaction between these polymorphisms and marine n-3 fatty acids on the incidence of breast cancer remains low.

Continuing with the premise that a high intake of n-3 fatty acids, especially LC fatty acids (EPA and DHA) can protect against prostate cancer, and in order to gain better understanding about their mechanisms and the potential genetic influence, Hedelin et al.\(^{(39)}\), carried out a population-based case-control study in Sweden. Bearing in mind that one of the proposed mechanisms by which n-3 fatty acids may affect carcinogenesis is through their suppressive effect on the biosynthesis of eicosanoids derived from arachidonic acid, cyclooxygenase-2 (COX-2), a key enzyme in eicosanoid synthesis, overexpressed in prostate cancer tissues, it may play a very important role. Therefore, the COX-2 gene could be important in modulating the effect of the n-3 fatty acids on the risk of prostate cancer. To test this hypothesis, they analysed 5 polymorphisms in the COX-2 gene in participants of the Cancer Prostate in Sweden Study. Fish consumption and the intake of n-3 fatty acids were measured through a validated FFQ. They found no association between total intake of n-3 fatty acids and prostate cancer risk, but did find an inverse association between fatty fish consumption and the risk of prostate cancer. They only found a statistically significant interaction between salmon-type fish intake or combined intake of salmon/herring/mackerel and the COX +6365 T/C polymorphism. Thus, among subjects who were heterozygous or homozygous for the variant allele (C), high intake of salmon-type fish was associated with a significantly decreased risk of prostate cancer (OR for once per week or more v. never = 0.28, 95% CI 0.18, 0.45). No association was found in carriers of the major allele.

Interestingly, the results of this study have been partially replicated by others, so increasing the consistency level of the interaction between n-3 fatty acids and the COX-2 gene polymorphisms. Fradet et al.\(^{(40)}\) in a case-control study that included aggressive prostate cancer cases in Cleveland, USA, assessed n-3 fatty acid intake through a FFQ and genotyped nine polymorphisms in the COX-2 gene. They found that an increased intake of LC n-3 was strongly associated with a
Interactions between dietary n-3 fatty acids and genetic variants

The decreased risk of aggressive prostate cancer, as well as a statistically significant interaction between the LC n-3 fatty acid intake and a COX-2 polymorphism (SNP rs4648310), so that in men with this variant SNP, the association of the LC n-3 fatty acids with lower risk of prostate cancer was very strong.

Fradet et al.\textsuperscript{(40)} did not find a similar pattern of interaction with rs5275. The SNP rs4648310 and rs5275 are located 2.4 kb apart and have weak linkage disequilibrium among Whites. The functional effect of each one of these variants on COX-2 activity is not yet known. However, the combined findings of Fradet et al.\textsuperscript{(40)} and Hedelin et al.\textsuperscript{(37)} support the hypothesis that the effect of LC n-3 fatty acids on the risk of prostate cancer may be modified by COX-2 genetic variation.

On the other hand, persistent oxidative stress and oxidative damage to the colon epithelium seem to play an important role in colorectal cancer. Polymorphisms in genes involved in the fatty acids-DNA damage/repair pathway, therefore, may play a role in determining the effects of fatty acids in causing DNA damage and subsequent colon cancer risk. XRCC1 (X-ray repair cross-complementing protein 1) and XRCC3 (X-ray repair complementing defective repair in Chinese hamster cells 3) are involved in the efficient repair of DNA and may play an important role in the repair or modulation of the effects of PUFA intake on cancer colo-rectal cancer risk. Stern et al.\textsuperscript{(41)} studied the effect of some polymorphisms in the XRCC1 and XRCC3 genes as unsaturated fatty acids effect modifiers on colorectal cancer risk in the USA. The XRCC1-194, XRCC1-399 and XRCC3-241 polymorphisms were determined. The ratio of dietary n-6/n-3 fatty acids was calculated. Although they did not find significant interactions between the polymorphisms and total PUFA intake on cancer risk, they found a significant interaction between the omega-6/ω-3 ratio and the XRCC1 genotypes on colorectal cancer risk. Subjects with the XRCC1 Trp 194 allele or the Arg 399 allele seem to have a higher risk of colorectal adenomas when ratio is high. No evidence of an XRCC3-241 effect modification on the effect of high intake ratios was observed. Bearing in mind that this is the first study to report a significant interaction between the n-6/n-3 fatty acid ratio and the risk of colo-rectal cancer, further studies are required to confirm the results. Moreover, many more genes other than the XRCC1 and the XRCC3 play a role in the fatty acids-DNA repair pathways. Thus, the simultaneous analysis of all relevant genes in the complex pathway will be useful in understanding these interactions better. Along these lines, Stern et al.\textsuperscript{(42)} carried out another nested case-control study on Chinese participants in the Singapore Chinese Health Study. 7 SNP in various DNA repair genes: XRCC1, Poly (ADP-ribose) polymerase (PARP), Oxoguanine glycosylase 1 (OGG1), and xeroderma pigmentosum group D (XPD) were analyzed. The authors reported that the PARP Val762Ala SNP modified the association between marine n-3 fatty acids and rectal cancer risk, with no evidence of interaction with colon cancer. A high intake of marine n-3 fatty acids notably increases cancer risk in 762-Ala allele carriers. The PARP protein plays an important role in maintaining genomic stability, apoptosis, and regulating transcription. The mechanism through which greater marine n-3 fatty acids could increase cancer risk in carriers of this genetic variant is not fully understood, but the fact that the authors have not found this same effect in total n-3 fatty acid intake suggests that the association must be mainly due to the LC EPA and DHA. In addition to cancer, several statistically significant interaction between n-3 fatty acids and genetic variants in determining other disease phenotypes have been found (metabolic syndrome, cardiovascular diseases, etc).\textsuperscript{(43–47)} These studies are also summarized in Table 2.

Interaction between n-3 fatty acids and genetic variants in determining intermediate and disease phenotypes in intervention studies

Table 3\textsuperscript{(49–54)} provides a summary of the main characteristics of intervention studies based on changes in food patterns, or addition of specific food or dietary supplement, all aimed to provide different levels of n-3-fatty acids. Given the large diversity of results and genotypes analysed, firm conclusions cannot be established. Intervention studies are few and far between and often carried out on small populations, so new intervention studies on larger samples and over longer periods of time are required.

Although intervention studies provide a higher level of scientific evidence than observational studies, they are more complex to undertake, especially on large samples. One of the limitations of most of the intervention studies carried out to date has been the fact that the sample size is small and the groups of the different genotypes have been heterogeneous in size because the studies were not basically designed to investigate the effect of a genetic variant or previously selected genetic variants, but to first undertake the intervention and then genotype the individuals. One proposal for improving this would be to undertake a priori the choice of the main genetic variants to be studied and select individuals for their genotype (either by a genetic variant or by a combination of relevant genetic variants) and include the individuals in the study depending on their genotype in order to form groups of similar size and with an adequate statistical power. We should incorporate better markers of dietary intervention compliance and discard individuals who do not comply with the intervention instead of analyzing ‘intention to treat’ data. Furthermore, it would be necessary to propose a parallel sample of replication in order to check the replication of results in different individuals from the same population and so increase internal validity. Later studies would also be required in order to confirm the external validity of the results in other populations.

Conclusions

There are scores of studies showing statistically significant interactions between n-3 fatty acid intake and/or their concentrations in different biological samples and various genotypes, resulting in differing effects of n-3 fatty acids on the disease analysed (dyslipidemias, metabolic syndrome, obesity, cancer or cardiovascular diseases). These results suggest that the effect of n-3 fatty acids on the various intermediate phenotypes and genotypes can be more effectively studied with the addition of specific food or dietary supplements, all aimed to provide different levels of n-3 fatty acids.
### Table 3. Intervention studies analyzing n-3 fatty acids and genetic variants in determining intermediate and disease phenotypes

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study population</th>
<th>Phenotype</th>
<th>Genetic variant</th>
<th>Interaction</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lindman et al. (49)</td>
<td>Intervention study in 219 subjects from the Diet and Omega-3 Intervention Trial on atherosclerosis (DOIT). Four groups: placebo capsules, placebo and dietary advice, very long chain (VLC) n-3 capsules, or VLC n-3 capsules and dietary advice combined.</td>
<td>Plasma coagulation factor VII (FVII), choline-containing phospholipids and triglycerides</td>
<td>FVII gene (R353Q polymorphism)</td>
<td>No</td>
<td>The observed effects of the intervention were independent of the R353Q genotype</td>
</tr>
<tr>
<td>Lindi et al. (50)</td>
<td>Intervention study in 76 men and 74 women in a controlled trial. Subjects were randomly assigned to consume either fish oil supplements (omega-3 fatty acids/d) or placebo capsules for 3 months.</td>
<td>Plasma lipids and lipoproteins</td>
<td>PPARG2 gene (Pro12Ala polymorphism)</td>
<td>Yes</td>
<td>PPARG2 polymorphism and n-3 fatty acids</td>
</tr>
<tr>
<td>Madden et al. (51)</td>
<td>Intervention study with fish oil supplementation in patients with claudication secondary to peripheral arterial disease. Fish oil supplementation for 12 weeks.</td>
<td>Inflammatory markers and ankle brachial pressure index</td>
<td>TNFA, IL1B and and IL-10 genes</td>
<td>No</td>
<td>Any of the genotypes examined affected the results</td>
</tr>
<tr>
<td>Nelson et al. (52)</td>
<td>Intervention using alpha-linolenic acid (ALA) in healthy adult males and females. The control subjects (27) were instructed not to alter their habitual diet and the ALA group (n = 30) was instructed to follow an enriched ALA diet by using flaxseed oil capsules.</td>
<td>Adiponectin and lipids</td>
<td>ADIPOQ gene (276 and 45 polymorphisms)</td>
<td>No</td>
<td>The effects of ALA on adiponectin were independent of the genotype</td>
</tr>
<tr>
<td>Madden et al. (53)</td>
<td>Intervention study in 111 healthy Caucasian men. Subjects consumed habitual diets while taking 6 g MaxEPA daily for 12 weeks</td>
<td>Plasma lipids</td>
<td>CD36 gene (polymorphisms: 25444G &gt; A, 27464del &gt; ins, 30294G &gt; C, -31118G &gt; A and -33137A &gt; G)</td>
<td>Yes</td>
<td>CD36 (25444G &gt; A) and EPA</td>
</tr>
<tr>
<td>Ferguson et al. (54)</td>
<td>LIPGENE dietary intervention cohort. 450 individuals with metabolic syndrome and dietary fat modification for 12 weeks</td>
<td>Biomarkers of cardiovascular risk and plasma fatty acid composition</td>
<td>NOS3 gene (polymorphisms: rs11771443, rs1800783, rs1800779, rs1799983, rs3918227, and rs743507)</td>
<td>Yes</td>
<td>NOS3 rs1799983 SNP and plasma n-3 PUFA status</td>
</tr>
</tbody>
</table>

and disease phenotypes is highly variable and depending on genetic factors. For each phenotype studied there is a great number of genetic variants that have been shown to have significant interactions. However, these studies were at first mostly designed to show gene-diet interactions, i.e. that the phenotypical effect foreseen for a certain genetic variant could be modulated by diet, specifically by PUFA intake. Although the result of the statistical interaction is the same, the methodological approach in the study design, in data gathering, in bias control and in the specific analysis of data is different if studies are planned as diet-genome interactions, i.e. how the expected results faced with a certain amount of nutrient consumed or dietary intervention undertaken is modulated by the genetic variants.

The difference between calling a study a gene-diet interaction study as opposed to a diet-genome interaction study is as follows: The first interaction studies between genetic variants and diet were undertaken in studies that had been designed to evaluate the effect of certain genetic variants on disease phenotypes. When dietary data were provided in those studies, gene-diet interactions were also investigated. This, however, could lead to a greater bias because diet is not measured specifically from the outset of the study with the best tools for guaranteeing maximum measurement validity, but using previously obtained data. In contrast, a diet-genome interaction study is a study which, from the outset, has the aim of studying possible modulations of diet due to genetic variants, setting off from a much more valid and accurate dietary measurement. Hence, new studies are required which set off from this diet-genome interaction instead of the traditional gene-diet interaction in order to obtain better quality results. It has also been noted that there is a very low level of results replication for gene-diet interactions. Most reports are unique, in the sense that no further studies confirm or refute the findings. Publication bias may contribute to this scenario, given the fact that if later studies do not find statistically significant results, there is a high probability of not being published. Most often, journals give little priority to articles that do not contain novel results, preferring to publish new results in which new genetic variants are involved even though they will never be replicated.

Furthermore, most findings are based on observational studies, whereas large-scale, carefully designed, intervention studies analysing omega-3-gene interactions are absent from the literature. The drop in cost and time required to do genetic analyses combined with improved statistical and computer power, should allow new interaction studies to undertake more robust experimental approaches including multiple gene variants thus providing complete view of these interactions.

In summary, based on the available evidence, titration of n-3 fatty acid intake based on genetic information with the purpose of preventing or treating disease is not warranted at this time. New intervention studies, designed specifically for that purpose will be required.

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References


