Statistical analysis of human microarray data shows that dietary intervention with n-3 fatty acids, flavonoids and resveratrol enriches for immune response and disease pathways

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(Submitted 17 July 2017 – Final revision received 8 November 2017 – Accepted 17 November 2017 – First published online 18 January 2018)

Abstract

n-3 Fatty acids, flavonoids and resveratrol are well publicised for their beneficial effects on human health and wellbeing. Identifying common, underlying biological mechanisms targeted by these functional foods would therefore be informative for the public health sector for advising on nutritional health and disease, food and drug product development and consumer interest. The aim of this study was to explore the potential effects of gene expression changes associated with n-3 fatty acids EPA and DHA, flavonoids and resveratrol on modifying biological systems and disease pathways. To test this, publicly available human microarray data for significant gene expression changes associated with dietary intervention with EPA/DHA, flavonoids and resveratrol was subjected to pathway analysis and significance testing for overlap with signals from genome-wide association studies (GWAS) for common non-communicable diseases and biological functions. There was an enrichment of genes implicated in immune responses and disease pathways which was common to all of the treatment conditions tested. Analysis of biological functions and disease pathways indicated anti-tumorigenic properties for EPA/DHA. In line with this, significance testing of the intersection of genes associated with these functional foods and GWAS hits for common biological functions (ageing and cognition) and non-communicable diseases (breast cancer, CVD, diabesity, neurodegeneration and psychiatric disorders) identified significant overlap between the EPA/DHA and breast cancer gene sets. Dietary intervention with EPA/DHA, flavonoids and resveratrol can target important biological and disease pathways suggesting a potentially important role for these bioactive compounds in the prevention and treatment of dietary-related diseases.

Key words: Flavonoids: Functional foods: Genome-wide association studies: n-3 Fatty acids: Resveratrol

Diet is an important factor in promoting health and preventing chronic diseases such as CVD, diabetes, obesity and cancer. The rapid growth of the global ageing population and the limited efficacy of available pharmacological therapies for age-related cognitive decline and neurodegenerative diseases means that there is an increasing interest and public demand for so called ‘functional foods’ that promote cognitive wellbeing and longevity1–3. At the other end of the scale a growing body of evidence supports that early nutrition during prenatal development, infancy and childhood affects both cognitive development and cognitive function and behaviour in later life4–7. The beneficial effects of certain food groups including plant foods and oily fish which provide high sources of plant bioactives (e.g. polyphenols, glucosinolates and antioxidant vitamins) and n-3 fatty acids, respectively, are well documented8–10. Many modern diets and food supplements are enriched with these bioactive compounds and claim to be beneficial to health through boosting the immune system, supporting cardiovascular function, protecting cells against oxidative stress and promoting healthy skin, teeth and bones11–17.

Addressing the synergistic effects of certain functional foods and supplements consumed in combination on health and wellbeing is of growing interest in the field of nutrition2,18,19. The purpose of this communication was to address potential common biological effects of the functional foods n-3 fatty acids EPA and DHA, flavonoids and resveratrol on different regulatory mechanisms including development, immune responses, metabolic processing and disease using a pathway analysis approach. Gene sets associated with these functional foods were also overlaid with genome-wide association studies (GWAS) hits for common diseases and biological functions. The findings of this analysis will have implications for public health,
disease intervention, therapeutic management, nutrition-related behaviours, marketing strategy, economic growth and food development.

Methods

Gene expression array database

Human microarray data for dietary interventions with n-3 fatty acids (EPA and/or DHA), flavonoids and resveratrol was downloaded from the ArrayExpress database (https://www.ebi.ac.uk/arrayexpress/). Study descriptions for the microarray data sets are detailed in Table 1. Studies included in the analysis were selected on the following criteria: (1) performed in healthy human subjects or subjects with a dietary-related disease but otherwise healthy, (2) double-blinded randomised controlled trials, (3) microarray-based gene expression data available at baseline and following treatment intervention for each subject. Gene expression changes between baseline measurements or placebo for cross-over studies (control group) and following treatment intervention (treatment group) within the same individuals were analysed using the Ingenuity Pathway Analysis (IPA) Core Analysis function (QIAGEN) under default parameters. Significance testing was performed using the following methods: (1) ratio of the number of differentially expressed genes from the uploaded data set that map to an IPA pathway divided by the total number of molecules that exist in the pathway. (2) Fischer’s exact test, used to calculate the probability that the association between the genes in the uploaded data set and the canonical pathway is explained by chance alone. P values of <0.05 were considered significant. (3) Benjamini-Hochberg procedure, used to calculate the false discovery rate and correct for multiple-testing. Adjusted P values of <0.05 were considered significant. (4) Fold change cut-off of 2> or <2 was considered significant. (5) Z score analysis, used as a statistical measure of the match between expected relationship direction and observed gene expression of the uploaded dataset. Positive and negative z scores indicate up-regulated and down-regulated pathways, respectively. In line with IPA cut-off values, z scores of 2>0 or <2 were considered significant.

Statistical overrepresentation testing of gene lists was performed using the publicly available PANTHER Classification System, http://www.pantherdb.org/. Over-represented pathways were determined using the binomial distribution test. P values of <0.05 were considered significant.

Results

Gene expression changes associated with dietary n-3 fatty acids EPA/DHA, flavonoids and resveratrol

The total number of significant gene expression changes associated with dietary intervention with the functional foods addressed in this study were 3403 for EPA/DHA (mean: 1213; range: 542–1827), 2703 for flavonoids (mean: 1480; range: 915–2,045) and 3464 for resveratrol (mean: 925; range: 555–1424). Gene lists representing all genes significantly regulated in response to these treatment groups are included in the online Supplementary Appendix S2. Significant overlap was observed between the gene sets for EPA/DHA and flavonoids (P = 4·67·10^-25) and flavonoids and resveratrol (P = 0·015), but not for EPA/DHA and resveratrol (P = 0·624). A total of 141 genes were common across the three treatment groups (online Supplementary Appendix S2, Table SA12). Statistical over-representation testing of these overlapping gene sets was performed using the PANTHER Classification System, which compares user defined gene lists to a reference gene list within the PANTHER database and determines whether a particular class of genes is over- or under-represented with respect to a particular biological process or pathway. Significantly over-represented pathways are represented in Fig. 1.
Table 1. Microarray study descriptions included in the pathway analysis

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Arrayexpress ID</th>
<th>Study description*</th>
<th>Study cohort</th>
<th>Supplementation description</th>
<th>Array ID</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-3 Fatty acids</td>
<td>E-GEOD-48368</td>
<td>Gene expression profile of PBMC after intake of fish oil for 7 weeks</td>
<td>Seventeen healthy subjects</td>
<td>Fish oil capsules providing a daily intake of 1.6 g EPA/DHA (0.7 g EPA; 0.9 g DHA)</td>
<td>Illumina HumanHT-12 V4.0 expression beadchip array</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>E-GEOD-12375</td>
<td>Gene expression profile of PBMC after intake of fish oil for 6 months</td>
<td>Twenty-three healthy elderly subjects (fifteen males, eight females); age 69-9 (range 67–76) years; BMI 26-5 (range 21–33.6) kg/m²; TAG 1-0 (range 0-4–2-0) mmol/l</td>
<td>Fish oil capsules providing a daily intake of 1.8 g EPA/DHA (1093 ± 17 mg EPA; 847 ± 23 mg DHA)</td>
<td>NuGO human Hs1a520180 array</td>
<td>(20)</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>E-GEOD-20114</td>
<td>Gene expression profile of PBMC after DHA supplementation for 90 d</td>
<td>Four hypertriglycerolaemic but otherwise healthy men; age 39–66 years; BMI 22–35 kg/m²; TAG 1.7–4.5 mmol/l</td>
<td>DHA oil capsules providing a daily intake of 3 g DHA and 0 g EPA</td>
<td>Affymetrix GeneChip Human Genome U133 Plus 2.0 array</td>
<td>(21)</td>
</tr>
<tr>
<td></td>
<td>E-GEOD-13899</td>
<td>Gene expression profile of monocytes after quercetin supplementation for 2 weeks</td>
<td>Four healthy subjects (two males, two females); age 24 (so 2.8) years; BMI 21.1 (so 1.9) kg/m²; systolic/diastolic BP 122/73 (so 5/5) mmHg; TAG 1.2 (so 0.4) mmol/l</td>
<td>Capsules providing a daily intake of 150 mg quercetin (naturally occurring polyphenol of the flavonoid subclass flavonol)</td>
<td>Affymetrix GeneChip Human Genome U133 Plus 2.0 array</td>
<td>(22)</td>
</tr>
<tr>
<td></td>
<td>E-GEOD-34145</td>
<td>Gene expression profile of PBMC following dietary intake of anthocyanins for 8 weeks</td>
<td>Three overweight female subjects; 53 (so 6) years; BMI 31.4 (so 4.7) kg/m²; systolic/diastolic BP 147/93 (so 18/9) mmHg; TAG 2.2 (so 0.7) mmol/l</td>
<td>Daily consumption of bilberry puree and dried bilberries equivalent of 400 g fresh bilberries (contain the highest levels of polyphenols, particularly anthocyanins of the flavonoid subclass flavonols)</td>
<td>Illumina Human-6 v2 Expression BeadChip</td>
<td>(23)</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>E-GEOD-42432</td>
<td>Gene expression profile of adipose tissue following 30 d resveratrol supplementation</td>
<td>Nine obese but otherwise healthy male subjects; age 40–65 years; 28–36 kg/m²</td>
<td>Double-blind randomised cross-over study; daily intake of placebo and 150 mg/d resveratrol (99 % resVida) with a 4-week washout period in between</td>
<td>Affymetrix Human Gene 1-1 ST Array [HuGene-1_1-st]</td>
<td>(24)</td>
</tr>
<tr>
<td></td>
<td>E-GEOD-41168</td>
<td>Gene expression profile of adipose tissue following 12 weeks resveratrol supplementation</td>
<td>Thirteen healthy, postmenopausal female subjects</td>
<td>Daily supplementation with 75 mg/d resveratrol</td>
<td>Affymetrix GeneChip Human Genome U133 Plus 2.0 Array</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>E-GEOD-41168</td>
<td>Gene expression profile of skeletal muscle (vastus lateralis) following 12 weeks resveratrol supplementation</td>
<td>Nine healthy, postmenopausal female subjects</td>
<td>Daily supplementation with 75 mg/d resveratrol</td>
<td>Affymetrix GeneChip Human Genome U133 Plus 2.0 Array</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>E-GEOD-32357</td>
<td>Gene expression profile of skeletal muscle (vastus lateralis) following 30 d resveratrol supplementation</td>
<td>Ten obese but otherwise healthy male subjects 52.5 (sex 2-1) years; BMI 31.5 (sex 0.8) kg/m²; systolic/diastolic BP 131/82 (sex 3-1/2-5) mmHg; TAG 1.9 (sex 0.2) mmol/l</td>
<td>Double-blind randomised cross-over study; daily intake of placebo and 150 mg/d resveratrol (99 % resVida) with a 4-week washout period in between</td>
<td>Affymetrix Human Gene 1-1 ST Array [HuGene-1_1-st]</td>
<td>(25)</td>
</tr>
</tbody>
</table>

PBMC, peripheral blood mononuclear cells; BP, blood pressure.

* Gene expression changes were analysed in the same individuals using baseline measurements versus treatment intervention unless stated otherwise.
Determining regulatory pathways affected by dietary n-3 fatty acids EPA/DHA, flavonoids and resveratrol

For pathway analysis, differential gene expression data for all significantly regulated genes under each of the treatment conditions was parsed into the IPA platform and enrichment analysis performed using the Core Analysis function. This provides an overview of biological relationships, mechanisms, functions and pathways relating to the uploaded data set based on log fold changes in gene expression. Gene lists were filtered to include genes with a log fold change of more than 0.5. To correct for multiple-testing, data sets were adjusted using the Benjamini–

**Fig. 1.** Enrichment analysis of overlapping gene sets associated with dietary interventions with n-3 fatty acids EPA/DHA, flavonoids and resveratrol. Gene expression data were downloaded from ArrayExpress (https://www.ebi.ac.uk/arrayexpress/). Studies included in the analysis are detailed in Table 1. Significant gene expression changes are represented as a Venn diagram showing treatment-specific and overlapping profiles across the three dietary interventions. For gene lists included in this analysis, see the online Supplementary Appendix S2 (Tables SA9–SA12). Overlapping gene sets (n-3 and flavonoids, 494 genes; n-3 and resveratrol, 420 genes; flavonoids and resveratrol, 349 genes; all treatments, 141 genes) were subjected to statistical overrepresentation testing using PANTHER pathway analysis software. Significantly over-represented pathways are listed for each of the overlapping gene sets. Bold font indicates common pathways across the different gene sets. †Withstood Bonferroni correction for multiple-testing. TGF, transforming growth factor; JAK/STAT, Janus kinase/signal transducer and activator of transcription; PDGF, platelet-derived growth factor; CCKR, cholecystokinin receptor; CRF, corticotropin releasing factor; 5-HT2, 5-hydroxytryptamine/serotonin, PKB, protein kinase B; mAChR, muscarinic acetylcholine receptor; TRHR, thyrotropin-releasing hormone receptor.
Dietary intervention on biological pathways

Dietary n-3 fatty acids EPA/DHA, flavonoids and resveratrol target disease pathways

To investigate the role of EPA/DHA, flavonoids and resveratrol in the prevention and/or treatment of disease, IPA Core Analysis was used to identify the top disease pathways and biological functions associated with significantly regulated gene sets for these treatment conditions. This identified processes relevant to tumorigenesis, inflammation and autoimmune disorders, Fig. 4(a). The net effect of gene expression changes was addressed using z scores which predict an increase (positive z score) or decrease (negative z score) in net changes was addressed using z scores which predict an increase (positive z score) or decrease (negative z score) in

Table 2. Common canonical pathways significantly regulated following dietary intervention with n-3 fatty acids (EPA/DHA), flavonoids and resveratrol*

<table>
<thead>
<tr>
<th>Functional groups</th>
<th>Canonical pathway</th>
<th>n-3 (EPA/DHA)</th>
<th>Flavonoids</th>
<th>Resveratrol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Immune response pathways</td>
<td>PPARα/RXRα activation</td>
<td>2.20 × 10⁻³†</td>
<td>1.99 × 10⁻²†</td>
<td>1.97 × 10⁻²†</td>
</tr>
<tr>
<td></td>
<td>IL-10 signalling</td>
<td>9.53 × 10⁻⁴‡</td>
<td>4.48 × 10⁻¹‡</td>
<td>8.69 × 10⁻⁴‡</td>
</tr>
<tr>
<td></td>
<td>Glucocorticoid receptor signalling</td>
<td>9.51 × 10⁻⁴‡</td>
<td>2.07 × 10⁻¹‡</td>
<td>5.22 × 10⁻¹‡</td>
</tr>
<tr>
<td></td>
<td>Natural killer cell signalling</td>
<td>5.33 × 10⁻¹</td>
<td>6.51 × 10⁻³‡</td>
<td>3.79 × 10⁻²‡</td>
</tr>
<tr>
<td></td>
<td>Leucocyte extravasation signalling</td>
<td>4.17 × 10⁻³‡</td>
<td>4.43 × 10⁻¹‡</td>
<td>8.70 × 10⁻⁴‡</td>
</tr>
<tr>
<td></td>
<td>Regulation of IL-2 expression in activated and anergic T lymphocytes</td>
<td>1.37 × 10⁻⁴‡</td>
<td>1.60 × 10⁻⁴‡</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>IL-6 signalling</td>
<td>4.75 × 10⁻²‡</td>
<td>3.01 × 10⁻¹</td>
<td>4.38 × 10⁻²‡</td>
</tr>
<tr>
<td></td>
<td>Protein kinase A signalling</td>
<td>3.36 × 10⁻⁴‡</td>
<td>3.26 × 10⁻⁴</td>
<td>4.11 × 10⁻⁴‡</td>
</tr>
<tr>
<td></td>
<td>NF-κB signalling</td>
<td>5.47 × 10⁻⁴‡</td>
<td>3.26 × 10⁻⁴</td>
<td>2.97 × 10⁻¹‡</td>
</tr>
<tr>
<td></td>
<td>HIPPO signalling</td>
<td>1.72 × 10⁻⁴‡</td>
<td>2.53 × 10⁻¹‡</td>
<td>1.58 × 10⁻²‡</td>
</tr>
<tr>
<td></td>
<td>Sumoylation pathway</td>
<td>2.29 × 10⁻⁴</td>
<td>7.85 × 10⁻⁴</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Systemic lupus erythematosus signalling</td>
<td>4.89 × 10⁻⁴‡</td>
<td>2.66 × 10⁻¹‡</td>
<td>3.38 × 10⁻¹</td>
</tr>
<tr>
<td></td>
<td>Hepatic fibrosis/hepatic stellate cell activation</td>
<td>2.79 × 10⁻⁴‡</td>
<td>–</td>
<td>2.51 × 10⁻⁴‡</td>
</tr>
<tr>
<td></td>
<td>Intrinsic prothrombin activation pathway</td>
<td>2.76 × 10⁻⁴‡</td>
<td>–</td>
<td>2.80 × 10⁻⁴‡</td>
</tr>
<tr>
<td></td>
<td>Creatine-phosphate biosynthesis</td>
<td>3.12 × 10⁻⁴‡</td>
<td>–</td>
<td>3.02 × 10⁻⁴‡</td>
</tr>
</tbody>
</table>

† Down-regulated pathway.

* Functional enrichment analysis of gene sets differentially regulated by dietary intervention with n-3 fatty acids EPA/DHA, flavonoids or resveratrol was performed using Ingenuity Pathway Analysis software. Fold change cut-off of &gt;0.5 and &lt;-0.5 log (differential expression) was applied. Statistical significance was determined using the Benjamini-Hochberg procedure. Net effects of gene expression changes on pathway activation or repression were determined using activation z scores (threshold, &lt;-2.0; &gt;2.0).

† Pathways that withstood multiple-testing correction (adjusted P &lt; 0.05)
pathway activation. Based on this analysis, EPA/DHA was suggested to have anti-tumorigenic properties (increased apoptosis of tumour cell lines; decreased aggregation of blood cells), flavonoids had both anti-tumorigenic (decreased growth of melanoma) and pro-tumorigenic properties (decreased apoptosis and necrosis of prostate cancer cell lines; increased cell viability of cervical cancer cell lines), and resveratrol was strongly enriched for pro-tumorigenic processes (decreased apoptosis of breast cancer cell lines; increased migration, invasion and proliferation of tumour cell lines) (Fig. 4(b)).

To further address how EPA/DHA, flavonoids and resveratrol may converge onto major disease pathways, gene expression changes associated with these functional foods were compared against GWAS hits for different biological functions and diseases. Hypergeometric distribution testing using the Gene-Overlap package in R was used to determine significant overlap between the GWAS data sets and genes associated with dietary interventions with EPA/DHA, flavonoids and resveratrol.
As illustrated in Fig. 5(a), significant overlap was observed between the two lists representing genes regulated in response to EPA/DHA and those implicated in breast cancer risk from GWAS. A total of thirty-two genes were identified as being common between the two gene lists. Pathway analysis of this gene set identified enrichment of genes implicated in metabolic processes (Fig. 5(b) and developmental pathways (Fig. 5(c)). No other significant associations were found between gene lists for the functional foods and diseases/biological functions tested (Fig. 5(a)).

Discussion

There is a growing interest in the therapeutic potential of n-3 fatty acids and polyphenol-rich foods in the prevention and treatment of chronic disease. The protective role of these functional foods has in part been attributed to their antioxidant and anti-apoptotic effects. However, the exact biological mechanisms underpinning the functional aspects of these bioactive compounds are not fully understood. In this communication, pathway analysis was performed to identify the major biological processes associated with genes targeted by dietary intervention with n-3 fatty acids EPA/DHA, flavonoids and resveratrol. Better understanding of the potential synergistic effects of these functional foods in modulating complex biological and pathological processes has important therapeutic implications for the prevention and management of disease.

Enrichment analysis of overlapping gene sets significantly regulated in response to dietary interventions with EPA/DHA, flavonoids and resveratrol identified apoptosis, IL signalling and angiogenesis as common pathways across all treatment groups (Fig. 1). In addition, EPA/DHA and flavonoids enriched for pathways relevant to cellular proliferation and migration, B and T cell activation, neuroendocrine signalling and cardiovascular function; flavonoids and resveratrol enriched for signal transduction, immune response and neuroendocrine pathways; and EPA/DHA and resveratrol enriched for pathways associated with neurodegeneration (Fig. 1 and 2). PPARα/β activation was identified as a significantly regulated pathway common to all three treatment interventions (Fig. 2). PPAR signalling was also found to be significantly associated with EPA/DHA (online Supplementary Appendix S3, Table SA13). n-3 Fatty acids,
Fig. 5. Significance testing of overlap between genes regulated in response to functional foods and genome-wide association studies (GWAS) hits for common diseases/biological functions. (a) Gene lists representing all genes significantly regulated in response to dietary interventions with n-3 fatty acids EPA/DHA, flavonoids and resveratrol (see Table 1 for study details) were compared against gene lists identified from GWAS (see the online Supplementary Appendix S1 for study details) for ageing, breast cancer, cognition, CVD, diabetes, neurodegeneration and psychiatric disorders. Numbers in brackets represent the number of genes within each gene list. GeneOverlap and GeneOverlapMatrix functions available in R were used to calculate and visualise significant overlap between the gene lists tested. Fischer’s exact test was used to calculate P values which are stated within each panel of the grid. Colour key represents OR values. * Significant overlap (P < 0.05). (b) and (c) Statistical overrepresentation testing was performed using the binomial statistics tool available through PANTHER to identify biological processes (b) and pathway classifications (c) for common genes associated with breast cancer and dietary intervention with n-3 fatty acids (EPA/DHA). Uploaded genes were compared with a reference list containing all human genes within the PANTHER database in order to statistically determine over- or under-representation of PANTHER classification categories using the binomial distribution test. Associated genes are listed on the right of the bar charts. Flav., flavonoids; Resv., resveratrol; Nb, nucleobase; HIF, hypoxia-inducible factor; mGluR, metabotropic glutamate receptor; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; IGF, insulin-like growth factor; PKB, protein kinase B.
flavonoids and resveratrol are known ligands of PPARγ\(^{53-55}\), which plays a key role in the regulation of apoptosis, cellular proliferation, glucose and lipid metabolism, inflammatory responses, oxidative stress, endothelial function and cancer\(^{56}\). We would therefore expect to see strong regulation of PPAR-specific target genes in our analysis. However, casual analysis for potential upstream regulators in IPA\(^{55}\) suggested only marginal involvement of PPARG and PPARA in the regulation of genes differentially expressed in the treatment intervention groups studied, Fig. 3(a). This may reflect the long-term and systemic nature of the studies included in our analysis; even if PPARs played a significant role in triggering gene expression changes at the start of treatment intervention, expression of direct PPAR targets are likely stabilised via multiple feedback mechanisms. Nonetheless, more long-term differential gene expression profiles triggered by these early induced changes are clearly represented in our functional network analysis of primary PPAR targets (Fig. 3(b)). On the other hand, continuous activation of receptors can eventually cause an adaptive cellular response and shutting down of the expression of the receptors and their downstream pathways. It would therefore be interesting to follow this line of investigation into interrogation of organismal and tissue responses to short- and long-term exposures to these bioactive compounds.

Analysis of disease pathways associated with the functional foods analysed in this study indicated anti-tumorigenic properties for EPA/DHA, Fig. 4(b), which is supported by the literature\(^{57-59}\). In contrast, flavonoids were linked to both anti- and pro-tumorigenic processes, whereas resveratrol was strongly enriched for pro-tumorigenic pathways. Resveratrol is well documented for its modulation of the cell cycle in models of cancer\(^{60-66}\). These conflicting results may reflect tissue-specific gene expression profiles (adipose vs skeletal muscle) or phenotypic variations across the study cohorts (overweight/obese male versus postmenopausal female) and warrant further investigation.

Our diet is influenced by many factors including age, sex, education, ethnicity and socioeconomic pressures. Psychological determinants of diet such as mood and stress levels are also very influential\(^{67-70}\). To address overlap between nutrition and key biological processes and common diseases potentially modified by dietary interventions, GWAS data sets for ageing, cognition, breast cancer, CVD, diabetes, neurodegeneration and psychiatric disorders were compared against gene expression changes associated with EPA/DHA, flavonoids and resveratrol. Significant overlap was observed between the EPA/DHA and breast cancer gene sets (Fig. 5), emphasising the anti-tumorigenic properties of this functional food.

There are several limitations of this study, most notably the comparison of small cohort trials with varied treatment interventions and methodologies, including, and not limited to, cohort size, subject variables, length of intervention, type and dosage of supplement tested, tissue type used for gene expression analysis, and type of microarray technology used for gene expression profiling. Correction for the effect of confounding variables such as age, sex, health status, genetic variation etc. on gene expression changes across the different study cohorts included in our analysis could not be accounted for in this study. A further limitation of this study is the absence of a matched control group; not every study included in this analysis had gene expression data for no-treatment intervention. Nonetheless, this study identifies several potentially important genes and regulatory pathways targeted through human dietary interventions with n-3 fatty acids EPA/DHA, flavonoids and resveratrol. This is exemplified through significant overlap of the EPA/DHA gene set, identified from pathway analysis as having an anti-tumorigenic profile, with GWAS hits for breast cancer.

In summary, gene expression changes associated with dietary n-3 fatty acids EPA/DHA, flavonoids and resveratrol converged onto important biological and disease pathways, including immune response and cell cycle regulation, suggesting a potentially important role for these bioactive compounds in the prevention and treatment of dietary-related disease. The findings of this study warrant further investigation into the synergistic effect of these functional foods in the management of non-communicable diseases.

**Acknowledgements**

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

A. W., P. Q., J. P. S. and J. P. Q. were involved in the study design. A. W. and O. V. were involved in the generation and interpretation of data.

None of the authors has any conflicts of interest to declare.

**Supplementary material**

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114517003506

**References**


