COURSE NOTES: Understanding genetics for improving health outcomes

Course Code: CEUGH

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Course Description

The course is intended to provide the tools that holistic nutritionists can use to deliver personalized healthcare to their clients, catered to the genetic makeup of the client. Genetics plays a very robust role in nutrition, detoxification, weightloss and overall health and wellness. Furthermore, genetics can be used not only to improve the health of the clients but also to develop DNA based nutritional plans that can potentially prevent development of chronic diseases. Course participants will gain knowledge on how genetic information can be used to deliver nutritional plans, weightloss strategies, detoxification plans, hormonal balance plans along with nutritional plans to prevent development of chronic disease such as diabetes and cardiovascular diseases.

IHN has partnered with Anantlife Canada Inc., a leader in clinical grade genetic testing for healthcare providers all over the world, to offer a Certified Genetic Testing Provider Certificate upon successful completion of the course. Successful completion of the course implies that the candidates have received the education and training to not only understand genetic concepts pertaining to diet, nutrition, detoxification, fitness, hormonal health and metabolic disorders but have also been trained on interpretation of the genetic testing reports along with development of a DNA based health plan for better health outcomes.

SESSION 2:

NUTRITIONAL AND DIETARY GENETICS: UNDERSTANDING HOW OUR GENES REGULATE OUR NUTRITIONAL REQUIREMENTS AND DIETARY INTAKE AND HOW CAN THIS INFORMATION BE APPLIED TO OFFER PERSONALIZED NUTRITION PLANS TO IMPROVE HEALTH?

Nutritional absorption is largely governed by two processes – cellular entry and conversion into bioactive forms. The two processes are encoded by DNA and as such our DNA influences our risks for developing nutritional deficiencies. Furthermore, the metabolism of dietary components are regulated by enzymes which are encoded by DNA as such our DNA influences how we metabolize dietary components along with the downstream effects. The readings herein are to provide an extensive understanding of how genes regulate nutritional requirements along with dietary intake.

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4. Nutritional genomics. A new approach in nutrition research

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Abstract. There is an increasing evidence that nutritional genomics represents a promise to improve public health. This goal will be reached by highlighting the mechanisms through which diet can reduce the risk of common polygenic diseases. Nutritional genomics applies high throughput functional genomic technologies and molecular tools in nutrition research, allowing a more precise and accurate knowledge of nutrient-genome interactions in both health and disease. Understanding the inter-relationships among genes, genes products, and dietary habits is fundamental to identify those who will benefit the most or be placed at risk by nutritional interventions. This chapter provides an overview of this novel nutritional approach, including the most relevant results of our recent research on the nutrigenomic effects of food polyphenols on cancer cells. Those studies would highlight the molecular mechanisms underlying the chemopreventive effects of those bioactive food compounds.

Introduction

Until recently, nutrition research concentrated on nutrient deficiencies and impairment of health. The importance of diet to sustain health,

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prevention and treatment of diseases has been known for a long time. The advent of genomics –high-throughput technologies for the generation, processing, and application of scientific information about the composition and functions of genomes – has created unprecedented opportunities for increasing our understanding of how nutrients modulate gene and protein expression influencing cellular and organismal metabolism and thus, ultimately impacting human health and well-being. Notably, the knowledge of the human genome has dramatically broadened the scope of studies in nutrition science [1-4].

Nutritional genomics is a relatively new and very fast-moving field of research and combines molecular biology, genetics, and nutrition [3, 5]. It provides a genetic understanding for how diet, nutrients or other food components affect the balance between health and disease by altering the expression and/or structure of an individual's genetic makeup. The conceptual basis for this new branch of genomic research is built on the following premises [1,6]:

- Diet and dietary components can alter the risk of disease development by modulating multiple processes involved with the onset, incidence, progression, and/or severity;
- Diet and dietary components can act on the human genome, either directly or indirectly, to alter the expression of genes and gene products.
- Diet and dietary components could potentially compensate for or accentuate effects of genetic polymorphisms.

The term nutritional genomics is frequently used as an umbrella term for two research specialties: **nutrigenomics** and **nutrigenetics**. However, it is important to note the difference between the terms nutrigenomics and nutrigenetics because although these terms are closely related they are not interchangeable. Nutrigenomics focuses on the effects of nutrients on genes, proteins, and metabolic processes, whereas nutrigenetics involves determining the effect of individual genetic variation on the interaction between diet and disease [2,7]. Thus, those working in nutrigenomics investigate the role of nutrients in gene expression, and those working in nutrigenetics determine how genetic polymorphisms (mutations) affect responses to nutrients [7,8]. Moreover, when reviewing scientific literature, other terms appear, such as epigenetics, transcriptomics, proteomics or metabolomics. All of them describe processes, new tools or situations of this emerging field of nutrition (Table 1). The key challenge is to determine

whether it is possible to utilize this information meaningfully to provide reliable and predictable personalized dietary recommendations for specific health outcomes.

Nutrigenetics and nutrigenomics hold much promise for providing better nutritional advice to the general public, genetic subgroups and individuals [11]. In the future, the integration of nutrition and genomics may lead to the enhanced use of personalized diets to prevent or delay the onset of disease and to optimize and maintain human health. The objectives of this chapter are to provide an overview of this novel nutritional approach. Moreover, we will also include the most relevant results of our research on the nutrigenomic effects of food polyphenols on cancer cells. In addition to the essential nutrients, such as calcium, zinc, selenium or vitamins, there are a variety of classes of nonessential nutrients and bioactive components, such as polyphenols, that seem to significantly influence health. Those bioactive components are known to modify a number of cellular processes associated with health and disease prevention, including carcinogen metabolism, hormonal balance, cell signaling, cell cycle control, apoptosis, and angiogenesis. Our studies are focused in highlighting the molecular mechanisms underlying the chemopreventive effects of those bioactive food compounds.

Table 1. Definitions of terms used in nutritional genomics [9,10].

Term	Definition
Nutrigenomics	Investigates the effects of nutrients and other food components on genes, proteins, and metabolic processes. Transcriptomics, proteomics and metabolomics are used in nutrigenomics research
Nutrigenetics	Investigates the effect of individual genetic variation on the interaction between diet and disease. Genomics are often used in nutrigenetics studies
Epigenetics	Investigates the genome modifications that are copied from a generation to another but not implying changes on DNA sequence
Transcriptomics	Investigates gene expression changes at the mRNA level in response to different stimuli. Utilizes variety of technologies, most commonly microarrays and next-generation sequencing
Proteomics	Analyses all the proteins in a biological system, their interactions and their functional states although effectively, usually only the most abundant subset of 300 or so proteins is relatively easily analyzed
Metabolomics	Investigates the metabolome that consists of all of non-proteinaceous, small molecules present in a biological system. Changes in the metabolome content reflect the biological responses to external stimuli (nutrients among others), which involves altered gene expression and protein production/ activity associated with metabolic pathways

1. Nutrigenetics

Nutrigenetics focuses on the effects that genetic variations have on the binomial diet/disease or on the nutritional requirements and recommended intakes for individuals and populations. To achieve its objectives, the methodology used in nutrigenetics includes the identification and characterization of genetic variants that are associated with, or are the responsible for a different response to certain nutrients or food components [6,11]. These variations generically designated as polymorphisms, including the polymorphisms of a single nucleotide (SNP, single-nucleotide polypmorphisms), differences in the number of copies, inserts, deletions, duplications and rearrangements or reorganizations. Undoubtedly, SNPs are the most frequent as they appear every 1,000 base pairs [12].

These differences may determine the susceptibility of an individual to have a disease related to diet or to one or some diet components, as well as to influence in the individual's response to diet changes. There is certain parallelism between nutrigenetics and phamacogenetics, although in the field of nutrition is more difficult to draw conclusions, since there are important differences between drugs and food components, such as chemical purity, number of therapeutic targets and duration of the exposure, among others [3, 9, 11].

One of the best-described examples of the effect of SNPs is the relationship between folate and the gene encoding for MTHFR (5,10-methylenetetrahydrofolate reductase) [13]. MTHFR has a role in supplying 5-methylenetetrahydrofolate, which is necessary for the re-methylation of homocysteine to form methionine. Methionine is essential to many metabolic pathways including production of neurotransmitters and regulation of gene expression. Folate is essential to the efficient functioning of this MTHFR. There is a common polymorphism in the gene for MTHFR that leads to two forms of protein: the wild type (C), which functions normally, and the thermal-labile version (T), which has a significantly reduced activity. People with two copies of the wild-type gene (CC) or one copy of each (CT) appear to have normal folate metabolism. Those with two copies of the unstable version (TT) and low folate accumulate homocysteine and have less methionine, which increases their risk of vascular disease and premature cognitive decline [14].

Thus, in people with low folic acid intake, higher serum homocysteine levels would be detected in TT homozygotes compared with other genotypes, which would lead them to an increased risk of cardiovascular disease (Figure 1). However, when the intake of folic acid in diet is higher, this increased

amount would compensate the DNA defect in people with the TT polymorphism, and homocysteine serum concentrations would not reach such high values and consequently not show hyperhomocysteinemia. According to this example of gene-diet interaction, a practical application for cardiovascular disease prevention would be to recommend a higher daily consumption of folic acid-rich food to those people with the TT genotype, since these individuals have higher folic acid requirements than the general population due to their genetic susceptibility.

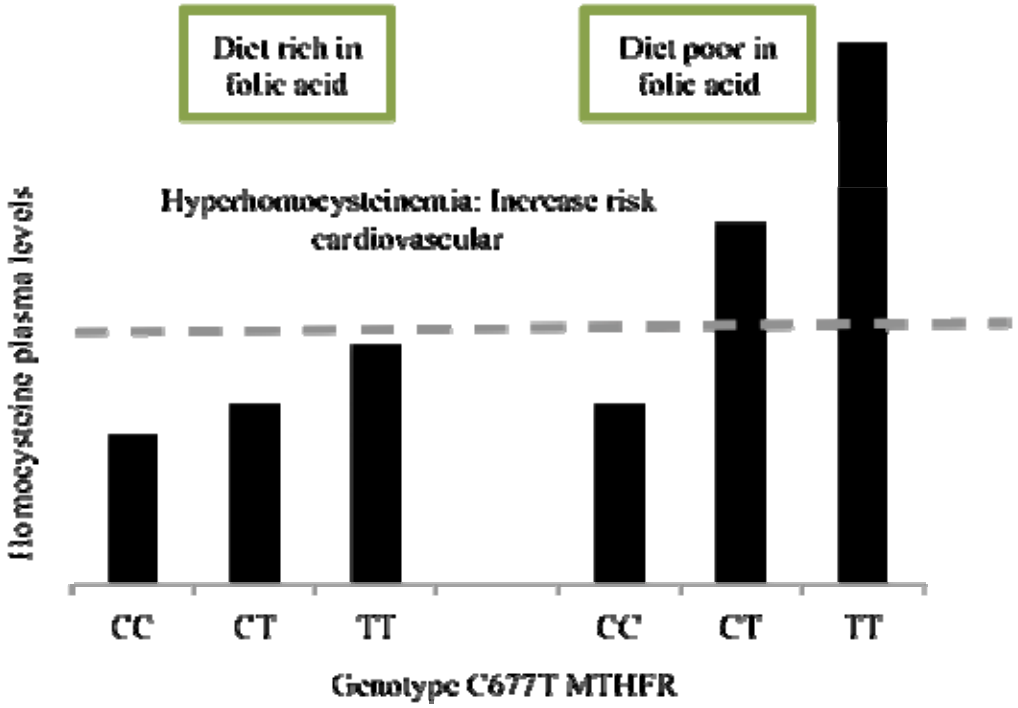

Figure 1. Gene-diet interaction. Folic acid intake may modulate the genetic risk of hyperhomocysteinemia conferred by the C677T polymorphism in the MTHFR gene. Hyperhomocysteinemia only would happen when the mutation occurs with a low folate intake [Adapted from 15].

Another of the genes on which a very active research has been developed is the one that encodes for the synthesis of the lipoprotein APOA1 [16]. APOA1 is the main component of plasmatic HDL and seems to play an important role in the transport of cholesterol. It has been reported that a polymorphism in the gene promoter the -75 A/G (substitution of guanine by adenine), has an influence on the individual's response to polyunsaturated fatty acids (PUFA) intake. Thus, women with the A/A genotype showed higher HDL-cholesterol levels in plasma after ingestion of PUFA, whereas those with genotypes A/G and G/G (wild type) did not show HDL-cholesterol

changes or even a certain decrease in response to the PUFA from diet (Figure 2). Therefore, for the individuals with the genotype A/A the ingestion of PUFA could be a good diet recommendation since it increases HDL. Those results illustrate the complexity of polymorphism-phenotype associations and underscore the importance of accounting for interactions between genes and environmental factors in population genetic studies.

The examples cited here and many others that can be found in the literature published until now [10,11,17-20] illustrate perfectly why nutrigenetics is also termed personalized nutrition, since its major goal is to identify and characterize genes, and nucleotide variants within these, that are associated (or account for) the differential responses to nutrients. In addition to providing a more rational basis for giving personalized dietary advice, the knowledge gained by applying genomic information to nutrition research will also improve the quality of evidence used for making population-based dietary recommendations. The sequencing of an individual's genome has fueled interest in the field of personalized medicine [21,22], but replicating and validating nutrigenetic studies need to remain a priority before personalized nutrition can be considered a worthwhile approach to improve human health [23].

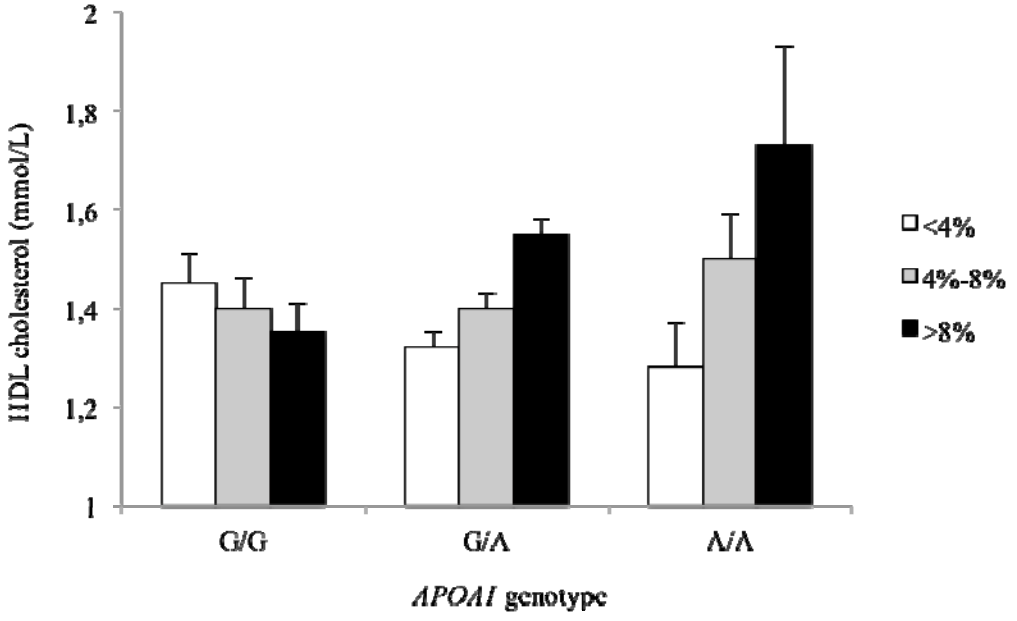

Figure 2. Effect of polyunsaturated fatty acid intake (>4%, 4-8% and >8% of energy) on high-density lipoprotein (HDL) cholesterol blood levels in women. Means were adjusted for age, body mass index, alcohol consumption, tobacco smoking, and intakes of energy, saturated fatty acids, monounsaturated fatty acids, and PUFAs [Adapted from 16].

2. Nutrigenomics

The term nutrigenomics was coined ten years ago to describe a branch of nutrition and food research that applies new profiling techniques for transcripts, proteins and metabolites to better understand the interplay of the genome with its nutritional environment. In this respect, nutrigenomics is still in its infancy and it will need time until it really delivers what was originally hoped [3,6,9].

The field of nutrigenomics harnesses multiple disciplines and includes dietary effects on genome stability (DNA damage at the molecular and chromosome level), epigenome alterations (DNA methylation), RNA and micro-RNA expression (transcriptomics), protein expression (proteomics) and metabolite changes (metabolomics), all of which can be studied independently or in an integrated manner [11, 24]. In this approach, nutrients, other food components, and even whole diets, are considered as "dietary signals" that are detected by "cellular sensors". These sensors, that are part of cellular signaling cascades, can affect, in turn, all the processes involved in cell function. Therefore, they influence the transcription, translation and protein expression and different metabolic pathways, which ultimately form the phenotype [25, 26].

Using the current genomic tools that include transcriptomics, proteomics and metabolomics, there are two approaches in nutrigenomic research. The first would identify genes, proteins or metabolites that are affected by the diet (nutrients or bioactive compounds) and determine which are the mechanisms involved in this interaction and, consequently, figure out the regulation pathways through which the diet induces these changes. In the second approach, early biomarkers are sought (genes, proteins or metabolites) that are linked with certain dietary compounds or to the whole diet [1,24]. Those biomarkers could act as a "warning signals" about changes in the homeostasis with could have implications for the health [10,11,24].

There are numerous examples [9,11,27,28] that illustrate the interaction between food components and the genome, from mammalian cells in culture to human studies. However, most applications are still of descriptive nature. As an example of a typical nutrigenomic approach research, we will explain our research which its main goal is to study mechanisms underlying the potential chemopreventive effects of a certain type of well-known food compounds called polyphenols.

Polyphenols are the most abundant antioxidants in the diet. Their main dietary sources are fruits and plant-derived beverages such as fruit juices, tea, coffee, and red wine. Vegetables, cereals, cocoa, chocolate, and dry legumes also contribute to the total polyphenol intake. Their total dietary intake could

be as high as 1g/d, which is much higher than that of all other classes of phytochemicals and known dietary antioxidants [29]. Despite their wide distribution in plants, the health effects of dietary polyphenols have come to the attention of nutritionists only rather recently. Current evidence strongly supports a contribution of polyphenols to the prevention of cardiovascular diseases, cancers, and osteoporosis and suggests a role in the prevention of neurodegenerative diseases and diabetes mellitus [30]. However, our knowledge still appears too limited to formulate recommendations for the general population or for particular populations at risk of specific diseases.

For many years, polyphenols and other antioxidants were thought to protect cell constituents against oxidative damage through scavenging of free radicals. However, this concept now appears to be an oversimplified view of their mechanism of action [31,32]. More likely, cells respond to polyphenols mainly through direct interactions with receptors or enzymes involved in signal transduction, which may result in modification of the redox status of the cell and may trigger a series of redox-dependent reactions [33]. Both antioxidant and prooxidant effects of polyphenols have been described, with contrasting effects on the cell's physiologic processes. As antioxidants, polyphenols may improve cell survival; as prooxidants, they may induce apoptosis and prevent tumor growth [30, 32]. However, the biological effects of polyphenols may extend well beyond the modulation of oxidative stress. One of the best-known examples involves the interaction of soy isoflavones with estrogen receptors and the effects of these compounds on endocrine function. These effects could explain the prevention by isoflavones of bone resorption among postmenopausal women [30]. A detailed understanding of the molecular events underlying these various biological effects is essential for the evaluation of the overall impact on disease risk and progression.

2.1. Coffee polyphenols and breast cancer: A transcriptomics approach

Coffee is one of the most popular and widely consumed beverages throughout the world. Recent meta-analyses demonstrate inverse associations between coffee intake and the risk of colon, liver, breast and endometrial cancer [34-37]. In prospective population-based cohort studies, the inverse association between coffee consumption and risk of cancer has also been showed. The group of Naganuma et al. [38] found that the consumption of at least one cup of coffee per day was associated with a 49% lower risk of upper gastrointestinal cancer in a Japanese population, while Wilson et al. [39] found that men who regularly drink coffee appeared to have a lower risk of developing a lethal form of prostate cancer. The lower risk was evident when consuming either regular or decaffeinated coffee.

It has been proposed that the inverse association between coffee intake and colon cancer could be explained, at least in part, by the presence of phenolic compounds in coffee [40]. Among the different phenolic compounds in coffee, the most abundant are hydroxycinnamic acids, which exist mainly in the esterified form. The best example is chlorogenic acid (5-caffeoylquinic acid). In fact, coffee is the major source of chlorogenic acid in the human diet; the daily intake in coffee drinkers ranges from 0.5 to 1 g, whereas coffee abstainers will usually ingest <100 mg/day. Studies have showed that approximately the 33% of ingested chlorogenic acid and 95% of caffeic acid are absorbed intestinally [41]. Thus, about two-thirds of ingested chlorogenic acid reaches the colon where it is probably metabolized to caffeic acid [42]. Bioavailability data suggest that the biological effects of chlorogenic acid would become apparent after its metabolism to caffeic acid, and hence studying the effects of this acid is necessary.

As mentioned before, there is enough evidence from epidemiological data supporting the theory that coffee seems to reduce the risk of certain types of cancer; however, the molecular mechanisms underlying the chemopreventive effects of coffee remain unknown. Using a transcriptomics approach, the effect at the molecular level of the main phenolic compound in coffee, caffeic acid, at concentrations equivalent to one cup of coffee on human colon cancer cells (HT29) was studied. Furthermore, the effect of coffee polyphenols was also evaluated in breast cancer cells.

Colon adenocarcinoma HT29 cells were incubated with caffeic acid at a concentration equivalent to one cup of coffee for 24 hours. It was previously determined that this concentration did not cause any cytotoxic effect in the cell incubations. Then, gene expression was analysed by hybridization to the GeneChip Human Genome U133A plus 2.0 microarrays from Affymetrix, containing 47,000 transcripts and variants. Quantification was carried out with GeneSpring GX v.11.5.1 software (Agilent Technologies), which allows multi-filter comparisons using data from different experiments to perform the normalization, generation of lists and the functional classification of the differentially expressed genes.

A list of differentially expressed genes by 1.3-fold with a p-value cut-off of <0.05 was generated. Upon incubation with caffeic acid, 12 genes were overexpressed whereas 32 genes were underexpressed. Among the overexpressed genes, 33% belonged to the Transcription factors category, 25% to Cell cycle, and 17% to Biosynthetic processes or Immune response. Within the underexpressed genes, again the category corresponding to Cell cycle was the most affected (30% of the genes) followed by Biosynthetic processes (15%) and Transcription factors (12%). Using these data, a Biological Association Network (BAN) was constructed using the Pathway

Analysis within the GeneSpring v.11.5.1, as described in Selga et al. [43]. Signal transducer and activator of transcription 5B (STAT5B) and Activating transcription factor 2 (ATF-2) appeared as highly interconnected nodes (Figure 3). In fact, STAT5B was overexpressed with respect to the control by 33,4% in cells treated with caffeic acid, whereas ATF-2 was found underexpressed in HT29 incubated with caffeic acid (26% decrease compared to the control).

The changes in mRNA expression of these two main (STAT5B and ATF-2) nodes were confirmed by RT-PCR and at protein level by Western blot analysis (Figure 4). The key function of STAT5B is to mediate

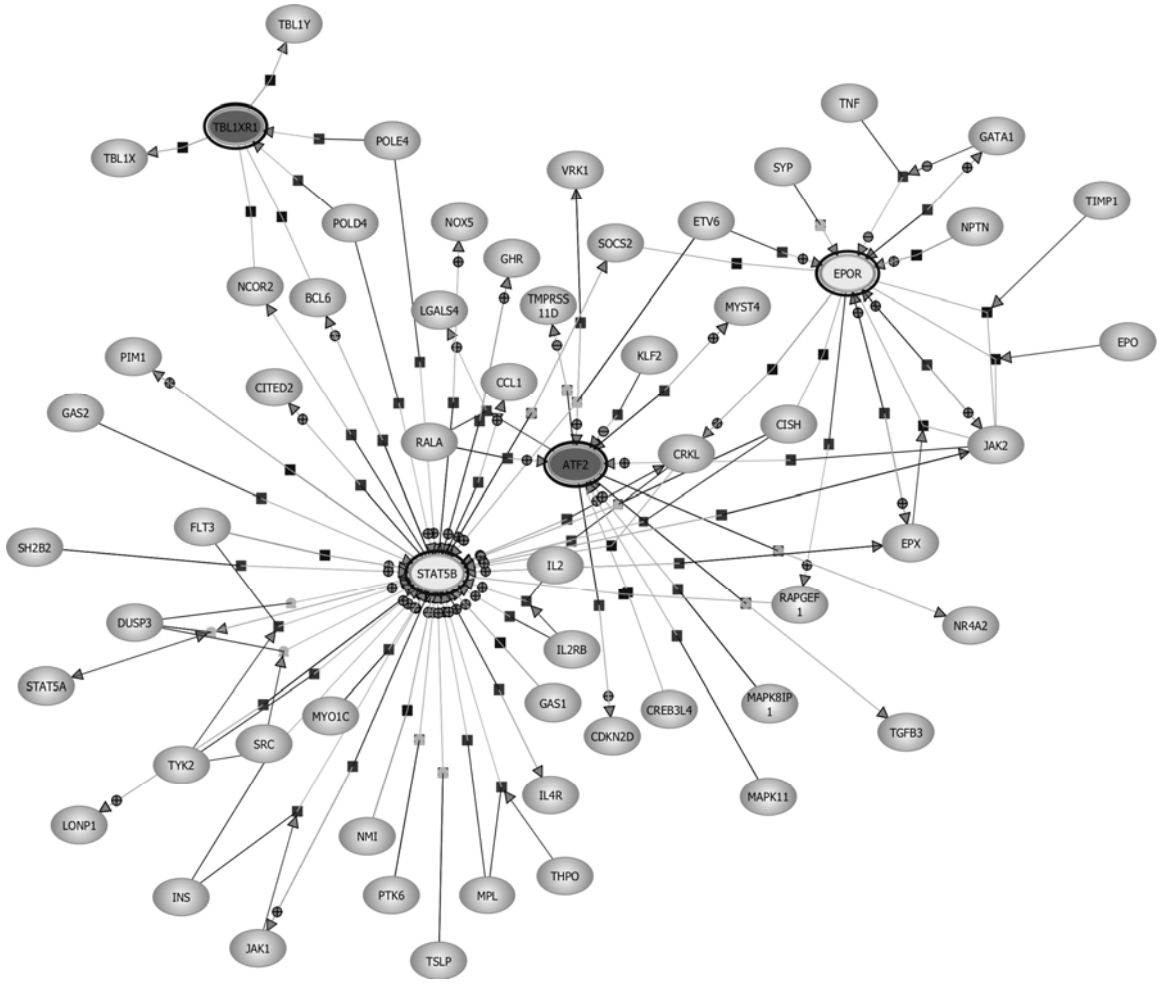

Figure 3. Biological Association Network (BAN) of differentially expressed genes under caffeic treatment. The BAN was constructed with the Pathway Analysis software within GeneSpring v11.5.1. An expanded network was constructed by setting an advanced filter that included the categories of binding, expression, metabolism, promoter binding, protein modification and regulation. Only proteins are represented. The BAN shows the node genes STAT5B and ATF-2 that were further studied.

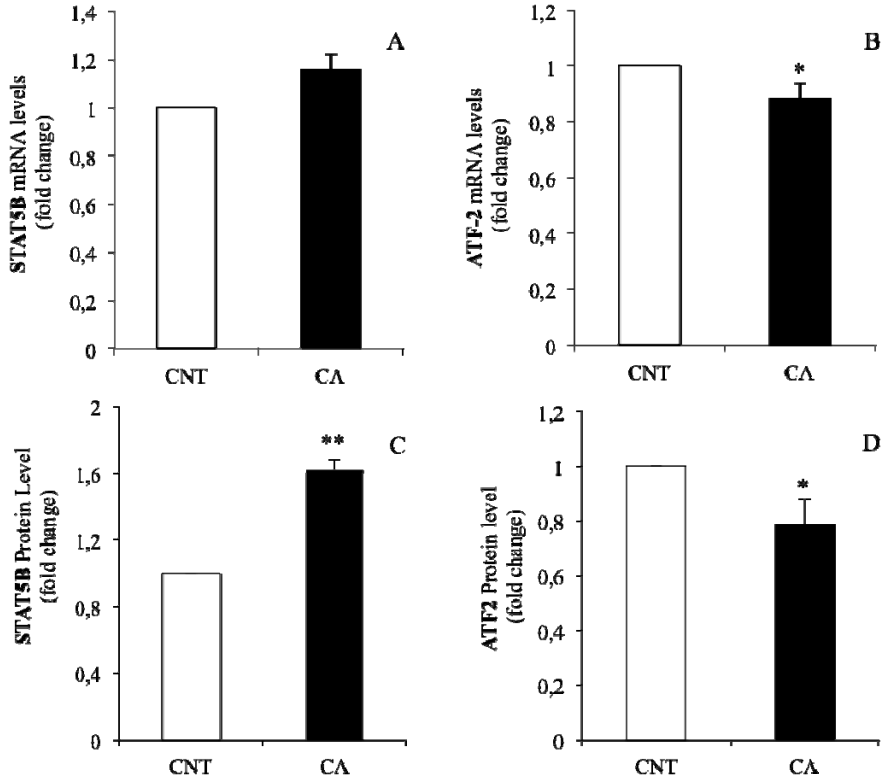

Figure 4. Quantification of mRNA and protein levels for STAT5B and ATF-2 in HT29 cells. The mRNA levels of STATB5 (A) and ATF-2 (B) were determined in control HT29 cells (empty bars) and cells treated with caffeic acid (CA, filled bars) by RT-Real Time. Results are expressed in fold-changes compared to the control, and are the mean + SE of 3 different experiments. *$p<0.05$ compared with the corresponding control. The protein levels of STAT5B (C) and ATF-2 (D) were determined in control HT29 cells (empty bars) and cells treated with caffeic acid (CA, filled bars) by Western blot. Blots were reprobed with an antibody against β-actin or tubulin to normalize the results. Results represent the mean ± SE of 3 different experiments. *$p<0.05$ and **$p<0.01$ compared with the corresponding control.

the effects of the Growth Hormone, as STAT5B-null mice failed to respond effectively to this hormone [44]. Modulation of STAT5 levels or transcriptional activity has already been described in cells treated with natural compounds such as nobiletin, acitrus flavonoid [45] thea flavins [46] and silibinin, a natural polyphenolic flavonoid which is a major bioactive component of silymarin isolated from *Silybum marianum* [47]. Activation of STAT5A/B in human breast cancer has been shown to positively correlate with the differentiation status of the tumour. STAT5 have been also shown to transcriptionally regulate E2-sensitive proliferative genes such as cyclin D1 and c-Myc [48] suggesting that STAT5 may play a role in E2-stimulated breast cancer growth. STAT5 activation has also been linked to regulating the expression of the cell cycle control protein cyclin D1 both directly and indirectly [48-50].

On the other hand, ATF-2 is a member of the ATF-cAMP response element-binding protein (CREB) family of transcription factors that can bind to the cAMP response element (CRE) found in many mammalian gene promoters [51]. ATF-2 exhibits both oncogenic and tumor suppressor functions [52)] CREs are found in several genes involved in the control of the cell cycle, e.g., the cyclin D1 gene and ATF-2 binding to this sequence stimulates the transcription of cyclin D1 [53]. ATF-2 has been correlated with proliferation, invasion, migration, and resistance to DNA-damaging agents in breast cancer cell lines.

Therefore, the two main nodes identified in our work regulate cyclin D1 transcription. Cyclin D1 is an important regulator of G1-S phase transition, and its expression in breast cancer cells is sensitive to estrogens and antiestrogens [54]. Cyclin D1 is over expressed at the mRNA and protein level in over 50% of the breast cancers either in the presence or absence of gene amplification and it is one of the most commonly over expressed proteins in breast cancer [55]. In order to know the influence that caffeic acid could have overcyclin D1 levels, since the expression of STATB5 and ATF-2 is modified by this phenolic compound, cyclin D1 levels in MCF-7 cells were analyzed upon incubation with caffeic acid by Western Blot. As shown in Figure 5, incubation of MCF-7 cells with caffeic acid led to a drastic decrease in the levels of cyclin D1 protein, together with an increase in the levels of STAT5B, but there was no decrease in the levels of ATF-2.

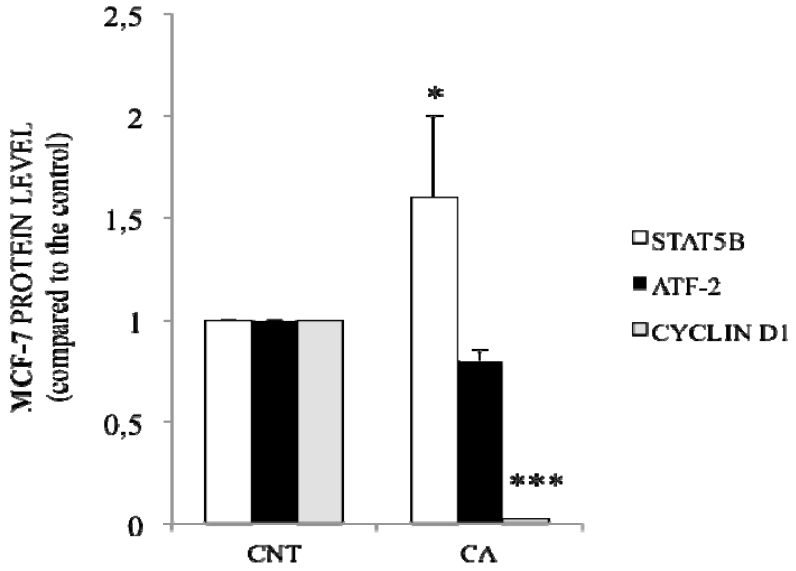

Figure 5. Expression of cyclin D1 upon incubation with caffeic acid in MCF-7 cells. The protein levels were determined in control MCF-7 cells (CNT) and in cells treated with caffeic acid (CA) by Western blot. Blots were reprobed with an antibody against β-actin to normalize the results. Results represent the mean ± SE of 3 different experiments. *$p<0.05$ and ***$p<0.001$ compared with the corresponding control.

It is believed that compounds that modulate cyclin D1 expression could have a role in the prevention and treatment of human neoplasia. For instance, flavopiridol, a synthetic flavonoid based on an extract from an Indian plant used for the potential treatment of cancer, induces a rapid decline in cyclin D1 steady-state protein levels [54]. Taking all these results together, inhibition of cyclin D1 expression appears to be a good approach for cancer treatment. In this direction our observation that coffee and caffeic acid are able to drastically reduce the expression of cyclin D1 in breast cancer cells could suggest that some coffee components could be used as a coadjuvant therapeutic tool in the treatment of breast cancer.

2.2. Cocoa polyphenols and changes in the CYP1A1 gene expression

Cocoa is rich in polyphenols. In fact, cocoa has the highest flavanol contents of all foods on a per-weight basis and is a significant contributor to the total dietary intake of flavonoids [56]. The main subclasses of flavonoids found in cocoa are flavanols, particularly the flavanol monomers catechin and epicatechin, and their oligomers, also known as procyanidins [57]. Many examples of the health benefits of cocoa consumption can be found in the literature [58].

Epidemiologic studies of cocoa intake and cancer risk are few, and those assessing overall mortality provide only weak support of the benefits of cocoa. However, human intervention trials indicate that cocoa favours intermediary factors in cancer progression—specifically, markers of antioxidant status [59]. Moreover, there is growing evidence that polyphenols may play a role in regulating apoptosis [60]. Apoptosis may be triggered intrinsically, through the mitochondrial pathway or extrinsically by death ligands and receptors. It is the external pathway that may potentially be modulated by bioactive food components. Flavanols found in cocoa have exhibited pro-apoptotic effects. Proanthocyanadins inhibited growth of human lung cancer cells in vitro and in vivo [61], and epicatechin synergistically enhanced apoptosis in lung cancer cells treated with epigallocatechin-3-gallate (EGCG) [62]. Cocoa polyphenols have also been found to inhibit the mutagenic activity of heterocyclic amines *in vitro* and *ex vivo* [63].

It has been reported that catechins from green tea could be effective in modulating estrogen-induced breast carcinogenesis, either interfering with receptor mediated pathways or reducing the production of genotoxic estrogen metabolites [64,65]. In our functional genomic study, we sought to evaluate the effect of cocoa flavonoids in a type of breast cancer cells (MCF-7), that are estrogen-receptor (ER)- dependent [66]. Estrogens are implicated in the initiation and promotion stages of breast cancer, and lifetime estrogen

exposure is a major risk factor for breast cancer [67]. Estrogens exert their carcinogenic effects by both estrogen receptor (ER)-dependent and independent mechanisms [68]. Most human breast cancers are initially positive for ER, and their growth can be stimulated by estrogens and inhibited by antiestrogens such as tamoxifen.

For that purpose, MCF-7 cells were incubated for 24h with a purified polyphenol cocoa extract (PCE). PCE was used as representative of the wide flavonoid spectrum (monomers and oligomers) present in cocoa and the concentrations used were not toxic. The differential gene expression analysis was done using PCR arrays. In particular, the expression profile of the 84 genes included in the Stress & Toxicity PathwayFinder™ PCR Array was analyzed in MCF-7 cells both control and treated with a PCE. It was observed that the exposure to PCE decreased the expression of serpine 1 and up-regulated the expression of the CYP1A1, GADD45A, GDF15, GPX1, RAD23A, TP53, and XRCC2 genes (Table 2).

Among those genes, CYP1A1 was chosen for further validation since: (a) it was one of the most overexpressed gene upon incubation with PCE, (b) its overexpression in response to polyphenols had already been described, and (c) it plays an important role in the oxidative metabolism of estrogens. CYP1A1 is a candidate gene for low-penetrance breast cancer susceptibility because it plays an important role in the metabolism of xenobiotics or carcinogens as well as in the oxidative metabolism of estrogens [2004]. CYP1A1 encodes aryl hydrocarbon hydroxylase (AHH) which catalyzes a

Table 2. List of under- and overexpressed genes in MCF-7 cells upon incubation with PCE for 24hours[1].

MCF-7 Gene symbol	Fold-up or down-regulation Test sample / control sample	p-value
CYP1A1	17.60	0.0001
GADD45A	4.20	0.0264
GDF15	2.60	0.0001
GPX1	4.25	0.0183
RAD23A	13.90	0.0394
SERPINE1	- 49.90	0.0216
TP53	2.26	0.0470
XRCC2	17.50	0.0356

[1]The expression of each gene was reported as the fold change obtained after each treatment relative to control after normalization of the data. A cut-off of 2-fold was chosen since small changes in gene expression may represent important changes downstream those differentially expressed genes. Lists of differentially expressed genes, with a p-value<0.05, were generated from three independent experiments.

hydroxylation reaction in Phase I metabolism as a first step to increase the polarity of different molecules. Some of these metabolites can be more active than the initial molecules and behave as electrophilic compounds, thus initiating or promoting tumorigenic processes. Additionally, other metabolites may behave as chemoprotectors, such as the result of 2-hydroxylation in E1 and E2 metabolism [70].

Therefore, the differential expression of CYP1A1 mRNA in control versus treated cells was validated by RT- Real Time PCR (Figure 6A). Next, we investigated whether the changes at the RNA level were translated into protein. PCE treatment for 24 h led to a very modest increase in CYP1A1 protein levels (1.2-fold). A time course incubation during 24, 48, 72 and 96 h led to an increase in CYP1A1 protein in MCF-7 cells of 3.9-fold after 48 h (Figure 6B). The difference between mRNA levels and the corresponding protein levels may indicate that many of the mRNA molecules do not reach

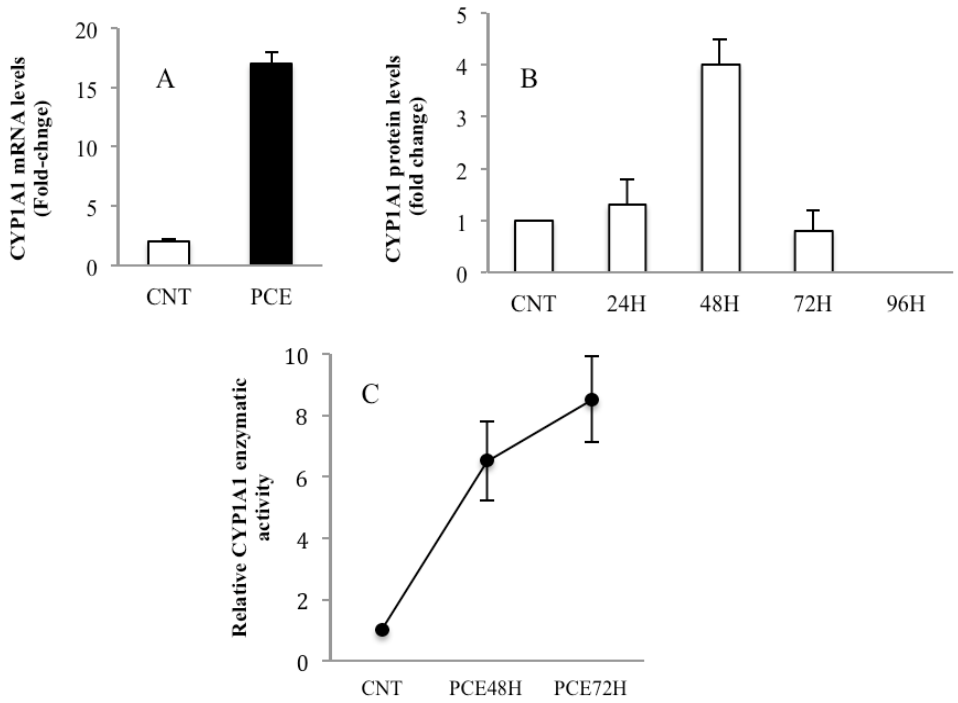

Figure 6. CYP1A1 overexpression in MCF-7 cells treated with PCE. (A) Determination of CYP1A1 mRNA levels. Results are expressed in fold changes compared to MCF-7 control and are the mean ± SE of 3 different experiments. (B) Determination of CYP1A1 protein levels. Results represent the mean ± SE of 3 different experiments. Significant differences at all time points were evaluated by ANOVA plus post hoc Bonferroni comparison. (C) Determination of CYP1A1 activity in MCF-7 treated cells. Results are expressed relative to the activity of the control and represent the mean ± SE of 3 different experiments. Significant differences at all time points were evaluated by ANOVA, plus post hoc Bonferroni comparison.

the translational machinery, probably because the translation mechanism is saturated in these conditions. Finally, CYP1A1 activity was determined upon incubation with PCE. An increase in CYP1A1 activity in good correlation with the observed increased in CYP1A1 protein levels was determined for both cell lines (Figure 6C).

The changes in CYP1A1 expression upon incubation with PCE could explain the antioxidant effect of flavonoids at the molecular level since this gene is involved in different oxidative pathways. Additionally, CYP1A1 overexpression might interfere with estrogen metabolism and the production of estrogen metabolites in breast cells. The increase in CYP1A1 activity may shift estrogen metabolism toward the production of 2-OHE2 (2-hydroxyoestradiol), a relatively non-genotoxic metabolite [71].

Finally, we wanted to test whether cocoa polyphenols would exert a synergistic effect in combination with Tamofixen (TAM) since it has been previously described in breast cancer cells. Thus, MCF-7 cells were incubated with increasing concentrations of TAM (10^{-6}–10^{-3}M) either alone or in combination with PCE (250 ng/µL). Then, cell viability was determined after 48 h. The presence of PCE, which did not cause significant cell death by itself, increased the cytotoxic effect of TAM in MCF-7 cells (Figure 7).

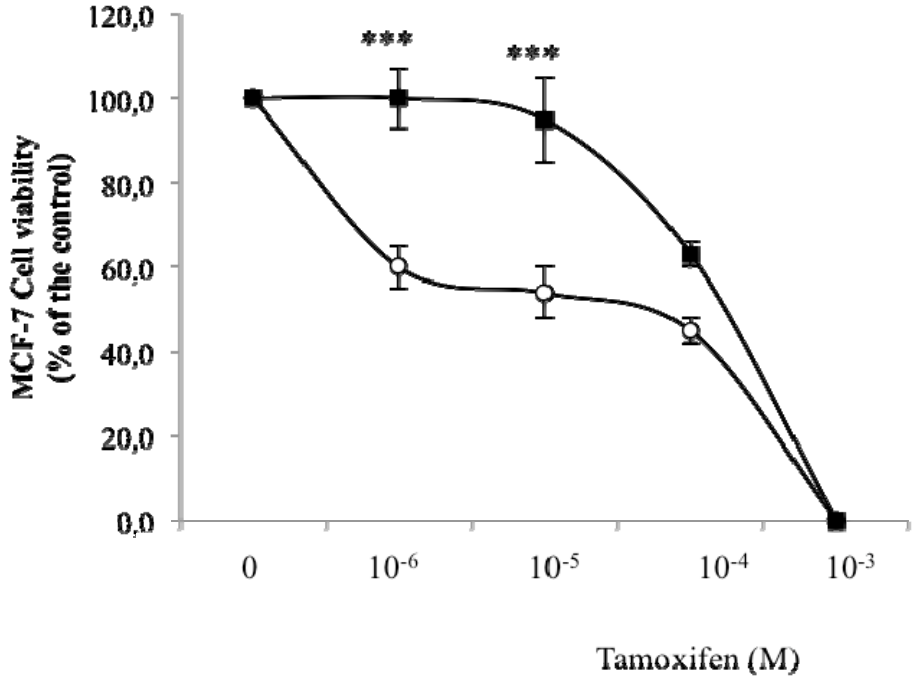

Figure 7. Effect of tamoxifen plus PCE on MCF-7 viability. Tamoxifen (TAM) either alone (filled squares) or in combination with PCE (250 ng/µL for 24H, empty circles). Results are expressed as % of living cells compared to the control only with DMSO (0.22%) and represent the mean ± SE of 3 different experiments. ***$p<0.001$.

The reduction in cell viability reached an increase of 44% when combined with 10^{-6}M TAM. Thus, in our conditions, the cytotoxic effect of TAM was enhanced by the combination with PCE in MCF-7 cells. The presence of PCE caused a synergistic effect, confirmed by the Chou-Talay method, which led to a decrease in cell viability of up to 40% in MCF-7 cells at tamoxifen concentrations that did not affect cell viability by themselves. A plausible explanation of the synergistic effect observed could be that the increase in estrogen metabolism, induced by the PCE on CYP1A1, could lead to the reduction in the levels of estrogens in mammary tumours, thus contributing to the cytotoxic effect of tamoxifen. Nevertheless, further *in vivo* studies are necessary to analyse the synergism between tamoxifen and cocoa and to establish the possible benefits of cocoa polyphenol consumption during breast cancer therapy.

3. Conclusions

Current global trends in food consumption may have an impact on disease progressions observed worldwide. The impact may occur because of gene regulation caused by nutrients, or by other unclear means that are yet to be discovered. The "omics" and associated technology will surely provide a greater understanding of the environmental and behavioral factors that influence phenotype and its relationship to health and wellness. It is highly likely that during the next decade the nutritional supplement and functional food industries will experience robust growth in response to advances in nutritional genomics research and its applications.

Parallel to this growth will be impressive progress in understanding the specific influence of certain food components on metabolic pathways and their role in health and disease. It will become increasingly less expensive to generate genetic information about individual persons, and such data are likely to redefine the current concept of preventive medicine. Moreover, through nutrigenomic research, new nutritional regulation of gene expression will hopefully come to light. If specific gene regulation by nutrients is identified in genes closely related to disease onset and progression, new arenas for disease prevention and potential for treatment will come to the foreground of nutrition and preventive medicine. Discoveries made in the field of nutrigenomics and nutrigenetics should translate into more effective dietary strategies to improve overall health by identifying unique targets for prevention.

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Genetics of Eating Behavior: Established and Emerging Concepts

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Abstract

Understanding why we eat and the motivational factors driving food choices is important to addressing the epidemics of obesity, diabetes and cardiovascular disease. Eating behavior is a complex interplay of physiologic, psychological, social, and genetic factors that influence meal timing, quantity of food intake, and food preference. Here we review the current and emerging knowledge of the genetic influences of eating behavior and how these relate to obesity with particular emphasis on the genetics of taste, meal size and selection, and the emerging use of functional magnetic resonance imaging to study neural reactions in response to food stimuli in normal, overweight and obese individuals.

Keywords

Eating behavior; Genetics; Obesity

INTRODUCTION

Understanding why we eat and the motivational factors driving food choices is important to addressing the epidemics of obesity, diabetes and cardiovascular disease, as food intake is a significant factor impacting the development and treatment of these disorders. Eating behavior is a complex interplay of physiologic, psychological, social and genetic factors that influence meal timing, quantity of food intake, food preference, and food selection. Active research involving the genetics of taste, food preference, pathological eating behaviors, meal size, and meal selection is rapidly expanding our understanding of how and why we eat. More recently, neural imaging modalities, specifically functional magnetic resonance imaging (FMRI), has emerged as a modality to effectively study eating behavior and genetics in fascinating ways. Here we review the current knowledge of the genetic influences of eating behavior with particular emphasis on the genetics of taste, meal size and selection, and the emerging use of FMRI as it applies to imaging neurophysiologic response to food stimuli. In this review, we focus primarily on obesity as a consequence of eating behavior, but other pathological disorders of eating behavior, including anorexia nervosa and bulimia nervosa, also have strong genetic, psychological, and environmental components.[1]

The rapid rise in obesity and associated co-morbidities (metabolic syndrome, coronary artery disease, sleep apnea, skeletal disorders, hyperlipidemia, and hypertension) over the past 30 years has led to the urgency of coming to a more complete understanding of the pathophysiology of obesity. The study of eating behavior attempts to define eating patterns

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and food preferences, and to explain why there is gravitation toward specific behaviors and food choices, and aims to develop approaches to bring about effective changes in modifiable behaviors. Knowledge of the biological mechanisms guiding eating behavior can provide effective treatment targets for obesity and associated disorders.

Rare monogenic genetic disorders involving hyperphagia and obesity have been identified.[2] Resulting from a deletion of the 11-13q region of chromosome 15, Prader Willi (PW) is characterized by hypotonia and poor feeding in early infancy, cognitive, motor and behavioral impairment, followed by insatiable hunger and the development of morbid obesity and diabetes during childhood.[3] PW patients rarely survive beyond the age of 25 to 30 years; the cause of death is often related to diabetes and cardiac failure. Monosomy 1p36 has also been associated with obesity and hyperphagia in a PW negative cohort.[4] Individuals with loss of function mutations of the leptin (*LEP*) gene on chromosome 7q31.3, or its receptor (*LEPR*) also display abnormal eating behavior and develop early-onset morbid obesity.[5–6] Leptin replacement can improve satiety and promote weight loss in leptin deficient individuals.[7] Leptin promotes α-melanocyte stimulating hormone(α-MSH) synthesis which promotes satiety.[8] α-MSH is bound by the melanocortin 4 receptor (MCR4) protein. *MC4R* mutations are associated with early onset obesity.[9–10] Discoveries of the genes and their respective proteins involved in these rare forms of obesity helps shed light on the pathways involved in regulating eating behavior and energy homeostasis. Although important, monogenic forms of obesity account for less than ten percent of today's obesity epidemic.[11]

Although rare genetic mutations cause dramatic hyperphagia, most common genetic variants have smaller effect sizes. The risk of obesity, metabolic syndrome, and other complications is increased by a variety of common genetic variants, and many of these are associated with specific eating behaviors. Research tools used to measure eating behavior include food logs, observation, food preference flash cards, labeled scaling, and more recently FMRI. A widely used research tool known as the Three-Factor Questionnaire (TFQ) has been used to quantify eating behaviors in normal-weight, obese, and in individuals with eating disorders.[12] This questionnaire uses a series of questions to measure three patterns of behavior: restraint, disinhibition, and hunger. Both high restraint and disinhibition scores are positively correlated with BMI.[13–14] Restraint is characterized by intentional avoidance of certain foods in order to control body weight, and is measured by response to questions on the TFQ such as "I avoid certain foods because they make me fat." Disinhibition is the tendency to overeat when surrounded by others who are overeating. Hunger measures the subjective sense of an individual's need to eat. Heritability and linkage analysis of eating behavior measured by the TFQ provides evidence that these behavior traits are heritable.[15–16] Although much remains to be understood about the genes regulating these behaviors, genetic influence of disinhibition has been linked to *neuromedin,* a factor mediating satiety, in a French Canadian cohort and to *TAS2R38*, a bitter taste receptor, in a cohort of Amish women.[16–17] *GAD* (glutamic acid decarboxylase) has also been linked to eating behavior. GAD decarboxylates glutamate into GABA (γ-aminobutyric acid), a major inhibitory neurotransmitter in the brain. Two specific *GAD* variants, rs7908975 and rs992990 have been reported to be associated with disinhibition and disordered food intake, specifically increased carbohydrate intake, in women.[18]

GENETICS OF TASTE

Taste affects food preference and food intake thereby directly influencing eating behavior. However, not all humans perceive taste in exactly the same way. The density of taste papillae on the tongue, genetic differences in taste receptors or sensitivity of taste receptors, constituents of saliva, and other factors all contribute to an individual's taste perception and

subsequent food preferences.[19] Differences in taste papillae density impacts taste sensitivity and are thought to be genetically determined[20]; however, the gene or genes responsible for this trait have yet to be identified. Differences in taste perception and preference influence food choices and have significant impact on nutrient and caloric intake.

Five tastes are recognized by humans: sweet, bitter, sour, salty, and umami— described as the taste of glutamate or the taste of amino acids and proteins. Food preference and intake is influenced by sweet and bitter taste. For example, individuals who possess enhanced perception of bitter taste tend to avoid certain foods, including specific fruit and vegetables. [21] Preference for sweet and high-fat food has been reported to decrease with increasing perception of bitter taste.[21–24] Evidence suggest bitter tasting ability may be related to body mass index (BMI), adiposity, and risk factors for CVD,[25–26] while the perceived sweetness of foods has been shown to be inversely correlated with BMI.[27] Bitter taste sensitivity has also been linked to variation in height among children, suggesting this trait may influence food selection and impact growth rate.[28–29] Individuals who are particularly sensitive to bitter compounds tend to avoid the bitter taste of beer and alcohol, and avoid cigarette smoking as well.[25,30] Bitter taste as well as preference for sweet and fat guide ingestive behaviors, and have been linked to obesity, and these food preference traits may in part be genetically determined.

Bitter, sweet and umami tastes are mediated by G-protein-coupled receptors (GPCR). Bitter taste receptors are encoded by 25–30 *TAS2R* genes, located on chromosomes 12p13, 7q34 and 5p15.31. The ligand specificity of TAS2Rs appears to be quite broad, consistent with their roles in detecting thousands of bitter-tasting compounds.[31] One of these, *TAS2R38* has been extensively characterized *in vitro, in vivo* and in human populations, and is responsive to the bitter stimuli phenylthiocarbamide (PTC), propylthiouracil (PROP), and to thiocyanates—bitter compounds found in brassia vegetables such as brussels sprouts and broccoli. Two common haplotypes of *TAS2R38* have been shown to influence perception of bitter taste and are significantly related to differences in bitter taste sensitivity,[32] preference for sucrose and sweet tasting foods and beverages, and to modestly lower risk of type 2 diabetes among participants of the British Women's Heart and Health Study.[33–34] While studies are not all in complete agreement, individuals most sensitive to the taste of PROP more often dislike bitter fruits and vegetables, such as grapefruit and kale. These low energy foods may be replaced by more energy dense foods among individuals more sensitive to bitter taste.[35] *TAS2R38* haplotype has been suggested to be predictive of obesity,[36] however, to date, studies involving large cohorts have failed to demonstrate convincing evidence for a direct relationship between *TAS2R38* and BMI in spite of evidence that polymorphisms in this gene influence ingestive behavior.[37] The majority of *TAS2R38* studies have been conducted in Caucasian populations, therefore further research is necessary to determine how well current findings can be generalized to other ethnic populations.

TAS2R5, another bitter receptor, may be an important regulator of ingestive behavior. This gene resides in a region of chromosome 7 that is significantly associated with a quantitative phenotypic marker of alcohol dependence called ttth 1. Furthermore, a single nucleotide polymorphism (SNP) located within a linkage disequilibrium block that includes *TAS2R5* accounts for this association.[38] A SNP in another chromosome 7 gene, *TAS2R16*, has been linked to alcohol dependence as well.[39] These findings suggest that genetic variation in TAS2R genes may be involved in regulating ingestive behaviors.

The receptors for sweet and umami taste are encoded by three *TAS1R* genes located on chromosome 1p36. Heteromeric TAS1R2:TAS1R3 taste receptors respond to sweet-tasting compounds such as sugars, high-potency sweeteners, and some D-amino acids, while TAS1R1:TAS1R3 heteromers comprise an umami taste receptor sensitive to L-amino acids.

[31] Both subunits of the sweet taste receptor bind sugar ligands, though they do so with distinct affinities and ligand-dependent conformational changes.[40–41] Although variability in both sweet and umami taste have been described, these traits are not as well defined as those of PROP tasting, and specific genetic variants responsible for variation in sweet and umami taste remain to be identified.

TAS1Rs and *TAS2Rs* are expressed in diverse tissue including brain, adrenal gland, pancreas, small intestine, retina, skeletal muscle, salivary gland and tongue.[42–44] Of particular interest is the observation that TAS1R and TAS2R receptors, as well as other proteins involved in taste transduction, are expressed in the gastrointestinal mucosa, where they modulate responses to ingested nutrients via glucagon like peptide-1 (GLP-1), cholecystokinin (CCK), and gastric inhibitory polypeptide (GIP).[44–45] GIP, GLP-1 and CCK regulate gut motility and appetite. Therefore, TAS1R and TAS2Rs may be integral to modulating both taste and ingestive behavior via mediating enteroendocrine secretion. Dotson et al demonstrated *TAS2R9* to be involved in GLP-1 secretion, with a loss of function mutation in the gene resulting in attenuated GLP-1 response to agonist.[46] In another study, Dotson et al have also shown genetic variation in *TAS2R38* to be associated with eating behavior in a cohort of Amish women.[17] Genetic variation in TAS1R and TAS2Rs may impact eating behavior via altered taste perception as well as via alteration in neuroendocrine signals impacting satiety. The observation that these receptors are involved in both taste and secretion of hormones involved in satiety tell us that these processes may be biologically entwined. Table 1 summarizes gene variants linked to eating behavior and taste.

MEAL SELECTION AND SIZE

Research into meal size and selection is especially complex as socioeconomic environment, learned eating behaviors, physiologic conditions such as depression, and even medical treatments can all influence appetite and food selection, independent of genetics; however meal quantity, frequency, and timing are thought to be at least in part under genetic control. The study of genetic variants in digestive neuroendocrine hormones, such as CCK, leptin and ghrelin, are providing new insights into how these hormones and their genetic variants may be involved in pathways regulating appetite and eating behavior.

Ghrelin, a 28-amino acid peptide, is primary produced by the stomach and pancreas and is involved in promoting meal intake and hunger through receptors in the hypothalamus.[47] Plasma ghrelin levels rise pre-meal and are suppressed by food intake.[48] *GHRL* is located on chromosome 3. The gene product is involved in growth hormone release, and post translational modifications yield the hormones ghrelin and obstatin. Obstatin opposes the effects of ghrelin and is responsible for satiety and decreasing food intake.[49] Many studies have been devoted to investigating *GHRL* variants with respect to obesity. A common variant, Leu72Met has been associated with obesity,[50–51] metabolic syndrome,[52] and binge eating.[53]

Leptin and CCK work in opposition to ghrelin to promote satiety. CCK is released in response to lipids and promotes rapid post-prandial satiety in contrast to the long term action of leptin.[54] In a large case-control study of 17,000 obese and normal weight women, common leptin variants, (rs4577902, rs2060736, and rs4731413), were associated with increased risk of extreme snacking behavior (top 5th percentile based on 11 question questionnaire), but not increased meal size.[55] CCK variants (rs6809785, rs7611677, rs6801844, and rs6791019) were found to be more associated with extreme meal size (top 5^{th} percentile based on estimated portion sizes using 28 picture cards) but not increased snacking behavior in the same study. This study suggests that genetic variation in genes encoding CCK and leptin may contribute to obesity risk by influencing satiety, and may

have independent effects. Additional studies are needed to further clarify the role of genetic variation in these genes to provide a better understanding of how they may modulate eating behavior.

FTO, fat mass and obesity-associated gene, has been highly associated with increased risk of obesity.[56] *FTO*, is localized to chromosome 16 and is expressed in adipocytes, the pancreas, and the hypothalamus, particularly in regions known to regulate appetite. *FTO* may contribute to obesity by down regulating adipocyte production of leptin.[57] A common variant, rs9939609 is associated with adiposity, and possibly satiety responsiveness. den Hoed et al demonstrated the A allele of rs9939609 to be associated with reduced post-prandial satiety, and may also contribute to excess caloric intake in a study of men and women of Western European descent with BMI's ranging from 19–31, (5 of 62 subjects had a BMI >30).[56] This study also analyzed post prandial response to hunger and the interaction among variants in leptin, the leptin receptor and methyltransferase genes. The authors concluded that the effect of the rs9939609 A allele on the postprandial response in hunger appears to be mediated by an epistatic interaction involving variants in a methyltransferase gene and the leptin receptor. In another study, Scottish children who were homozygous or heterozygous for the rs9939609 A allele also demonstrated increased energy intake without associated energy expenditure. Of interest, all the children ate approximately the same weight of food, but those children with the rs9939609 A allele consumed more energy-dense foods.[58] The authors of this study concluded that this *FTO* variant confers a predisposition to obesity and may play a role in the control of food intake and food choice, perhaps involving a hyperphagic phenotype or a preference for energy-dense foods. Tanofsky-Kraff replicated these findings in a cohort of 289 children and adolescents, suggesting that *FTO* may indeed contribute to preference for higher fat intake and large meal size.[59] Although provoking, these findings should be interpreted with some caution, as at least one study has shown rs9939609 not to be correlated with increased risk of obesity,[60] however the current accumulated evidence clearly implicates *FTO* as having a significant impact on food intake and obesity.

Genetic variations in *FTO*, leptin, the leptin receptor and ghrelin, genes involved in the neuroregulation of food intake, appear to contribute to obesity risk by influencing satiety and hunger, and may contribute to increased caloric intake. Larger and more genetically diverse cohorts need to confirm these observations. Functional studies of the impact of these variants on gene expression or action are also needed. Improving our understanding of the mechanisms whereby these genes interact and their potential molecular cross talk may provide novel targets for developing treatments for individuals with reduced satiety in response to meals. Table 2 summarizes the current knowledge with respect to genetic variants linked to meal selection and size.

Functional Magnetic Resonance Imaging

A variety of cognitive pathways are involved in motivation and control of eating behavior. The new use of neuroimaging techniques, specifically functional magnetic resonance imaging (FMRI), to demonstrate specific neural reactions in response to food stimulus is revolutionizing the study of eating behavior. Other imaging techniques, such as position emission tomography (PET) studies have been previously used to investigate neural responses to taste and identify neural pathways involved in eating behavior.[61] FMRI has previously been well-established in identify pathology in studies of schizophrenia,[62] Alzheimer's disease,[63] and many other areas of neuroscience research. Nearly all FMRI studies utilize blood oxygen level dependence (BOLD) to identify areas in the brain that demonstrate increased glucose uptake and therefore increased activity in response to specific stimuli. Eating behavior research utilizing FMRI has focused on BOLD changes in specific brain regions in obese compared to normal weight individuals.

Eating behavior FMRI studies have shown that a fasting state increases cortical activation among lean individuals,[64] increases preference for high calorie foods in obese individuals,[64–66] and that obese men have attenuated post-prandial brain reactions to satiety which may explain excess caloric intake.[67] Ghrelin infusion in normal weight volunteers produced increased BOLD response to food pictures in the amygdala, orbitofrontal cortex, anterior insula, and striatum, areas of the brain involved in activating ingestive behavior, and elicited increased self-reports of hunger.[68] Likewise, patients with lower leptin levels secondary to weight loss or secondary to genetic leptin deficiency have increased BOLD activity in brain areas involved in emotional, cognitive, and sensory control of food intake in response to food stimuli, which subsequently normalize with leptin infusion.[7,69]

Neurophysiologic processing in response to food is largely accomplished in the left hemisphere, specifically in the dorsal and ventral striatum, fusiform gyri and insula— the latter two known as the "primary gustatory complex."[64] Feeding is associated with dopamine release,[70] and the amount of dopamine release positively correlates with perceived food pleasure.[71] Obese individuals have lower striatal concentrations of the D2 dopamine receptor,[71] findings suggesting that the lower concentrations of this G protein coupled receptor may evoke overeating in obese individuals in order to produce a reward response. An alternative interpretation is that dopamine receptors may be downregulated in response to excessive food stimuli. Martin et al demonstrated that brain regions involved in pathways of food reward in obese individuals exhibited increased BOLD activation, specifically the limbic region and prefrontal region, both which have high concentrations of dopamine receptors.[72] Obese individuals also had greater memory for foods in the fasted state. Fasted obese individuals have also been shown to exhibit higher pre-meal activation of the anterior cingulated cortex and medial prefrontal cortex, areas of the brain implicated in motivational processing.[72] These findings have been supported by Haase et al who also noted increased BOLD activation in the prefrontal and limbic regions in response to taste stimuli among fasting obese individuals.[73] Although these studies are promising, they remain limited by small numbers of participants and many of these studies have focused largely on obese women. Because MRI equipment can withstand limited study subject body weight, these studies are restricted to the study of subjects whose body weights can be accommodated by the MRI equipment. Despite these limitations, FMRI has been used successfully to shed light into pathways involved in eating behavior and to demonstrate important functional difference in brain imaging among obese individuals.

Of particular interest are innovative studies combining genetic studies with FMRI to investigate eating behavior. Combining these modalities may help uncover inter-relationships among genetics and the neurophysiologic pathways involved in food response and eating behavior. Felsted et al hypothesized that polymorphisms in genes involved in the neurophysiology of feeding and reward processing would demonstrate differential responses in brain regions known to be involved in food reward.[74] They chose to investigate a particular variant, TAQ1A,[75] a restriction fragment length polymorphism located on *ANKK1* (ankyrin repeat and protein kinase domain-containing protein 1), a regulatory gene downstream of the dopamine D2 receptor, and to perform functional magnetic resonance imaging to measure neural response to the ingestion of palatable and caloric milkshakes in 26 healthy subjects (24 women and 2 men). The TAQ1A variant has previously been implicated in having a role in obesity and eating behavior, particularly with respect to the relationship between neural response to food and prospective weight gain.[71] Individuals with the A1/A1 or A1/A2 allele of TAQ1A are more likely to be obese, and have 30–40% fewer dopamine receptors.[71] In the Felsted study, either a milkshake or a tastless, odorless liquid was randomly dripped into the mouths of the subjects while subjects rested within the MRI device. The investigators attempted to control for confounding variables that may influence food response. Participants were all matched for BMI, hunger rating, and for

psychological factors such as impulsivity, addiction, and eating style assessed through a variety of psychological and food intake surveys. No subject reported taking prescription or over-the-counter medications. This study elegantly demonstrated that individuals possessing the A1 TAQ1A allele had decreased BOLD response to a milkshake in midbrain, thalamus, and orbital frontal cortex regions, regions of the brain involved in regulating eating behavior, even though all participants rated similarly the perceived pleasantness and familiarity of the milkshake.[74] These findings suggest that individuals possessing the A1 allele might be predisposed to overeating as they experience attenuated neural reward response to food. Whether this variation in response to food stimulus is due to diminished number of dopamine receptors is yet to be determined. This study is the first of our knowledge to directly demonstrate that individuals with a specific genetic variant have measurable neural changes directly correlated with eating behavior in responsiveness to a food stimulus. These findings provide hope for the future development of treatment aimed at modulating food induced sensitivity to pleasure and satiety centers in genetically susceptible individuals.

CONCLUSION

Clinical applications and future directions

Eating behavior is a complex trait with both genetic and environmental influences. While sequencing an individual's entire genome is expensive, this process will become less costly and more rapid in the future. Personalized medicine, tailoring pharmacologic and behavioral therapy to an individual's genetic code, is an emerging practice. Applications of research of the genetics of eating behavior may lead to individualizing therapies targeting specific genetic mutations and behavioral interventions addressing eating behaviors. For example, once the role of specific gene variants in pathways involved in specific behaviors or food responses are well established, treatment could be individualized toward modifying these behaviors (for example carbohydrate craving, unrestrained or binge eating, comfort eating, food addiction) and pharmacologic modalities developed to modify molecular pathways involved. Individuals with a TAS2R38 variant associated with enhanced bitter taste might be counseled to select healthy foods that might be more palatable or instructed regarding methods of food preparation to make bitter vegetables more palatable. While current research is limited, preliminary studies hold promise toward these ends. Authors of a nutra-genomic study involving customized treatment with a nutraceutical based on the study subjects' genetic profile report improvement in weight loss, sugar craving reduction, appetite suppression, snack reduction, reduction of late night eating among study participants who received therapy tailored toward their genetic profile.[76]

The risk of obesity, metabolic syndrome, and related complications is increased by a variety of common genetic variants, and many of these are associated with specific eating behaviors. Although rare genetic mutations cause dramatic hyperphagia, common genetic variants usually are responsible for smaller effect sizes. It is likely, however, that genetic susceptibility toward aberrant eating behavior and obesity may be overcome by practicing healthy behaviors. This principle was demonstrated among individuals harboring the common TCF7L2 variant which is associated with increased risk of developing type 2 diabetes mellitus (T2DM). In the Diabetes Prevention Study, individuals with the at risk variant who were randomized to intense lifestyle interventions including prudent diet, weight loss and physical activity, demonstrated reduced progression to T2DM in spite of their genetic predisposition.[77] Studies involving *FTO* variants also demonstrate that genetic predispositions to obesity can be overcome by prudent diet and exercise.[78–79] With additional research individuals and physicians will have more tools to help identify susceptible individuals and to guide therapy and treatment. The study of the genetics of eating behavior and its interplay with obesity is undergoing rapid progress, and new

techniques, including FMRI, are changing how behavioral research is performed and providing new insights into mechanisms of eating behavior. While it is premature to know if developing pharmacologic therapy targeting the A1 allele of TAQ1A or other alleles discussed in this review will contribute to substantial changes in eating behavior or weight loss treatment, at this point in time, it may be helpful for an individual to be aware of his or her genetic susceptibilities of becoming overweight and to practice prudent nutritional behaviors before becoming overweight or obese.

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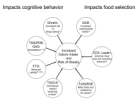

Figure 1.
Genes identified with common variants influencing eating behavior with potential impact on obesity

Table 1

Common variants associated with variation taste and ingestive behavior

Tastes	Chromosome	Gene	Influence on Ingestive behavior	Reference
Sweet	1p36	TAS1R2, TAS1R3	Unknown	Nie et al. (2005)[41], Nie et al. (2006)[40], Scott et al. (2005)[31]
Umani	1p36	TAS1R1, TAS1R3	Unknown	Scott et al. (2005)[31]
Bitter	12p13, 7q34, 5p15.31	TAS2Rs: TAS2R38, TAS2R5, TAS2R16	Vegetable avoidance, increased fat and sweet intake, disinhibited eating behavior among women Alcohol dependence	Kim et al. (2003)[32] Drewnowski et al. (1997)[21] Mennella et al. (2005)[33] Timpson et al. (2005)[34] Dotson et al. (2008)[46] Lin (2005)[38], Hinrichs (2006)[39]

Table 2

Common variants associated with meal selection and size

Hormone	Gene variants	Physiologic effect of gene product	Contributions to eating behavior	References
CCK	rs6809785, rs7611677, rs6801844	Rapid post-prandial satiety	Extreme meal size	de Krom et al. (2007)[55]
Leptin	rs4577902, rs2060736, rs4731413	Promote satiety	Extreme snacking behavior	de Krom et al. (2007)[55]
Ghrelin	Leu72Met, 51GLN	Promote meal intake and hunger Metabolic Syndrome Obesity	Binge Eating	Monteleone et al. (2007)[53] Hinney et al. (2002)[51] Korbonits et al. (2002)[50] Steinle et al. (2005)[52]
FTO	rs9939609	Downregulates leptin, suppress satiety	Reduced post-prandial satiety, increased caloric intake	den Hoed et al. (2009)[56] Cecil et al. (2008)[58] Tanofsky-Kraff et al. (2009)[59]
GAD	rs7908975, rs992990	Promote GABA, regulate food intake	Increased carbohydrate intake	Choquette et al. (1998)[18]

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NUTRITION AND GENETICS
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FOREWORD

Characterisation of the human genome and identification of the pivotal role that nutrients play in gene expression evolved into the science of nutrigenomics.

As a new science "nutrigenomics" brings with it new terminology, novel experimental techniques and a fundamentally new approach to nutrition research such as high throughput technologies that enable the global study of gene expression in a cell or organism. This monograph aims to provide the reader with an introduction to the new science and its potential.

Until recently, nutrition research concentrated on nutrient deficiencies and impairment of health. The advance of nutrigenomics has created unprecedented opportunities to deepen our understanding of how nutrients modulate gene expression, protein biosynthesis and metabolism. Scientists face the challenge to provide comprehensive answers to questions such as:

- Which components of the diet have important health promoting effects?
- How, where and when are these effects exerted?
- Can some of these components also have adverse effects?
- How much and in what form and combination do we need to eat such components to obtain the maximum health benefit with minimum risk?
- How do individuals' dietary recommendations vary depending on their genetic profile, age, gender and lifestyle?

Nutrigenomics-aided research should ultimately provide a sound basis for dietary management of maintenance and protection of health, eventually positioning nutrients in the context of individual genetic background.

This may sound somewhat futuristic and the relative importance of gene-nutrient interactions on polygenic diseases is still at a very early stage as well as the understanding of the complexity of genetic regulation, including redundancy of pathways and the role of epigenetic modifications.

The greatest potential for benefit from dietary modification is likely to be the protection of health. Nutrigenomics will facilitate the identification of biomarkers that play a role in the initial physiological changes at the onset of disease providing indicators to measure the effectiveness of dietary interventions.

We are confident that this Concise Monograph will help readers to gain insight into the exciting area of nutrigenomics, its terminology, its technology, and its potential for nutrition science.

Els de Groene
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1. NUTRITION DEVELOPMENTS

The 20th century is the period during which nutrition 'came of age'. Many significant developments took place that commenced with the isolation and coining of the word 'vitamin' in 1912 and the appreciation of the important role that vitamins play in nutrition.

In 1929, linoleic acid was shown to be the first essential fatty acid in experiments with rats. Later, linolenic and arachidonic acids were shown to partially relieve deficiency symptoms. The group subsequently became known as the essential fatty acids. As the century progressed, roles for fibre, antioxidants and other micronutrients were discovered. The concept of reference intakes of nutrients developed (determined on the basis of the average population requirement plus twice the standard deviation) and recommended daily amounts were established for the main nutrients across all age groups.

Just as important as the development in nutrition science is the change that has occurred in people's nutritional status. In the early part of the 20th century, the major concern was under-nutrition with overt deficiency symptoms frequently seen. In the second half of the century, in Western societies, the major concern had moved towards over-nutrition with the rising incidence of obesity and diabetes. In addition, there was an improved understanding of the links between nutrition and chronic disease(s), for example heart disease, stroke and cancer. As these diseases overtook infectious diseases as the major causes of death, a greater awareness of the need for moderation in food intake developed.

The last decade of the 20th century saw a further nutritional development. This was the use of nutrients or foods - the so-called functional foods - to promote a healthy body and to help avoid disease.

A key realisation has been that nutrients are now not only important to ensure nutritional adequacy, but can also help to maintain and improve health.

The development of functional foods, and nutritionists' improved understanding of the potential role of these in the diet, has helped informed consumers to make healthful dietary interventions that contribute to preventive healthcare.

The 21st century will witness a major step forward in nutrition science prompted by the recent characterisation of the human genome. The identification of genes and gene sequences can help unlock a whole new area of nutrition research.

Nutrient or nutrient/metabolic signals hold a pivotal role that governs the expression of genes encoding the proteins required for energy metabolism, cell differentiation and cell growth.

Possibly, future generations will be able to identify the link between an individual's genetic code and predisposition to dietary related illness and/or sub-optimum physiological performance, in essence enabling them to map their own individual health and making nutrition interventions to help maintain it.

2. GENE STRUCTURE AND FUNCTION

2.1 Introduction

The genetic material that we acquire from our parents consists of a collection of DNA nucleotide sequences that make the back-bones of the 23 pairs of chromosomes (*see Box 1: Chromosomes and Figure 1*) present in the nucleus of the cells throughout our body. Within each chromosome, the genetic material is organised into sequences known as genes (*see Box 4: Gene*).

Genes encode the proteins responsible for our structure and metabolic functions (*see Box 4: Genetic Code*).

Genes are turned on and off according to metabolic signals that the nucleus receives from internal factors, for example hormones, and external factors, for example nutrients, which are among the most influential of environmental stimuli.

Early in evolutionary development, the nutrients that organisms ingested functioned as primitive signals that turned on and off pathways of synthesis or storage during periods of starvation or excess. As simple

BOX 1

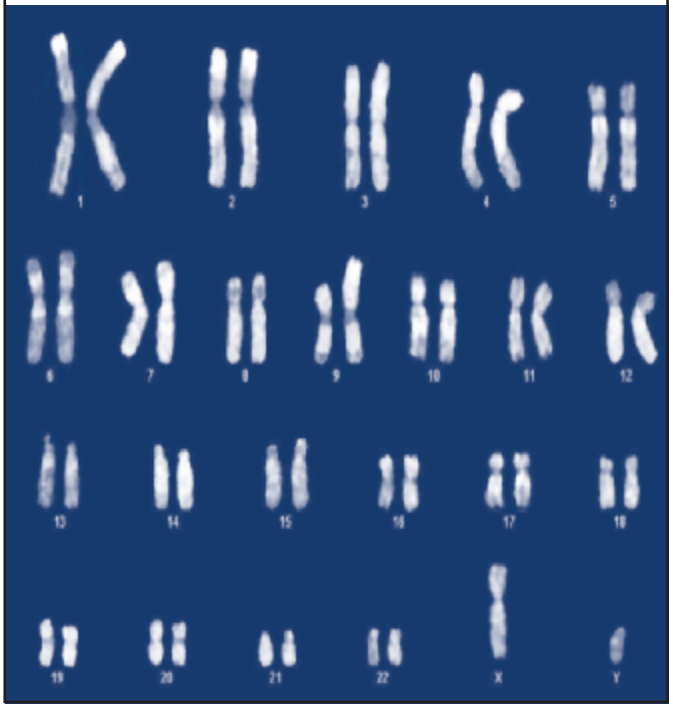
Chromosomes

Chromosomes are linear double stranded DNA molecules present in the nuclei of eukaryotic cells. Different organisms have different numbers of chromosomes. A normal human cell contains 46 chromosomes - two copies of each of chromosomes 1 to 22 - plus, in the case of a cell from a woman, two copies of the X chromosome or, in a cell from a man, one copy of the X chromosome and one copy of the Y chromosome.

The human chromosomes vary in length from 47 million to 246 million base pairs of deoxyribonucleic acid (DNA) sequence (see Box 2: DNA).

The DNA in the chromosomes is normally present in the nuclei in the form of chromatin – DNA complexed with proteins. Prior to cell division the entire sequence of the DNA in each chromosome is replicated, so that for each chromosome two identical sister chromatids are present. During mitosis (cell division) the chromatin structure of each chromosome becomes highly condensed forming discrete structures, visible by microscopy, which possess distinct characteristic morphologies, and structurally and functionally distinct regions.

FIGURE 1. Human chromosomes



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BOX 2

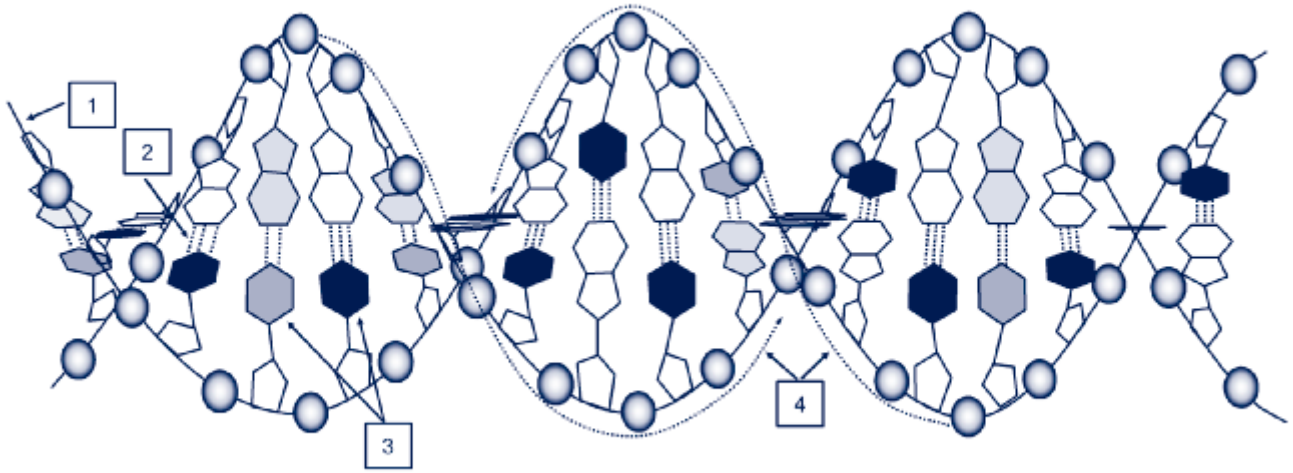
Deoxyribonucleic acid (DNA)

Deoxyribonucleic acid (DNA) is the repository of all genetic information in the cell. It is a long linear polymeric molecule made up of nucleotide building blocks. Each nucleotide comprises a deoxyribose (a sugar) and phosphate group and one of four different bases, adenine (A), guanine (G), cytosine (C) or thymine (T). The deoxyribose and phosphate groups form the backbone of the polymer. It is the sequence of the bases that carries the genetic code. In most cells DNA is present in a double-stranded form and the two strands are held together by hydrogen bonding between bases (base pairing) on opposite strands. The nucleotides always pair as C and G or A and T. The two strands are twisted round a common axis to form a double helix.

organisms developed into more complex forms of life they retained the ability to respond to nutrient or nutrient/hormonal signals that govern the expression of genes encoding the proteins of energy metabolism, cell differentiation and cell growth.

The central role that nutrients play in governing the cell content of different proteins has been further investigated and a recognition of their role as regulators of gene transcription, nuclear RNA processing, mRNA stability and mRNA degradation (*see Box 3: Ribonucleic Acid*) has emerged (*see Chapter 5*).

FIGURE 2. DNA double helix



1 - Deoxyribose and phosphate back bone; 2 - Hydrogen bonds; 3 - Nucleotide bases, adenine, guanine, cytosine or thymine; 4 - Double helix.

Source: Elliott, R.M., Bacon, J.R. and Bao,Y-P. (2004). Chapter 1 "Nutritional Genomics", pp 1-25, in *"Phytochemicals in Health and Disease"*, Editors Bao, Y-P. and Fenwick, R. with permission from Taylor and Francis Group.

BOX 3

Ribonucleic Acid (RNA)

RNA is a nucleic acid that is structurally similar to DNA, but it differs in three principal ways. Firstly, the sugar component of the nucleotide building blocks is a ribose rather than a deoxyribose. These molecules include two –OH groups, which make them more reactive, but less flexible. Secondly, the base uracil is used in place of thymine, so that the code consists of A,C,G and U rather than the A,C,G and T in DNA. Thirdly, unlike DNA, RNA is generally single stranded, but can form a duplex with a complementary strand of either DNA or RNA. In eukaryotic cells the major RNAs are involved in all stages of protein synthesis and many types of RNA are involved in regulatory, catalytic and other processes in the cell.

2.2 The Genome

The genome is essentially the genetic fingerprint of an organism.

The genome is the entire DNA sequence of an organism. It is the palette of information that an organism can call on to ensure its own survival and growth, and that it can pass on to its own progeny.

The Human Genome Project is the largest ever international collaboration in biology. The result has been that the sequence of three billion chemical coding units in human DNA is now known. The next challenge is to identify each of the sequences of codes that are responsible for a specific activity or outcome.

Although the just fewer than three billion base pairs have been sequenced, most genes have not yet been definitively identified. It has been estimated that in human cells some 30-40,000 genes exist, although more recent estimates suggest 24,500 – and even this may be an over-estimate. This number is surprisingly small, as a simple worm is considered to have 17,000 genes.

There are sequence similarities between many genes of related functions in different species, and these often appear in a similar order along chromosomes. For example, organisational similarities are apparent between the human and mice genomes. The sequence of the human chromosome 22 has been compared to that of the mouse chromosome. It was found that over 80% of the human sequence that contains genes includes regions that have direct counterparts on the mouse chromosome.

Genomic similarities also exist between plant species. When wheat and rice were compared, many of the genes and their order on the chromosomes were identical, although the wheat genome is 40 times greater than that of rice.

The genomes of many organisms contain more than just sets of genes. Within the entire genomic sequence there can be stretches of DNA that are not known to code for anything. Such non-coding DNA appears to account for 90-95% of human DNA. DNA which has no known function in the cell or that does not appear to code for proteins is known as “junk DNA”, but this designation may become inappropriate as future research may identify its role.

Genomes are not completely static. Genes may mutate during the reproductive process and genes from parents are shuffled and produce a new combination in their offspring. These new and different sequences and combinations may confer specific advantages, for example, encourage taller offspring which may be an advantage in crops or animals. For thousands of years farmers and latterly plant breeders have been identifying

BOX 4

Gene

A gene is defined as the smallest indivisible unit of heredity.

The totality of an organism's genes provides the instruction book for all inheritable characteristics and directs the production of specific proteins. In molecular terms, a gene consists of a sequence of DNA that carries all the genetic information necessary to produce the specific product (contained within the coding region) for that gene and to do so in an appropriately regulated manner (controlled by the non-coding region of the gene). The non-coding regions, called promoter regions, can respond to factors such as diet and determine how much RNA is made from that gene.

The number and order of the nucleotides in the coding sequence determine the individuality and the functionality of the gene and also the identity of the product it encodes, either a functional polypeptide chain or RNA molecule.

In some cases a gene may share a similar or identical region of sequence(s) with another gene. If the similar sequences lie in the non-coding sequence, this may indicate similarities in the way these genes are regulated or the product processed. If the similar sequences are within coding regions, this may indicate that the gene products share some common function such as a common enzymic activity or the ability to bind to DNA.

Genetic Code

The "code" in which information for the synthesis of proteins is contained lies in the nucleotide sequence of the coding region of a gene. Prior to protein synthesis this coded information is first reproduced by the process of gene transcription in messenger RNA, also called mRNA. The code carried by the mRNA is then translated into one or more polypeptides. Each amino acid of a polypeptide is encoded by a particular sequence of three nucleotides (called a codon) in the mRNA. The polypeptide synthesis begins at an initiator codon in the mRNA and the translating machinery (ribosome) reads the information in adjacent non-overlapping triplets moving along the mRNA from this point. The codon does not interact directly with the corresponding amino acid, as the amino acid to be added to the growing polypeptide must be linked through an adapter molecule transfer RNA, also called tRNA. A number of tRNAs are produced that are specific for each type of amino acid. Each of these contains a triplet sequence (anti-codon) complementary to one of the possible codons for that amino acid.

The four bases A,C,G,U can generate 64 possible triplet combinations; 61 of these encode 20 amino acids (which means in essence most amino acids are encoded by two or more triplets). For example, the amino acid phenylalanine is coded by UUU and UUC and valine by GUU, GUC, GUA and GUG. The remaining three codons UAA, UAG and UGA are nonsense, "stop" or "termination" codons, which signify the end of the polypeptide chain.

and cultivating specific crops that have a competitive advantage through their changed genome.

Not all changes in the genome are beneficial; some changes will result in immediate death, or others engender poor growth and premature death of the plant, animal or organism.

As well as the natural processes by which spontaneous genetic differences arise, a range of technologies are available that permit specific targeted changes to be made in genomes.

Genetic modification (*see Box 5: Genetic Modification*) is one technique that can be used to remove, modify or add

BOX 5

Genetic Modification

Genetic modification (also called genetic engineering or gene technology) is used to describe the process by which the genetic make-up of an organism can be altered by removing, modifying or adding gene(s) to a living organism to create a new combination of DNA codes. This may be achieved by introducing genetic material from a different species, by adding additional copies of a gene or genes originating from the same organism (sometimes engineered so that they can be regulated by different processes compared with the original genes), or by deleting copies of genes. GM technologies have proven to be extremely powerful tools for fundamental research in addition to their use in crop production. Current genetically modified crops include soya, maize and tomatoes.

Not all genetic modification technology involves inserting DNA from other organisms. Plants and microorganisms may be modified by removing or "switching-off" particular genes. Genes can be "switched off" (alternately known as gene silencing) by inserting an inactive or partial copy of a gene already present or by inserting an "antisense" copy of the gene. Research is underway to use this methodology to switch off genes that encode allergenic (allergy-causing) proteins in foods.

gene(s) to a living organism to create a new combination of DNA codes.

There are two main ways in which the genetic makeup can be modified. Directly, by altering the expression of existing genes. For example, it is possible to prevent, or *knockout*, the normal expression of some existing genes. This allows investigations of the function of particular genes. For instance, knockout mice are being widely used in research on cystic fibrosis, breast cancer, colon and other cancers in humans. Secondly, by adding new (*foreign*) sequences of DNA. Physically inserting the DNA coding for a gene with a desired effect into the DNA of another animal, is termed *gene transfer* and the animals receiving the foreign DNA are called *transgenic* animals. DNA is chemically identical across species, and the genetic codes for producing particular proteins are the same across species. This means that it is possible to transfer genes not only within species, but also between species, and sometimes even between different classes of organism. For instance, bacterial and viral DNA has been introduced to a range of food crops to confer insect and virus resistance.

Looked at another way, modification is the specific effect of man's intervention on gene structure, where genomics studies the expression of the genes and the sequencing of the whole genome.

Once the genome sequence has been modified, the resulting new combination of DNA replicates itself in the same natural way that all organisms cut and copy their DNA and therefore becomes an integral part of the building blocks of the organism.

The study of genomics is acquiring knowledge not only of the gene sequences, but also determining what genes do. Genetic modification relates more specifically to the alteration of gene(s) and the impact of that alteration.

The definition that is used throughout this document is that genomics refers to "the holistic study of biomolecules" and comprises the study of all nucleotide sequences including structural genes, regulatory sequences and non-coding DNA sequences of the chromosome.

2.3 DNA replication

DNA replication is the first step in cell division. The process of DNA replication starts with a new strand of DNA being synthesised on each of the pre-existing strands. Each of these pre-existing strands acts as a template. Each new strand is complementary to, not a copy of, the original strand.

Initiation of the DNA replication begins at one or more special sites known as an ori (origins). Initiation of replication involves the recognition of an ori site by various initiation factors and enzymes. As no known DNA polymerase can initiate synthesis of a new DNA strand there is a requirement for a starter series of nucleotides known as a primer.

DNA synthesis is usually primed by a short strand of RNA, which is transcribed on to the DNA template. During this process, ribonucleotides in the RNA strand base pair with nucleotides in a template strand and inorganic phosphate is released.

Termination of DNA synthesis occurs when the entire duplex is replicated. Replication is a complex and carefully regulated process involving a number of different proteins; incorrect replication can lead to e.g. cell death or cancer.

2.4 Transcription and RNA

Transcription conveys the message from DNA ("the library") to the cell factory making proteins.

Transcription is the process by which a RNA strand is formed from a DNA template. Where messenger RNA (mRNA) is the RNA that is produced, this subsequently acts as the template on which amino acids are assembled for the purpose of protein synthesis.

Transcription can be regulated by dietary components and their metabolites that can influence the cell environment. The location of these products within the cell, any of the intermediates of their synthesis and their subsequent rate of degradation can also influence the transcription process.

Initiation and termination of transcription are important control points for the regulation of gene expression.

With any one gene, normally only one of the two strands of DNA acts as a template. The transcriptome contains the totality of RNA species produced from the genome of an organism. The transcriptome can be separated into several different types of RNA including mRNA, as described above.

The role of tRNA during protein synthesis is to act as the adapter molecule matching amino acids to their codons on the mRNAs (*see Box 4: Gene*).

Ribosomal RNAs (rRNA) combine with ribosomal proteins to form ribosomes, the cellular machines that read the code carried by mRNAs and work with tRNAs to produce proteins from this code.

In addition to these three main categories of RNA, a number of, generally small, RNA molecules have been recognized, which possess novel regulatory or enzymatic activities.

2.5 Translation and protein

While the DNA may be the information archive of the cell, it is the proteins that do the work of the cell and ultimately dictate biological processes and cellular fates.

Protein regulation is a factor of major importance in translating the genome into function. Rates of protein synthesis, localisation of proteins within and outside the cell, degree and positions of phosphorylation and/or glycosylation, rates of degradation and many other processes determine the activity of different proteins.

Generally the proteins in the cell do not exist in isolation, but form a cellular protein network that consists of protein interactions and pathways that connect in finely tuned orchestration. Proteins coalesce into networks and circuits in response to specific stimuli.

As stimuli fluctuate and feedback loops return information, newly formed protein networks rapidly break apart. Consequently the population state of protein networks is constantly changing within each living cell.

The amino acid sequence of a protein is the primary determinant of its (3-D) shape. It is this shape and the surface presentation of amino acids that enable highly selective lock and key recognition between protein partners and metabolites in the communication circuit, as well as substrate specificity and enzyme activity.

Examples of post-translational modifications that alter 3-D protein structure are phosphorylation, cleavage, glycosylation and lipidation.

As multiple stimuli impinge on the living cell, hundreds of protein-signal networks are constantly changing. However, it is possible to map the state of key nodes in known protein networks and relate this back to cell function. Investigators measure the ratio between activated (e.g. phosphorylated or cleaved) and the inactivated form of the key signal proteins and from this can be estimated the status of a signal node at the time the proteins are extracted from the cell.

For example, a fast growing tumour cell may have a higher proportion of activated signal proteins within pathways that stimulate cell growth or suppress cell death.

In a diseased cell the protein network is disrupted, deranged or locally hyperactive compared with that in a healthy cell.

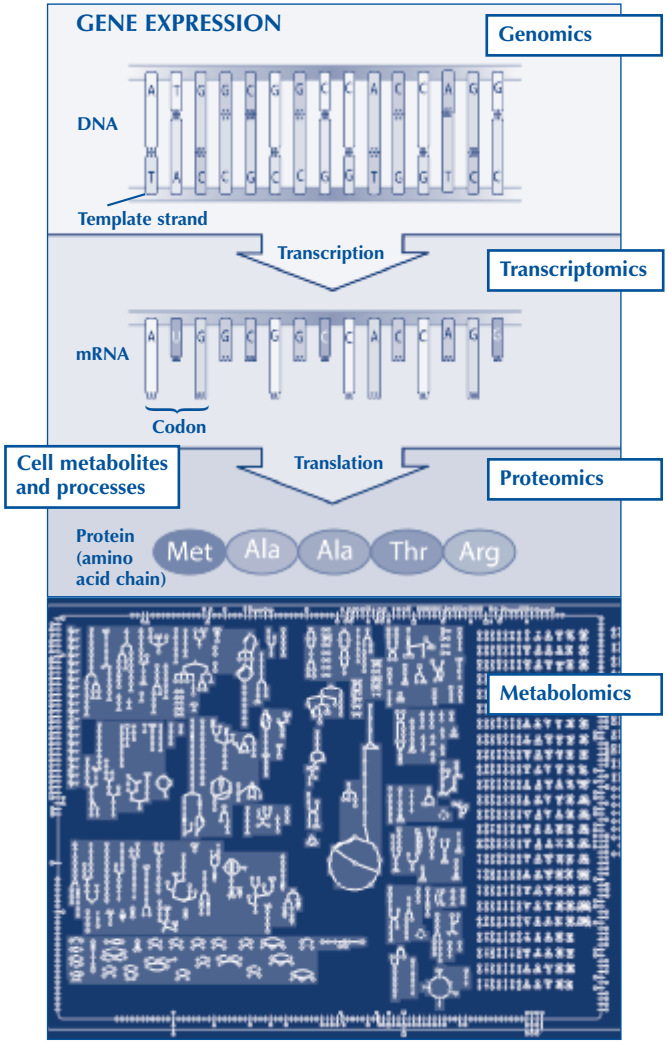
2.6 Metabolic processes in the cell

Processes and metabolic regulation in individual cells or tissues give rise to a complete set of metabolites in the cell. These are generally low molecular weight molecules and include the intermediates of metabolism in the cell (the totality of all such metabolites is termed the metabolome).

Metabolomics investigates metabolic regulation and fluxes in individual cells or tissues. The metabolites derive from a broad range of functions in the cell and are the final stage of biological activity along the line from gene to mRNA, to protein, to function, to phenotype. The metabolites are usually rapidly converted in enzyme-controlled or chemical reactions and provide the building blocks for larger molecules or transient energy storage.

The identification and quantification of the metabolites and the reactions they are involved in are important in the context of systems biology. Metabolic profiles can be derived from tissue, cellular and extra-cellular fluid samples, and because of the literally thousands of compounds involved, pose the greatest analytical challenge to the investigator. One of the strategies adopted is the sub-division of the metabolome into classes of compounds with similar chemical properties, while undertaking parallel analysis to help to visualise a greater portion of the metabolome.

FIGURE 3. The steps in gene expression and the 'omics' descriptor



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3. THE NEW TECHNOLOGIES

The sequencing of the human and other genomes has led to the development of a whole new scientific methodology. These new areas of scientific study usually include the 'omics' suffix (*see Figure 3*).

The characterisation of one gene can immediately provide an insight into the gene function in a related species. The deposition of the draft sequence of the mouse genome in 2002 has been particularly useful in this respect. Sequences from this and other organisms can be compared in order to find commonality; other tools for developing an understanding of gene function are under development.

Once it became apparent that there was a large number of genes with unknown functions, the development of large scale, high throughput technologies that could assign functions to genes was necessary.

Definitions for the technologies adopted are:

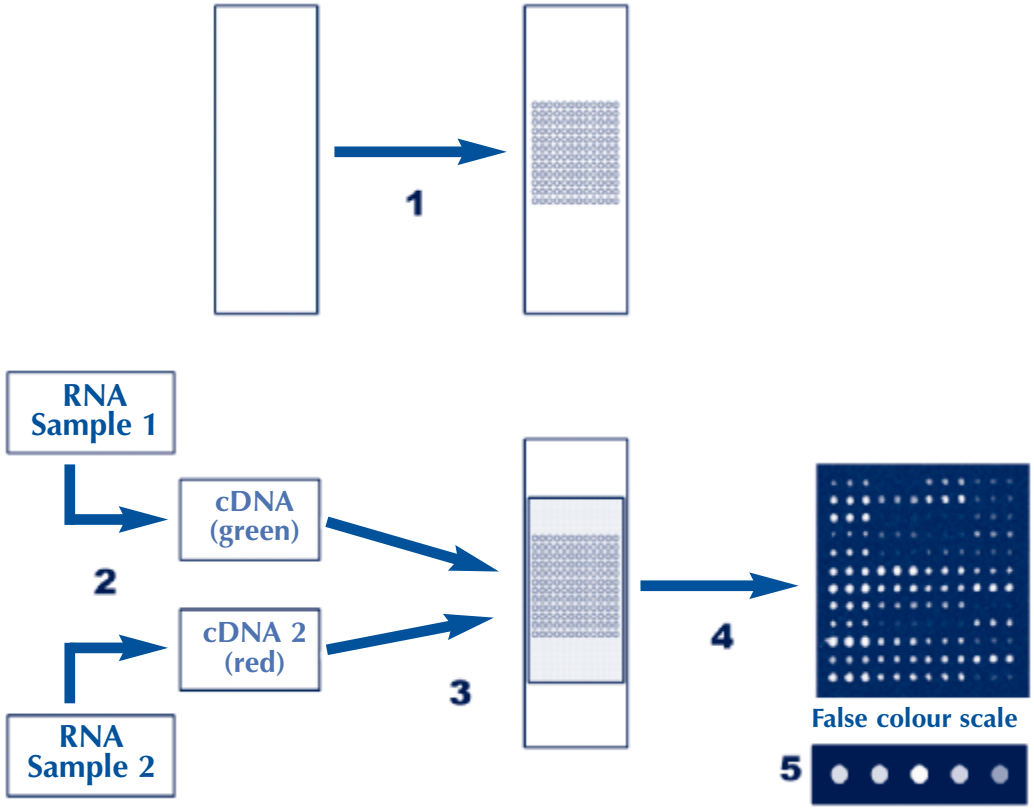
- Transcriptomics is the study of activity of all the genes in response to changing conditions and, in essence, is a study of gene expression at the level of the mRNA.

  DNA arrays are the most widely used tool for measuring the relative amounts of the thousands of RNA species within cellular or tissue samples (*see DNA Microarray: Box 6 and Figure 4*).

- Proteomics is the study of the totality of the proteins that can be expressed within an organism.

Currently, the most widely used technologies for proteomics are two-dimensional gel electrophoresis (2D gel electrophoresis) to separate the proteins in a complex mixture isolated from cells or tissues, and specialised mass spectrometry techniques as protein identification tools (*See Figure 5*).

FIGURE 4. DNA chip or Microarray (A small device for detecting the presence or activity of many genes simultaneously)



Microarray analysis. DNA or oligonucleotides are printed onto specially coated slides (1) and covalently bound. Two RNA samples are reversed transcribed (2) and either green or red fluorescent dyes incorporated into the cDNA products. The two fluorescent cDNA populations are combined and hybridized to the array under a cover slip (3). The slide is washed and fluorescence imaged using a microarray laser scanner (4). The fluorescent signals from the two dyes are presented together with a false colour scheme (5) in which cDNA present in only one of the two samples appear red or green and those present in both appear in varying shades of orange and yellow.

Source: Elliott, R.M., Bacon, J.R. and Bao, Y-P. (2004). Chapter 1 *"Nutritional Genomics"*, pp 1-25, in "Phytochemicals in Health and Disease", Editors Bao, Y-P. and Fenwick, R. with permission from Taylor and Francis Group.

BOX 6

DNA Microarrays

DNA microarrays or gene chips allow the activity of a large number of genes at the level of the mRNA to be measured simultaneously.

DNA chips contain multiple short DNA sequences, each unique to one gene, synthesised directly on silicon chips enabling the expression analysis of thousands of genes within a single experiment. The production of these slides is an industrial process, which provides good reproducibility.

The material investigated is the mixed RNA population or can be isolated RNA extracted from the cells or tissue of interest. Labelled copies of these RNA molecules are produced enzymatically *in vitro*. These bind to their corresponding DNA sequence on the DNA chip where they are detected using fluorescent reagents. This type of experiment provides a "snapshot" of gene activity.

For other microarray formats, scientists generally produce their own DNA arrays derived from known or unknown DNA clones. A commonly used method for copying specific sequences of DNA nucleotides is polymerase chain reaction (PCR). Typically PCR products or synthetic oligonucleotides for thousands of genes are deposited on one microarray. RNA is isolated from two samples – a control and treated. DNA reverse copies of these RNA samples are produced (each of the two carrying a different fluorescent label) and both are hybridised to the same microarray. Fluorescent densities of both markers are then measured for each separate spot resulting in thousands of data points. Analysis of the large data set results in an overview of the activities of most metabolic pathways and biological processes.

The microarray allows researchers to determine which genes are active at that specific time and under specific circumstances. Microarray techniques have been very useful in understanding how bacteria work. For example, DNA corresponding to all of the few thousand genes that a bacterial genome contains can be placed on a single microarray to demonstrate which genes are active during infection. The information obtained can be used to design new antibiotics or treatments to combat the infection.

New techniques under development include protein arrays, "two-dimensional" column chromatography and one-dimensional protein separation technologies.

- Metabolomics is the study of the complete set of metabolites that an organism produces. It investigates metabolic regulation and fluxes in individual cells or tissues, in response to specific environmental changes.

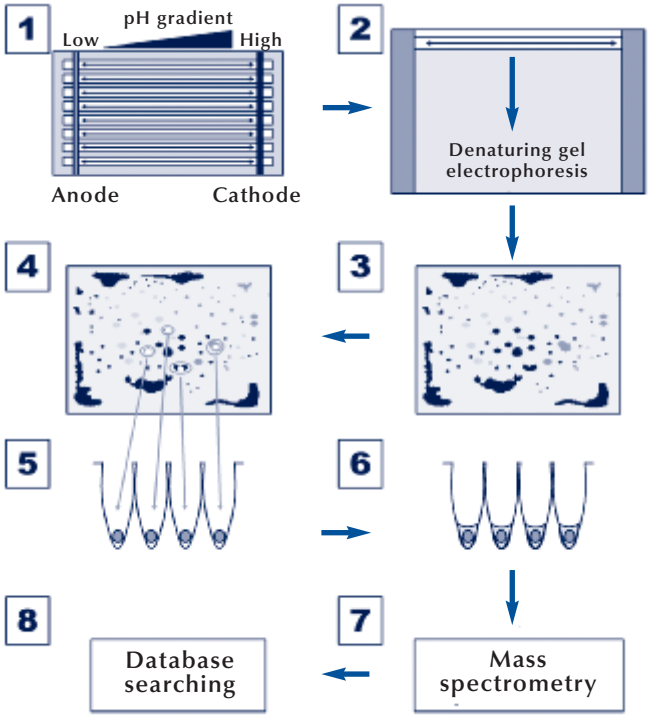
In common with transcriptomics and proteomics it involves the non-targeted determination of all metabolites present under specific environmental conditions. The analysis and interpretation of the data that is derived from the comparison of different cell conditions is achieved by the use of bioinformatics (*see Box 7: Bioinformatics*).

Some researchers use the term metabolomics to refer to both simple (cellular) and complex (whole tissue or organism) systems, others distinguish between metabolomics studies that are in simple systems only and metabonomics in complex systems. In metabonomics, systematic biochemical profiles and regulation of function are determined in whole organisms by analysing biofluids and tissues.

A metabolomics experiment provides quantitative information on which pathways are being used by an organism, and whether they are operational in a specific compartment.

As the turnover of many metabolites is very fast with half-lives of less than a second, it is important that the metabolism in the cell is stopped instantaneously at the moment of sampling. Regulation of transcription, translation and enzyme activities are only directly affected by the metabolite in its immediate environment. Therefore, to obtain an accurate picture,

FIGURE 5. Proteomics Process



Proteomics process. 2D gel electrophoresis first separates proteins along a strip gel and a second gel separates proteins by size. The aim is to produce a protein map for a particular cell or tissue. Protein extracts are subjected to isoelectric focusing on strip gel (1). Each strip gel is then placed onto a slab gel and the samples subjected to denaturing gel electrophoresis at right angles (second dimension) to the direction of isoelectric focusing (2). The protein is visualised using a suitable staining method (3). Individual protein spots of interest are identified, excised from the gel (4) and transferred into a tube (5) for treatment with proteolytic enzymes (6). The peptide fragments produced are analysed by mass spectrometry to determine their amino acid content (and if necessary sequence) (7). This information is used to search protein databases (8) to identify each excised protein.

Source: Elliott, R.M., Bacon, J.R. and Bao,Y-P. (2004). Chapter 1 *"Nutritional Genomics"*, pp 1-25, in "Phytochemicals in Health and Disease", Editors Bao, Y-P. and Fenwick, R. with permission from Taylor and Francis Group.

metabolites need to be determined separately in the different compartments of the cell, for example, in cytoplasm, mitochondria, extra-cellular matrix, cell membrane etc.

Ideally, the metabolome of a cell is determined by non-invasive techniques such as NMR or IR. Although current techniques are not very sensitive and do not always separate individual metabolites, there will no doubt be future advances in the technology. In the meantime, conventional analytical methods like HPLC, GC and gel electrophoresis using a combination of different columns and detectors are used for the analysis of the metabolome.

At the present time, there is only a limited number of researchers with the facilities required to do such specific studies, so until now there are few reported examples of metabolomics in human subjects. Most examples have involved the metabolic profiling of individuals, where large-scale analyses of body fluids have been used to diagnose for metabolic disorders or exposure to xenobiotics.

With the development of computer algorithms it is also possible to correlate other physiological parameters with the physiological status of the organism. In this way, metabolomics is being expanded and can incorporate physiological parameters such as pH, oxido-reduction potential and growth characteristics in the computer analysis. This approach may then be used to understand the secondary effects of changes in metabolism, for example to identify secondary metabolites that may induce food spoilage, pathogenic bacteria, or enzyme induction that may have adverse effects.

BOX 7

Bioinformatics

Bioinformatics is the science that handles the huge demand for the analysis and interpretation of biological data and is essential for the management of data in modern biology and medicine.

It is specifically defined as the application of the tools of computation and analysis to the capture and interpretation of biological data. The bioinformatics toolbox includes computer software programs and the internet. The ever-increasing amount of data from the human genome project necessitated the development of computer databases that can assimilate large amounts of data quickly, and transform them into formats that can be interrogated by non-specialists. A key requirement is for sequence analysis of DNA and proteins. Two significant websites that provide freely available access are detailed below:

The National Centre for Biotechnology (www.ncbi.nlm.nih.gov) provides BLAST (basic local alignment search tool), which is an integrated database retrieval system that is capable of searching databases for genes with similar nucleotide structure and hence allows comparison of an unknown DNA or amino acid sequence with thousands that are already logged within the database. The resulting search is sorted on the basis of maximum similarity.

The European Bioinformatics Institute archives all gene and protein genome study data from all studies on all organisms. As part of a joint venture with the Sanger Centre they provide free access to the database Ensembl (www.ensembl.org). This database produces and maintains automatic annotation of the human and other genomes and can assemble and analyse genes and other features of interest to medical or nutritional researchers.

It is considered that bioinformatics will make a major contribution in identifying susceptibility genes and illuminate the pathways of the pathogenesis involved in illness. These susceptibility genes may be influenced by environmental or nutritional factors that will provide an opportunity for targeted therapy. Potential targets in cancers were recently developed from gene expression profiles. An example of a therapeutic advance was the development of the novel designer drug – imatinib mesylate (Gleevec), which interferes with an abnormal protein made in chronic myeloid leukaemia. The ability to identify and target specific genetic markers by using bioinformatic tools facilitated the development of this drug.

Already the study of genetic disorders is shifting from investigation of single genes in isolation to the understanding of cellular networks of genes and their complex interactions. Bioinformatic tools will help molecular scientists and clinical researchers integrate their skills to capitalise on the huge biological databases now available.

This range of technologies is just beginning to be used in nutrition science, but their potential is demonstrated by the rapid adoption of the technologies by pharmaceutical and clinical research.

The final 'omics to mention at this stage is nutritional genomics or nutrigenomics.

- Nutrigenomics is the all-encompassing study of the genome-wide influences of nutrition.

This area of study is likely to be extensive, as the nutritional environment of the cell is constantly changing thereby posing an additional challenge for researchers. It is likely that this area of research will identify the key to understanding such crucial questions as inter-individual variations in food intake and response to nutrients, providing the information "to map" individual health.

4. VARIATION IN HUMAN POPULATIONS

4.1 Introduction

Since the time of Gregor Mendel, an understanding of patterns of inheritance has been established. The variation between individuals within a population can be related to the package of genes they have inherited from their parents. For example, the probability of having blue eyes or blond hair can be related to our parents' genes, just like other obvious physical traits such as skin colour, stature and hair type can be linked to heritage.

In some cases, a trait will only be present in an individual if the specified gene or genes is present in the form of identical alleles (*see Box 8: Allele*); such a condition is known as homozygous.

In the 19th century, Charles Darwin introduced the concept of the survival of the fittest, indicating that some alleles may provide a selective advantage compared to others. We are all too familiar with the disappearance of the dinosaurs and the many other animal and plant species unable to compete in the changing world they found themselves in.

Genetic polymorphism (*see Box 9: Single Nucleotide Polymorphisms*) is the basis of this variation from individual to individual. DNA polymorphism is defined as a difference from the generally accepted gene sequence, and occurs in at least 1% of the general population.

Genetic variance can readily be identified among human populations. An example of this is the survival of a few individuals in isolated tribes where most died following exposure to the colds, flu and measles viruses brought by pioneers and travellers from the West.

Differences in genetic susceptibility exist between certain individuals or populations with regard to the major causes of ill health - diabetes, coronary heart disease and cancers. For example, it has been established that there is a significantly higher incidence of Type II diabetes among Pima Indians. The offspring of Pima Indians, particularly

BOX 8

Allele

An allele is two or more alternative forms of a given gene. Alleles are concerned with the same trait or characteristic, but the product or function coded by a particular allele differs from that coded for by other alleles of that gene. When the members of an allelic pair occupy corresponding positions (loci) on a pair of homologous chromosomes and the alleles are genetically identical, it is said to be homozygous. If the alleles are genetically different, the organism is heterozygous with respect to that particular gene.

BOX 9

Single Nucleotide Polymorphisms (SNPs)

Single Nucleotide Polymorphisms (SNPs, pronounced 'snips') are the commonest form of genetic variability and relate to a single nucleotide substitution in a DNA sequence, for example, ACGT could be replaced by AGGT. SNPs occur roughly every 1000-2000 nucleotides in the human genome, and to date 22,000 have been sequenced and their relationship to the gene determined.

those that are obese have a 50%, or greater, chance of also becoming diabetic, and worryingly are doing so at a younger age than their parents.

There is evidence that obesity is influenced by genetic factors. The obesity gene map includes over 300 genes, markers and chromosomal regions that have been associated or linked to human obesity.

There are also well-established genetic links to conditions such as haemophilia, sickle cell anaemia and familial hypercholesterolemia where one or more SNPs have been identified.

Other aspects of metabolism may be influenced by genetic factors. An example is the control of energy expenditure in infants and the extent of suppression of inflammatory response following fish oil supplementation are both dependent on a SNP that affects genotype for pro-inflammatory cytokine TNF-α.

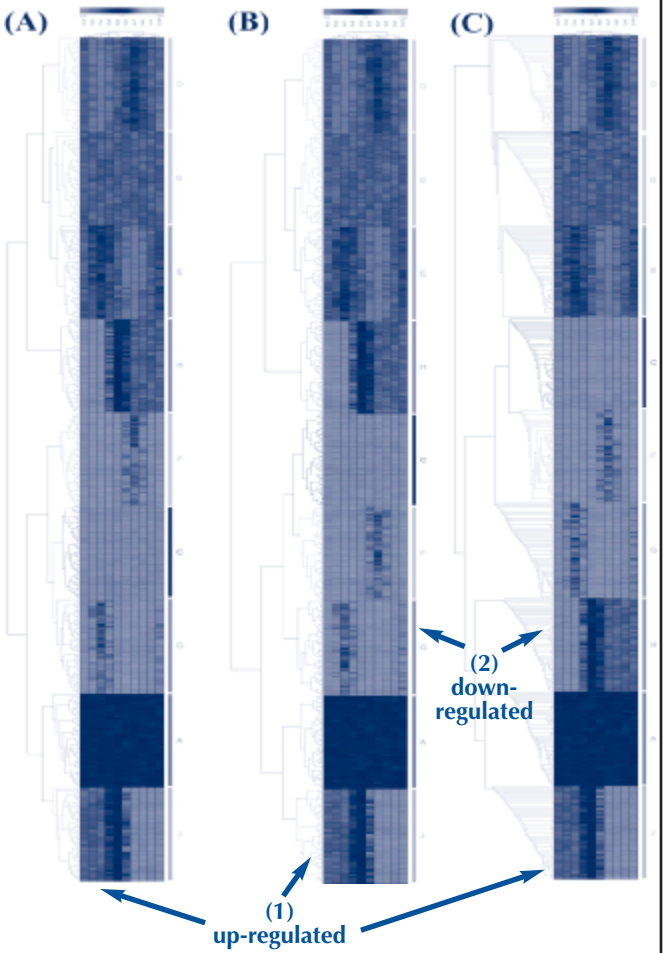
Recently, several extensive genetic polymorphism databases have been developed that allow high throughput genetic screening. These not only enable the study of inter-individual genetic screenings, but can also help to identify future areas for closer scrutiny in nutrition and clinical research.

4.2 Finding genes associated with disease

Some of the initial research carried out has been in the search for genes associated with complex diseases. This commences by finding the chromosomal location of the genes for disease susceptibility using linkage analysis. The principle of this approach is shown in Figure 6.

Initially families in which sibling pairs are affected with the disorder are typed with DNA polymorphisms (common variations in the DNA sequence), in order to

FIGURE 6. Linkage analysis



Linkage analysis. Genes in the demonstration data set were subjected to hierarchical clustering:
(A) average linkage
(B) complete linkage
(C) single linkage
Genes that are up-regulated appear mid-blue (1). Those in light blue colour are down-regulated (2). This method of clustering groups/genes by reordering the expression matrix allows patterns to be easily visualised.

Source: Reproduced with permission from Nature Publishing Group. © Quakenbush, 2001 *Nature* June 2001, vol 2, p424.

identify a polymorphism that is co-inherited with the disease. If a substantial number of the alleles of the polymorphism are shared in the affected sibling pairs then the polymorphism is probably linked (closely) to a gene that engenders susceptibility to that disease. To find polymorphisms requires 200-300 sibling pairs in which 300-400 polymorphisms that are evenly spaced along the human genome are evaluated. This process is known as a genome scan.

This approach has been used to map susceptibility genes for a number of chronic diseases. However, there is some degree of inconsistency in the results, with linkages being reported by one research group not being replicated by another group. This may be due to lack of statistical power in the studies undertaken or a false positive in the original data set. It may also be a consequence of the gene/environment interaction, which may alter susceptibility in one population and not another. There may also be different susceptibility genes in different populations. Until a study has been replicated by at least one other independent large-scale population study, a degree of caution is required.

Once a linkage has been confirmed, the search for the critical gene in a region of 20-30 million base pairs can begin – a little like looking for a needle in a haystack.

5. RELATIONSHIP BETWEEN NUTRITION, GENES AND HEALTH

5.1 Introduction

The importance of the role that nutrients play in modulating the expression of genes encoding the proteins of energy metabolism, cell differentiation and cell growth has already been described above.

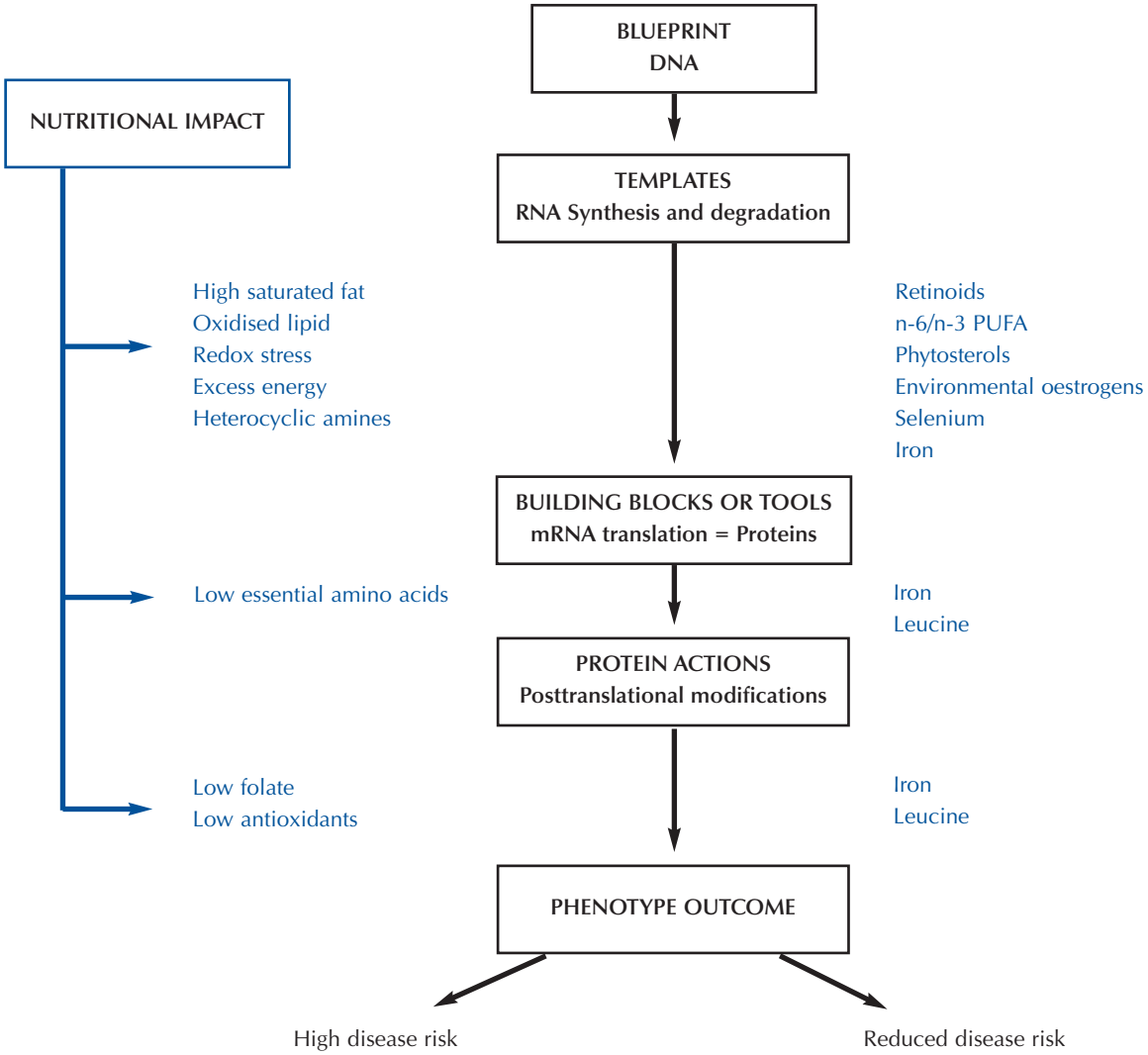
Dietary-derived regulators of gene expression may be nutritive (e.g. fatty acids, iron or selenium) and non-nutritive (e.g. phytochemicals) components of food, metabolites of food components (e.g. eicosanoids, retinoic acid) result from the cooking process (e.g. heterocyclic amines in cooked meats), or end products of intestinal bacterial metabolism (e.g. short-chain fatty acids).

The simplest interpretation of nutrient control of gene expression is that the reading of the genomic blueprint and its translation into functional proteins can be modulated by a single food component.

In most cases, relationships are more complex and often involve diet-diet (e.g. fatty acid and retinoids) or diet-hormone (e.g. fatty acids and thyroid hormone) interactions.

Regardless of the type of diet-gene interaction, nutritive and non-nutritive components of food influence the abundance and function of cellular proteins by governing gene expression at a variety of levels (*see Table 1 and Figure 7*).

FIGURE 7. Pathway of protein expression showing where regulation occurs by nutrients



Shown in blue are where nutrients can impact

Source: Clarke, S.D. (2001). The Human Genome and Nutrition. In Bowman, B.A. and Russell, R.M. ed. Present Knowledge in Nutrition, 8th ed., ILSI Press, Washington, DC. Reproduced with permission from ILSI Press.

TABLE 1

Points in the pathway of protein expression regulated by dietary constituents

Targeted Site	*Examples of Nutrient Regulator*
Gene transcription	Fatty acids, glucose, cholesterol, retinoids, vitamin D
mRNA stability	Fatty acid, glucose, selenium, iron
mRNA processing	Polyunsaturated fatty acids, glucose
mRNA translation	Iron, amino acids
Post-translational modification	Vitamins and minerals

5.2 Diet and gene transcription

One key determinant of protein abundance is the rate at which the mRNA template is synthesised. This rate is determined by the binding of transcription factors to specific DNA recognition sequences generally located within a specific region known as the 5′-flanking region of the gene.

An example of nutrient regulation of the abundance of a transcription factor is the cholesterol regulation of sterol regulatory element-binding protein-2 (SREBP-2) and the polyunsaturated fatty acid (PUFA) regulation of SREBP-1.

Precursor-SREBP molecules are located in the membrane of the endoplasmic reticulum. The active form of the molecule is released by a two-stage cleavage. The release of the mature SREBP is highly dependent on cholesterol concentration and possibly the fatty acid composition of the endoplasmic reticulum.

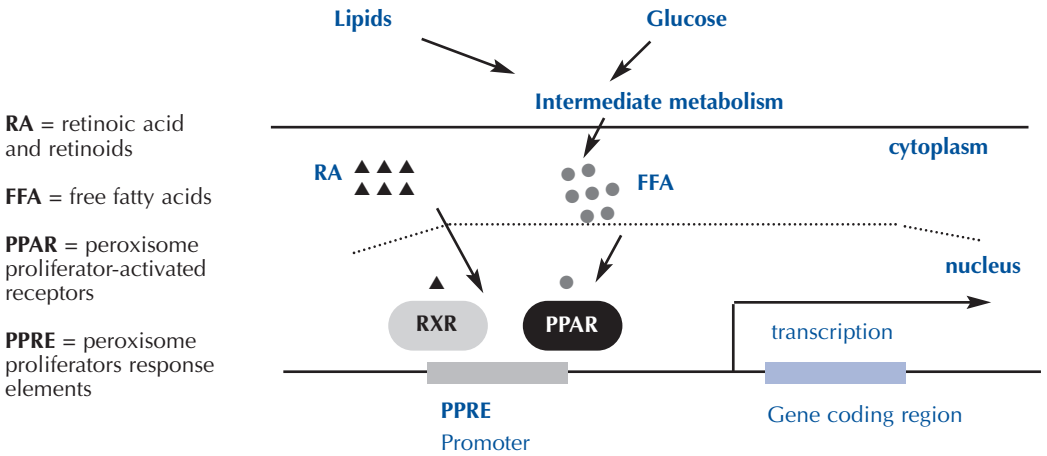
When the cholesterol or PUFA content of the endoplasmic reticulum is high the release of SREBP is slow, but when the endoplasmic reticulum is depleted of cholesterol or PUFA, release of mature SREBP increases.

Changes in the release of SREBP are paralleled by comparable changes in the transcription of cholesterogenic and lipogenic genes.

Dietary constituents also exert a strong influence on the affinity that a transcription factor has for its DNA recognition sequence. Lipophilic factors and their metabolites frequently modulate transcription factor DNA-binding activity. A broad family of steroid-like receptors that include retinoid receptors, vitamin D3 receptor, and peroxisome proliferator-activated receptors (PPARs) has attracted attention due to their role as regulators of genes involved in cell differentiation, lipid and energy metabolism, inflammatory response, atherosclerotic plaque formation and cancer. Activation factors for PPAR include n-3 and n-6 fatty acids, conjugated linoleic acids, prostaglandins, leukotrienes and oxidised fatty acids. A generalised scheme for the PPAR signalling pathway is given in Figure 8.

Protein phosphorylation and dephosphorylation, regulated by the activity of specific protein kinases and phosphatases, also modulate the DNA activity of many transcription factors. In addition to functioning directly on kinases and phosphatases, dietary factors may influence DNA-binding activity by affecting the redox state of the cell. Anti-oxidants such as vitamin E may protect the cell against oxidative stress and prevent the initiation of the kinase stress pathway. Alternatively, antioxidants such as glutathione may increase the DNA-binding activity of transcription factors by protecting their oxidative status.

FIGURE 8. A generalised scheme for the PPAR signalling pathway



A general scheme of PPAR signaling pathway. Agonists for the system include free fatty acids, deriving from glucose or lipid intermediary metabolism, and retinoic acid and retinoids, binding to PPAR and RXR, respectively. The heterodimeric complex bound to the respective agonists is thus able to bind PPRE in the promoter region of responsive genes, thus driving transcription. See text for further details.

Source: De Caterina, R.D., Madonna, R., Hassan, J. and Propcopio, A.D., (2001). Nutrients and Gene Expression, in Nutrition and Fitness: Diet, Genes, Physical Activity and Health. ed Simopoulos, A. P. and Pavlou, K.N. *World Rev Nutr Diet.* **89**,23-52. Reproduced with permission from S. Karger AG.

Some nutrients appear to regulate the movement of mRNA into the cytosol. Glucose and PUFA are at least two of the nutrients found to modulate the mechanisms of mRNA processing.

5.3 Diet and mRNA stability

The cellular content of mRNA transcripts depends on cytosolic signals that determine the stability of a given mRNA.

Dietary examples of this regulation include the stabilisation of fatty acid synthetase mRNA by glucose, glutathione peroxidase by selenium and destabilisation of the transferrin receptor mRNA by iron. For example, when cellular levels of iron are low, the iron regulatory proteins bind iron and increase transcript stability. Conversely, the binding of iron accelerates degradation of the transferrin receptor mRNA.

5.4 Diet and mRNA translation

Synthesis of a protein from the mRNA template requires binding of ribosomes and subsequent reading of the message. Some dietary factors affect this process by blocking the ribosome binding which will alter the affinity for the initiation site or the rate of peptide elongation. Amino acid scarcity is one of the key factors that may slow or terminate peptide elongation.

As detailed above, iron status can also inhibit the translation of the ferritin transcript.

Research defining the full scope of the role that diet can play in mRNA translation is in its infancy and attention to date has focused on individual amino acids. However, as the translation of the transcript relies on a wide array of proteins, including enzymes such as kinases and phosphatases, and ribosomal proteins, a wider dietary role is implicated.

5.5 Diet and post-translational modification of proteins

Once translated, many proteins undergo further modification. The array of post-translational modifications includes proteolytic cleavage, phosphorylation-dephosphorylation, acetylation, acylation, methylation and glycosylation. Each of these processes has the potential to be regulated by dietary constituents, and any defects in the post-translational mechanism may result in major changes in cellular functioning or metabolism.

The binding of a vitamin or mineral cofactors to a protein and the subsequent conversion from an inactive apoenzyme to an active holoenzyme is another common post-translational modification for a number of enzymes. Examples include thiamin addition to pyridoxine dehydrogenase and manganese insertion in arginase.

5.6 Nutrition and gene polymorphism

The function and relative abundance of a protein can be altered by genetic mutations that may affect any of the numerous steps involved in converting the genetic code into a protein.

The most obvious outcome of gene polymorphism is when a change in a nucleotide sequence occurs that results in the protein product of the mRNA template losing its function or having altered substrate affinity (*Section 5.6.1*).

A more subtle but potentially just as, or even more, important gene polymorphism occurs when the variation in DNA sequence is in the non-transcribed region of a gene where the control switches for governing gene transcription, mRNA stability, or rate of translation are affected (*Section 5.6.2*).

Several genetic polymorphisms with a nutritional significance have already been identified; some of these are detailed in Table 2.

5.6.1 Gene polymorphisms affecting proteins

A SNP (*see Box 9: SNP*) has been identified that influences the dietary folate requirement.

The affected gene codes for a key enzyme methylene-tetrahydrofolate reductase (MTHFR) and the polymorphism replaces a single cytosine with thymidine. This in turn changes a codon that encodes alanine instead of valine. This single and apparently minor change in amino acid sequence reduces the thermostability of the enzyme. Those individuals homozygous for the allele demonstrate lowered MTHFR activity and elevated plasma homocysteine. Folate supplementation appears to lower plasma homocysteine levels, which is beneficial, as elevated homocysteine levels have been associated with atherosclerosis.

This finding is important, as it demonstrates that single nucleotide changes may have a significant effect on the expression and function of protein.

One of the most common inherited disorders with nutritional implications is familial hypercholesterolaemia, caused by mutations in the low-density lipoprotein (LDL) receptor gene template.

TABLE 2

Examples of known cellular processes and genetic polymorphism with direct consequences for nutrition

Cellular Process	*Gene with known polymorphisms*	*Nutrition/health Impact*
Folate metabolism	Methylene tetrahydrofolate reductase, cystathione beta-synthase, methionine synthase, glutamate carboxy-peptidase III	Risk of neural tube defect, Down's syndrome, CVD and cancer
Iron homeostasis	Hereditary haemochromatosis, linked gene HFE and transferrin receptor	Effect on iron requirements, anaemia, and iron overload
Bone health	Vitamin D receptor, oestrogen receptor, type I collagen	Effect on bone metabolism, osteoporosis, mediation of calcium and phosphorus translocation
Lipid metabolism	Apolipoprotein (AIV, B, C3, E), low density lipoprotein receptor, lipoprotein lipase	Effect on blood cholesterol and other cardiovascular risk factors
Immune function	HLA (MHC), tumour necrosis factor α and other cytokines	Susceptibility to various food allergies (such as coeliac disease) and modified susceptibility to cancer through diet

Source: Derived from Elliott, R. and Ong, T.J. (2002). Nutritional genomics, *BMJ* 324, 1438-42, with permission from BMJ Publishing Group.

The mutations lead to impaired LDL clearance and individuals display elevated cholesterol levels of greater than 7.76 mmol/L. Approximately 1 in 500 people are heterozygous (one allele mutated) for these mutations and individuals that are homozygous have exceedingly high cholesterol levels very early in life and shortened life spans.

A second common polymorphism related to lipoproteins is the apolipoprotein (apo)-E gene. Apo-E is involved in lipid transport and the receptor-mediated uptake of chylomicron and very low density lipoprotein (VLDL) remnants. Apo-E synthesis and secretion is increased when diets high in saturated fat are consumed. An example of the impact of polymorphisms in this gene is detailed below.

There are three major variants of apo-E; the normal form is apo-E3. In apo-E2, a cysteine has replaced arginine 158 and in the apo-E4 variant, cysteine 112 is replaced by arginine. The relative occurrence in the US population is circa 60% for the homozygous allele, 56%, 1% and 2% for alleles E3, E2 and E4 respectively. Population studies indicate that heterozygous allele is carried by 23% in the case of E3/E4, 12% for E3/E2 and 3% for E2/E4. Both E2 and E4 homozygotes demonstrate impaired lipoprotein metabolism. In the case of E2 hyperlipoproteinemia results due to defective binding to the apo-E receptor and with E4 elevated total cholesterol and LDL levels are seen.

LDL receptor, apo-E and MTHFR are only three of the many gene polymorphisms that affect human health and the development of heart disease.

5.6.2 Gene polymorphisms affecting the level of protein expression

The polymorphisms that occur in the untranscribed region of the gene produce more subtle effects because they limit the level of expression of a protein rather than altering the protein *per se*.

Polymorphisms of this type have been identified by DNAase foot-printing and simple DNA sequence comparisons. A common example of this change is in the apo-B gene, where cytosine may be replaced by thymidine at position 516. This substitution increased gene transcription by 40% and healthy middle-aged men who were homozygous for the thymidine allele have 12% higher LDL levels.

A polymorphism in the regulation of the hepatic lipase gene is caused by a SNP at the 514 nucleotide; a change from cytosine to thymidine results in a major difference in the response of HDL to dietary fat intake.

Similarly, a single nucleotide change at position 491 of the apo E gene significantly increased gene transcription, which was associated with an increased risk of the development of Alzheimer's Disease.

5.7 Nutrition and Epigenetics

Epigenetics refers to modifications of the genome, not involving alterations in the primary DNA sequence, but including an alteration of the DNA itself by, for example, methylation and posttranslational modification of the octet of histone proteins around which the DNA is wrapped.

DNA methylation occurs on cytosines (C) within the molecule, particularly where the cytosine base is followed by a guanine base and the dinucleotide may be methylated at the 5′ position of cytosine. When DNA methylation occurs in assemblies of cytosine/guanine dinucleotides in the promoter region of the gene, often gene silencing results with no subsequent mRNA or protein production.

Rogue methylation is part of the ageing process and is involved in the development of a number of diseases including cancers, cardiovascular disease and nerve cell or tissue degenerative disorders such as Alzheimer's disease and Parkinson's disease.

Rogue methylation is influenced by dietary factors and drugs and is shown to be reversible, although the impact that food components may have is not yet understood. At some future time it may be possible to develop nutrition regimes that help to maintain normal methylation and so promote longer-term health.

Chemical modification of the protein (histone) tails that protrude from the histone bundles, around which DNA is wrapped within the chromatin, has been described as histone "decoration". Although not fully understood these chemical modifications are thought to be epigenetic signals that regulate gene expression. There is a close interplay between histone decoration and DNA methylation. It is believed that histone decoration is one of the ways in which the genome integrates both intrinsic and extrinsic signals that result in the modulation of gene expression and modification of the phenotype.

6. CONSEQUENCES AND POTENTIAL

6.1 Introduction

The application of genomic technologies to nutrition and biochemical research techniques provides a powerful tool to understand the mechanisms by which individual foods or nutrients modulate processes occurring within the tissues of the body.

Scientists face the challenge to provide comprehensive answers to questions such as:

- Which components of the diet have important health promoting effects?
- How, where and when are these effects exerted?
- Can some of these components also have adverse effects?
- How much and in what form and combination do we need to eat such components to obtain the maximum health benefit with minimum risk?
- How do individuals' dietary recommendations vary depending on their genetic profile, age, gender and lifestyle?

Answering these questions will require collaboration between groups of scientists with diverse specialisms such as molecular biologists, geneticists, nutritionists, clinicians and bioinformaticians. In fact, the subject is so huge that in many cases a global approach to data use and sharing will be required to increase the scope of understanding.

This poses one of the challenges to the development of this area, as groups of traditional and 'omics scientists must learn to collaborate and communicate within multi-skilled teams of scientists.

Another barrier will be the high cost of entry into this specialist field. This is due to the immense requirement for data handling, integration of data from different sources and techniques, the lack of sufficiently sophisticated tools for data interrogation and modelling and logistical difficulties between co-operating groups

There are, however, specific areas where progress has already been made.

6.2 Nutrition and gene polymorphism

Defining nutrient requirements from DNA sequences may be somewhat futuristic. However, DNA sequencing could be used to screen for specific gene polymorphisms e.g. the Apo-E alleles. Effective dietary advice for those with the E3 allele that have elevated cholesterol would be different to the dietary advice given to those with either the E2 or E4 alleles. Using this technology would allow nutrient requirements of particular groups of individuals to be tailored more specifically.

As many polymorphisms identified appear to be linked to increased disease susceptibility, better understanding of the mechanisms involved would allow scope for better targeting of more appropriate dietary advice to the relevant population sub-groups.

However, understanding the relative importance of gene-gene and gene-environment interactions for polygenic diseases is still at an early stage.

For example, in osteoporosis, twin and sibling studies indicate that genetic factors are the main determinants of bone mineral density and structure, typically accounting for 50-85% of phenotypic variance, with environmental factors accounting for the rest. Although some genetic polymorphisms have been linked to variations in bone mineral density, these associations are still contentious and it seems more likely that several genetic polymorphisms each make a small contribution to the genetic component of osteoporosis.

Identifying appropriate candidate genes that mark disease risk in these circumstances becomes substantially more complicated and the risk of finding spurious results is increased.

The best strategy for resolving the genetic and environmental contributors to such polygenic disorders remains unclear at this stage.

Future use of DNA polymorphisms could be to investigate the genome for sequence information that defines the variation in nutrient absorption and use. However, the bioinformation requirements of such an approach are enormous and still in the relatively early stages of development.

A second requirement is for basic biological research that can correlate nutritional outcome with the gene polymorphism.

Where links are established between nutrients or dietary practice and SNP, it is conceivable that people at risk could be identified early in life, if rapid and inexpensive screening methods were available. This would enable a lifelong dietary approach that may improve both longevity and quality of life.

6.3 Gene and food bioactives

Food bioactives such as naturally occurring phytochemicals found in many fruits, vegetables, spices, and tea can also play a significant role in gene expression. Functional genomics techniques could effectively be used for identifying the effect of the novel functional food or food component (often described as nutraceuticals) on global gene expression and cell function, without having to make assumptions about what to look for in terms of risk.

The same approach is being used to establish the safety of genetically modified food and food ingredients.

Research is already underway to identify the chemopreventive effect of model food components by comparing the effect on protein and RNA expression within the relevant cell lines. It is anticipated that these model food components will affect different mechanisms involved in colon carcinogenesis. Once a mechanism and marker genes are linked, it should be possible to gain an understanding of the prophylatic mechanism of the food component under study.

6.4 Genomics in the development of biomarkers

The greatest potential for benefit from dietary modification is likely to be in terms of the maintenance or protection of health.

At the present time, biomarkers of disease risk rely on the measurement of a single or few nutrients, genes, proteins or metabolites and often measure parameters that indicate that the degenerative process is underway if not well advanced. For example, an elevated blood cholesterol concentration may indicate that considerable atherosclerosis has already taken place. In addition, as

many biological processes are multifactorial, a single biomarker may not accurately reflect the process under study.

Nutritional genomics offers the possibility of measuring genome-wide changes in gene expression resulting from changes in diet or possibly a single food component. Specific effects on gene expression would provide the focus to seek for links in the disease development process.

Alternatively, the disease state could be monitored to identify the genes involved in its early development. This would involve studying various tissues at different stages of disease development, which will allow more relevant markers at the DNA, RNA or protein level to be identified. These molecular biomarkers will permit early identification of pivotal changes between health maintenance and disease onset and progression.

This work may be complicated by the fact that some components in foods may be protective in one area at a specific time and cause adverse effects at another. An example of such a food is soya protein and its component phytoestrogens, which appear to offer varying degrees of protection to the breast health at different life stages and at different stages of breast cancer development.

7. ETHICS AND SOCIAL ISSUES

Crucial to consumer acceptance of the products, or services resulting from nutrigenomic developments, is the way in which they are communicated and by whom.

The information needs to clearly identify consumer benefits and to address any of their concerns and should be communicated by individuals or groups that the consumers trust to inform them on scientific issues.

Communicating any diet health message is an area fraught with difficulty, requiring much consideration to be given to the actual words used to communicate the message. Even after paying attention to how the message is communicated there is often poor uptake in large sectors of the population, who seem unwilling or unable to relate to the notion that today's diet will influence future health and well-being.

A barrier to greater exploitation of genetics in the area of nutrition and health is likely to be consumer-led fear or uncertainty about the consequences of characterisation of the genome and the identification of mutations of highly penetrant genes, e.g. those responsible for familial forms of cancer, and specific SNPs known to impact on health. This fear is based on the assumption that such consequences may have implications on an individual's ability to obtain employment, finance or insurance. It is important that those involved in the technologies address these concerns and communicate the benefits and the safeguards that are in place.

Clearly advantages for the individual can be identified and there is the potential for positive action through nutrition, for example, as detailed previously in those carrying the Apo-E3 allele (*see Section 6.2*). Under these circumstances genotyping is likely to be less controversial. Gene-specific advice or products could be used with responders or non-responders as appropriate to allow better targeting of resources and effort.

The greater benefit would be in the context of disease prevention where the knowledge of an individual's genetic profile, encoded by their unique pattern of SNPs, could be used to tailor specific risk-reducing actions involving diet or other factors that could reduce the risk of disease and improve the quality of life.

However, although an attractive proposition, there is little research to support the proposition that individual targeting would provide the motivation for change. Further research is required into factors that motivate behaviour change and whether these are in themselves influenced by genotype before this approach could be recommended.

The confidentiality of data relating to individuals' genetic map is also an area for concern; some people will question the extent to which such information should or can remain anonymous.

Clearly, as advances are made in nutrigenomics, all of these issues need to be addressed and reviewed on a regular basis. This will ensure that the innovative technologies and products that develop take due account of changing public reaction, consumer concerns and ethical issues. A further concern must be to maintain the value of this emerging science while it is still in its infancy. Over-promising the ability of nutrigenomics could cause it to be undermined or dismissed by consumers.

There is also a greater need for a holistic view as more and more detailed information is generated and a global approach will be desirable.

GLOSSARY

Allele: Two or more alternative forms of a given gene. All alleles are concerned with the same trait or characteristic, but the product or function coded by a particular allele differs from that coded for by other alleles of that gene.

Amino acids: Building blocks of proteins. Typically 20 different amino acids are commonly used by the cells to make proteins.

Atherosclerosis: A degenerative disease of arteries in which there is a thickening caused by an accumulation of material (plaque) beneath the inner lining, eventually restricting blood flow. The material characteristically contains cholesterol and macrophage cells.

Bioinformatics: The evolving science that handles the huge demand for the analysis and interpretation of biological data.

Cardiovascular disease: Any one of numerous abnormal conditions characterised by dysfunction of the heart and blood vessels.

Cholesterol: A lipid (sterol) made in the body from acetyl-CoA and present in the diet; a constituent of cell membranes (especially in nervous system tissues) blood and atherosclerotic plaques.

Chromosomes: In the cell nucleus, DNA is tightly packed with particular proteins into structures called chromosones. Different organisms have different numbers of chromosomes. A normal human cell contains two pairs of 23 chromosomes. Packaging into chromosomes enables the organised assortment of genes into daughter cells upon cell division, as well as playing a role in controlling gene expression.

Codon: The sequence of three nucleotides in mRNA that encodes for each amino acid of a protein.

Coronary heart disease (CHD): A condition in which the main coronary arteries supplying the heart are blocked or restricted and are no longer able to supply blood, and therefore oxygen to the heart muscle (myocardium), which may then quickly die. The main cause of reduced blood flow is the accumulation of plaques in the arterial walls, a disease known as atherosclerosis. The blockage of an already narrowed artery is thrombosis.

Deoxyribonucleic acid (DNA): Deoxyribonucleic acid (DNA) is the repository of all genetic information in the cell. It is a long linear polymeric molecule made up of nucleotide building blocks. Each nucleotide comprises a deoxyribose (a sugar) and phosphate group and one of four different bases, adenine (A), guanine (G), cytosine (C) or thymine (T). Each DNA molecule consists of two strands in the shape of a double helix.

DNA Microarrays: DNA microarrays or gene chips allow the activity of a large number of genes at the level of the mRNA to be measured simultaneously.

Enzyme: A protein produced by living cells that regulates the speed of chemical reactions that are involved in the metabolism of living organisms, without itself being altered in the process. Also called a "biological catalyst".

Epigenetics: Modifications to the genome, not involving alterations in the primary DNA sequence, but including alteration of the DNA by processes such as methylation.

Gas chromatography (GC): a technique for separating a mixture of molecules that involves the vaporising of the sample in a suitable carrier gas, often helium, hydrogen or nitrogen.

Gene: The segment of DNA on a chromosome that contains the information necessary to make one protein. A gene is the smallest indivisible unit of heredity.

Genetic code: The "code" in which information for the synthesis of proteins is contained. It lies in the nucleotide sequence of the coding region of a gene.

Genetic: Inherited; a genetic disease is one that is inherited and potentially transmitted through a faulty gene.

Genetic modification: The techniques for removing, modifying or adding genes to a living organism. Also called "gene splicing", "recombinant DNA (rDNA) technology" or "genetic engineering". "Within-species" genetic modification is essentially similar to traditional breeding methods (except that it is much speedier and much less haphazard). Through "trans-species" modification, results are obtained that would not be obtained by traditional breeding methods.

Genome: The genetic fingerprint of an organism that contains all the nucleotide sequences including structural genes, regulatory sequences and non-coding DNA sequences of the chromosome.

Genomics: "The holistic study of biomolecules" and comprises the study of all nucleotide sequences including structural genes, regulatory sequences and non-coding DNA sequences of the chromosome.

Heterozygous: Where the members of an allelic pair are genetically different, it is heterozygous with respect to that particular gene.

High performance liquid chromatography (HPLC): A technique for separating a mixture of molecules that involves using very high pressures to force a liquid sample through a tightly packed column of particles; separation occurs on the surface of the particles by an adsorption process.

Histone: Protein bundles rich in the amino acids arginine and/or lysine around which DNA is wrapped within the chromatin.

Homozygous: Where the members of an allelic pair occupy corresponding positions (loci) on a pair of homologous chromosomes and the alleles are genetically identical, it is said to be homozygous.

Hypercholesterolaemia: Concentrations of cholesterol in the blood higher than normal (or reference) values. Causes include dietary and genetic.

Infra-red absorption spectroscopy (IR): A technique that measures the vibrations of molecules; each molecule has a unique internal frequency that can be used to determine what functional groups are in a sample.

Lipoproteins: Particles composed of specialised proteins and lipids including triglycerol, cholesterol and phospholipid. They enable (water-insoluble) lipids to be carried in the blood plasma. LDL and HDL are lipoproteins.

Low density lipoprotein (LDL): Plasma lipoproteins containing high concentrations of lipids (so low density compared to that of water), including cholesterol. Increased concentrations are a risk factor for coronary heart disease.

Metabolome: The complete complement of low molecular weight molecules including the intermediates of metabolism in the cell.

Metabolomics: The study of the entire complement of metabolites in the cell including those involved in metabolic regulation and fluxes.

Metabonomics: A variant of metabolomics described as a systems approach to examining the changes in the hundreds or thousands of low molecular weight metabolites in an intact tissue or biofluid.

Mutation: The change in DNA sequence caused by damage by a mutagen, or by errors in cellular processes that may occur during cell division. Some mutations have no effect on the function of the genes in which they occur, while others inactivate or change the activity of the genes. Some mutations are detrimental to the organism, a few are beneficial. Mutations are a source of variation between individuals and are a driving force of evolution.

Nuclear Magnetic Resonance (NMR): A technique that uses an electromagnet or superconducting magnet to determine the structures, confirmations and interactions of molecules, usually small molecules with a molecular weight <2000.

Nutrigenomics: The study of the genome-wide influences of nutrition – the application of genomics technologies in nutritional sciences and food technology.

Ori: The special site(s) on the chromosome where the initiation of DNA replication begins.

Promoter: A nucleotide sequence within the non-transcribed region of the DNA of a gene that regulates the process of transcription. Transcription is commonly initiated at a position within the promoter sequence.

Protein: Polymers (chains of linked units) of amino acids. The uniqueness of individual proteins depends on their length and the order of amino acids within the proteins.

Proteome: The full cellular content of proteins.

Proteomics: The study of proteomes.

Ribonucleic Acid (RNA): A nucleic acid that is structurally similar to DNA involved in all stages of protein synthesis and in regulatory, catalytic and other processes in the cell. It differs in three main ways: the sugar component of the nucleotide building blocks is a ribose, the base uracil is used in place of thymine, so that the code consists of A, C, G and U, and it is generally single stranded.

Ribosomes: The cellular machines that read the code carried by mRNAs and work with tRNAs to produce proteins from this code.

RNA: The RNA that combines with ribosomal protein to form ribosomes.

mRNA: Any RNA that functions as a template for the assembly of amino acids during protein synthesis.

tRNA: The RNA that during protein synthesis acts as the adapter molecule matching amino acids to their codons on mRNA.

Reverse transcription polymerase chain reaction (RT-PCR): An experimental method used for understanding gene expression and that provides information on RNA quantification and conformation.

Single Nucleotide Polymorphisms (SNPs): The commonest and smallest form of genetic variability, where a single nucleotide substitution occurs in a DNA sequence.

Transcription: Transcription is the process by which a RNA strand is formed from a DNA template.

Transcriptome: The complete complement of RNA species produced from the genome of an organism.

Translation: The stage where mRNA guides the assembly of the polypeptide chain that results in protein synthesis.

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REVIEW

Nutritional genomics and personalized diet

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Abstract Nutritional genetics is considered as the combination of nutrigenomics and nutrigenetics. Nutrigenomics is establishing the effects of ingested nutrients and other food components on gene expression and gene regulation. It will also determine the individual nutritional requirements based on the genetic makeup of the person (personalized diet) as well as the association between diet and chronic diseases which will help to understand the etiologic aspects of chronic diseases such as cancer, type-2 diabetes, obesity and cardiovascular disease (CVS). Nutrigenetics on the other hand identifies how the genetic makeup of a particular individual co-ordinates his or her response to various dietary nutrients. It also reveals why and how people respond differently to the same nutrient. The present review will focus upon interaction of genetic background and diet with regard to development of such life threatening chronic conditions as obesity, cardiovascular disease (CVD), and cancer that are responsible for the majority of deaths in developed Western countries.

Contents

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1. Definitions

Nutritional genetics is not a single field, but is considered as the combination of two-nutrigenomics and nutrigenetics [1].

1.1. Nutrigenomics

Nutrigenomics is establishing the effects of ingested nutrients and other food components on gene expression and gene regulation, i.e., to study diet-gene interaction in order to identify the dietetic components having beneficial or detrimental health effects [1,2]. It will also determine the individual nutritional requirements based on the genetic makeup of the person (personalized diet) as well as the association between diet and chronic diseases which will help to understand the etiologic aspect of chronic diseases such as cancer, type-2 diabetes, obesity and cardiovascular disease (CVS) [2]. Nutrigenomics will also identify the genes involved in physiological responses to diet and the genes in which small changes, called polymorphisms, may have significant nutritional consequences and the influence of environmental factors on gene expression [3].

1.2. Nutrigenetics

Nutrigenetics on the other hand identifies how the genetic makeup of a particular individual co-ordinates his or her response to various dietary nutrients. It also reveals why and how people respond differently to the same nutrient [4].

Together these two approaches promise to deliver a critical part of the scientific knowledge needed to understand how diet affects the individual humans [1] and eventually nutrigenomics will lead to evidence-based dietary intervention strategies for restoring health and fitness and for preventing diet-related disease [5].

2. Gene diet disease interaction

2.1. Nutrigenetic diseases

Ninety seven percent of the genes have known to be associated with human diseases result in monogenic diseases. Modifying the dietary intake can prevent some monogenetic diseases [6], e.g., in phenylketonuria (PKU) food containing the amino acid phenylalanine, including high protein food such as fish, chicken, eggs, milk, cheese, dried beans, nuts, and tofu must be avoided. In case of defective aldehyde dehydrogenase enzyme, alcohol must be avoided. Patients having galactosemia (lack of a liver enzyme to digest galactose) should avoids diets which contain lactose or galactose, including all milk and milk products while in case of lactose intolerance (shortage of the enzyme lactase) patients should avoid milk and milk products [7].

2.2. Nutrigenomic diseases

Diseases and conditions that are known to have genetic and/or nutritional components are candidates for nutrigenomic studies to determine whether dietary intervention can affect the outcome. Differences in genetic makeup or genotype are factors in gastrointestinal cancers, other gastrointestinal conditions or digestive diseases, inflammatory diseases, and osteoporosis. Nutrient imbalances are factors in aging, alcoholism/substance abuse, behavioral disorders, cancer, cardiovascular disease (CVD), chronic fatigue, deafness, diabetes, immune disorders, macular degeneration, multiple sclerosis, neurological disorders, osteoporosis, Parkinson's disease and stroke [7]. Diseases that are known to involve in the interactions between multiple genetic and environmental factors such as diet include, many cancers, diabetes, heart disease, obesity and some psychiatric disorders [7].

Therefore, both disciplines aim to unravel diet/genome interactions; however, their approaches and immediate goals are distinct. Nutrigenomics will unravel the optimal diet from within a series of nutritional alternatives, whereas nutrigenetics will yield critically important information that will assist clinicians in identifying the optimal diet for a given individual, i.e., personalized nutrition [8].

The following five tenets of nutritional genomics serve as a conceptual basis for understanding the focus and promise of this emerging field [3]:

1. Under certain circumstances and in some individuals, diet can be a serious risk factor for a number of diseases.
2. Common dietary chemicals can act on the human genome, either directly or indirectly, to alter gene expression or structure.
3. The degree to which diet influences the balance between healthy and disease states may depend on a person's genetic makeup.

4. Some diet-modulated genes (and their normal, common variants) are likely to play a role in the onset, incidence, progression, and/or severity of chronic diseases.
5. Dietary intervention based on the knowledge of nutritional requirements, nutritional status, and genotype (i.e., personalized nutrition) can be used to prevent, mitigate, or cure chronic disease.

3. Single nucleotide polymorphism (SNP)

Most of the genes have small sequence differences – polymorphisms – that vary among individuals. Single nucleotide polymorphisms (SNPs) are the most common type of variation [4].

The single nucleotide polymorphisms consortium is mapping polymorphic regions of the genome that control individual phenotypic differences among the human population. The importance of this genetic variation to the varying needs for and physiological responses to the particular nutrients was stated by Ames [9]. Missense single nucleotide polymorphisms occur about 1 in every 1000 bases in expressed genes, so one expects that there will be many more polymorphisms to be found in micronutrient and dietary studies. Specific genetic polymorphisms in human populations change their metabolic response to diet and influence the risk patterns of disease as SNPs are similar to variations in a recipe. Each gene is a recipe for a specific protein or group of proteins that either regulate biological functions or serve as structural building blocks for tissues (e.g., collagen). Some SNPs change the recipe for the gene so that either a different quantity of the protein is produced or the structure of the protein molecule is altered [3].

These genetic polymorphisms lead to alteration of the response to the dietary components by influencing absorption and metabolism. Epigenetic events can induce changes in DNA methylation pattern and thus influencing over all gene expression that can be modified in response to the food components. Many dietary constituents affect post translation events and many account for at least part of the variation in response to the dietary components [10].

One of the best-described examples of the effect of SNPs is the relationship between folate and the gene for MTHFR – 5,10-methylenetetrahydrofolate reductase. MTHFR has a role in supplying 5-methylenetetrahydrofolate, which is necessary for the re-methylation of homocysteine to form methionine. Methionine is essential to many metabolic pathways including production of neurotransmitters and regulation of gene expression. Folate is essential to the efficient functioning of this MTHFR. There is a common polymorphism in the gene for MTHFR that leads to two forms of protein: the wild type (C), which functions normally, and the thermal-labile version (T), which has a significantly reduced activity. People with two copies of the wild-type gene (CC) or one copy of each (CT) appear to have normal folate metabolism. Those with two copies of the unstable version (TT) and low folate accumulate homocysteine and have less methionine, which increases their risk of vascular disease and premature cognitive decline [11].

4. Nutrigenomics and chronic disease

The present review will focus upon interaction of genetic background and diet with regard to development of such life threatening chronic conditions as obesity, CVD, and cancer that are responsible for the majority of deaths in developed western countries [3]. The nature of these interactions is indeed very complex.

4.1. Nutrigenomics and obesity

Obesity is the commonest nutrition-related disorder and is the core element of a group of metabolic abnormalities (metabolic syndrome) which also commonly includes insulin resistance and hyperinsulinemia, hypertension, impaired glucose tolerance, and noninsulin-dependent diabetes mellitus [12]. Also obesity and associated metabolic anomalies dramatically increase the risk of developing a variety of chronic diseases including CVD and cancer [13,14]. However, individual susceptibility to obesity strongly depends on the genetically determined patterns of energy balance regulation [15].

Multiple polymorphic genes encoding central and peripheral determinants of energy intake and expenditure have been revealed over the past decade. Food intake control may be affected by polymorphisms in the genes encoding taste receptors and a number of peripheral signaling peptides such as insulin, leptin, ghrelin, cholecystokinin, and corresponding receptors [15]. Polymorphic central regulators of energy intake include hypothalamic neuropeptide Y, agouti-related protein, melanocortin pathway factors, CART (cocaine- and amphetamine-regulated transcript), some other neuropeptides, and receptors for these molecules. Potentially important polymorphisms in the genes encoding energy expenditure modulators (alpha- and beta-adrenoceptors, uncoupling proteins, and regulators of adipocyte growth and differentiation) are also known [15].

4.2. Nutrigenomics and CVD

CVD is the primary diet-related chronic disease of the modern time and the inflammation is emerging as underlying many chronic disorders including CVD. CVD can be characterized as a group of multifactorial conditions associated with obesity, atherosclerosis, hypertension, and thrombosis. All of these pathologic entities are known to be closely related to both genetic factors and environmental influences. Diet is considered as one of the environmental influences and a strong relationship between diet composition and CVD risk is well established [16–19].

Obesity per se is a major cardiovascular risk factor, thus polymorphic genes involved in energy balance control certainly provide ''favorable'' or ''unfavorable'' background for the development of CVD [15].

Atherosclerosis constitutes the key element in the pathogenesis of CVD and it can be regarded as a complex combination of lipid transport and metabolism disorder with chronic inflammation [16,20]. Permanently elevated plasma levels of total cholesterol, LDL cholesterol, and triglycerides predispose to the development of atherosclerotic plagues, whereas increased high density lipoprotein (HDL) cholesterol levels appear to be protective [15]. Genetic variation in genes encoding for apolipoproteins, some enzymes and hormones can alter individual sensitivity to develop the cardiovascular diseases. Some of these variants are susceptible for dietary intervention, for example: Individuals with the E4 allele in the apolipoprotein E gene show higher low-density lipopro-

tein-cholesterol(bad cholesterol) levels with increased dietary fat intake compared with those with the other (E1, E2 and E3) alleles receiving equivalent amounts of dietary fat [21].

ApoA1 is primarily found in the HDL particles. AG to A transition in the promoter of APOA1 gene is associated with increased HDL-cholesterol concentration but the results across studies are not consistent [22]. Ordovas et al. [23] found that the allele A was associated with the decreased serum HDL levels. The genetic effect was reversed, however, in women who ate more polyunsaturated fatty acids (PUFA). In men, this type of fat effect was significant when alcohol consumption and tobacco smoking was considered in the analysis. Also specific polymorphism in genes encoding lipid transport proteins, their receptors, and lipid-processing enzymes and inflammation related proteins were shown to be associated with the characteristic changes in blood lipid concentrations [24–28].

One polymorphism(-5′4 cc) in the hepatic lipase gene is associated with an increase in protective HDL levels compared with the TT genotype (common in certain ethnic groups such as African–Americans) in response to high fat diet [21].

4.2.1. Hypertension

Arterial hypertension constitutes an important pathogenetic element in CVD. It is now well understood that numerous genetic factors are involved in blood pressure regulation and some genetic patterns can be responsible for raising blood pressure, which characterizes essential (primary) hypertension [29]. Hypertension is one of the components of the obesity-associated metabolic syndrome [12], and influence of dietary factors altering energy homeostasis appears to predispose to blood pressure elevation. It is well known that the loss of weight in hypertensive obese individuals usually leads to simultaneous blood pressure decrease [30].

Sodium chloride is the only dietary risk factor well defined to predispose to hypertension. However, blood pressure responses to increases and decreases in dietary salt intake may be heterogenous, as only about 15% have sodium-sensitive hypertension. For the other 85%, eliminating salt from the diet has no effect on their blood pressure [31].

Polymorphic genes implicated in blood pressure regulation include renin-angiotensin system genes including those encoding angiotensinogen (*AGT*), angiotensin converting enzyme (*ACE*), and aldosterone synthetase (*CYP11B2*) [29]. However, no evidence of the interactions between polymorphic variants of these genes and dietary factors is available. On the other hand sodium transport/metabolism-related genes such as those encoding epithelial sodium channel (ENaC) subunits, adducin, and 11B-hydroxysteroid dehydrogenase are certainly of interest, given well-proven association between dietary salt intake and hypertension [31]. There are also some reports associating human hypertension with polymorphisms in some G-proteins (G protein_subunit, GNAS1) and adrenergic receptors but evidence is not sufficient [15]. So nutrigenomics is addressing why some people can control their hypertension with diet, whereas others require drugs.

4.2.2. Arterial thrombosis

Thrombosis of arteries affected by atherosclerosis constitutes the main mechanism leading to acute coronary and cerebrovascular syndromes. Impaired balance of multiple factors constituting blood coagulation system can lead to hypercoagulative state increasing thrombosis probability. Both the environmental and genetic factors are involved. Diet, especially excessive fat ingestion can trigger postprandial hypercoagulative state [32]. Gene polymorphisms affecting hemostasis (as genes encoding platelet surface glycoproteins, and coagulation factors) have been implicated [33,34]. Blood coagulation is counterbalanced by the anticoagulant and fibrinolytic systems that also include polymorphic factors [34].

4.2.3. Homocysteine metabolism

Hyperhomocysteinemia is now regarded as an independent risk factor in the development of cerebrovascular and coronary heart disease as well as venous thrombosis [35].

5. Nutrigenomics and cancer

Cancer is a process composed of multiple stages in which gene expression, and protein and metabolite function begin to operate aberrantly [36]. In the post-genomic era, the cellular events mediating the onset of carcinogenesis, in addition to their modulation by dietary factors, has yielded important information in understanding of this disease [37]. Inherited mutations in genes can increase one's susceptibility for cancer. The risk of developing cancer can be markedly increased if there is a gene-diet interaction. Studies of twins show that the likelihood of identical twins developing the same cancer is less than 10%, indicating that the environment plays an important role in cancer susceptibility [7].

Evidence of genome and epigenome damage biomarkers, in the absence of overt exposure of genotoxins, are themselves sensitive indicators of deficiency in micronutrients required as cofactors or as components of DNA repair enzymes, for maintenance methylation of CpG sequences and prevention of DNA oxidation and/or uracil incorporation into DNA [38].

Diet considered as a source of either carcinogens (intrinsic or cooking-generated) present in certain foods or constituents acting in a protective manner (vitamins, antioxidants, detoxifying enzyme-activating substances, etc.) [39]. It is clear that carcinogen metabolism-affecting polymorphisms may modify probability of contact between carcinogens and target cells, thus acting at the stage of cancer initiation [15].

Influences of polymorphisms of gene encoding factors involved in hormonal regulation are most strongly manifested in hormone dependent tumors such as breast, prostate, ovarian and endometrial cancers. Polymorphisms in sex hormone receptor genes comprising those encoding estrogen receptor, progesterone receptor, and androgen receptor have been shown to be associated with cancer risk modulation [15]. Dietary factors can certainly interact with hormonal regulation. Obesity strongly affects hormonal status. At the same time some food components, such as phytoestrogens are known to be processed by the same metabolic pathways as sex hormones [40], thus their cancer-preventive effect can be modulated by the polymorphisms mentioned here.

5.1. Diet and increased risk of cancer

There are various examples of the effects of diet on cancer risk.

There is an increase risk of colorectal cancer with high consumption of red meat [21]. N-Acetyl transferase (NAT) is a

phase II metabolism enzyme that exists in two forms: NAT1 and NAT2. Several polymorphisms exist in NAT1 and NAT2, some of which have been associated NAT capabilities of slow, intermediate, or fast acetylations. NAT is involved in acetylation of the heterocyclic aromatic amines found in heated products especially well cooked red meet. During cooking of muscle meat at high temperature some aminoacids may react with creatinine to give heterocyclic aromatic amines (HAA). HAA can be activated through acetylation to reactive metabolites which bind DNA and cause cancers. Only NAT2 fast acetylators can perform this acetylation. NAT fast acetylator genotype had a higher risk of developing colon cancer in people who consumed relatively large quantities of red meat [21].

A combination of excess body weight and physical inactivity are estimated to account for one fifth to one third of several of the most common cancers, specifically cancers of the breast (postmenopausal), colon, endometrium, kidney and esophagus (adenocarcinoma) [41].

Specific dietary irritants, such as salts and preservatives have been suggested as being carcinogens for gastric cancer [42].

C667T polymorphism in MTHFR gene which reduces enzymatic activity is inversely associated with occurrence of colorectal cancer. Low intake of folate, vitamin B12, vitamin B6 or methonine are associated with increased risk for cancer in CC or TT phenotype of MTHFR gene [43].

It was reported that a stronger relationship existed between the risk of developing hepatocellular carcinoma in Sudanese population and consumption of peanut butter with aflatoxins with the glutathione S-transferase M1 null genotype compared to those lacking the genotype [44].

5.2. Diet and cancer prevention

Cancer prevention studies have shown that all of the major signaling pathways deregulated in different types of cancer, are affected by nutrients. Pathways studied include: carcinogen metabolism, DNA repair, cell proliferation/apoptosis, differentiation, inflammation, oxidant/antioxidant balance and angiogenesis [45]. So far, more than 1000 different phytochemicals have been identified with cancer-preventive activities [46].

Dietary fibers have a protective effect against bowel cancer [7].

Long chain polyunsaturated fatty acids (LC-PUFA) beneficially affect physiological processes including growth, neurological development, lean and fat mass accretion, reproduction, innate and acquired immunity, infectious pathologies of viruses, bacteria and parasites; and the incidence and severity of virtually all chronic and degenerative diseases including cancer, atherosclerosis, stroke, arthritis, diabetes, osteoporosis, neurodegenerative, inflammatory, and skin diseases [47–51].

Fish oil, rich in omega-3 fatty acids, inhibits the growth of colonic tumors in both invitro and invivo systems [52–54].

Bioactive components present in fruits and vegetables can prevent carcinogenesis by several mechanisms such as blocking metabolic activation through increasing detoxification. Plant foods can modulate detoxification enzymes as flavonoids, phenols, isothiocyanates, allyl sulfur compounds, indoles, and selenium [55,56]. As a result carcinogen activation, covalent adducts with the individual nucleic acids of DNA or RNA are formed. It has also been found that reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl radicals attack DNA bases, resulting in potential mistranscription of DNA sequence [57]. Such disruptions can interfere with DNA replication and thus produce mutations in oncogenes and tumor suppressor genes. ROS can also result in breakage of DNA strand, resulting in mutations or deletions of genetic material [58].

6. Ethical, legal and social issues in nutrigenomics

Nutrigenomics raises ethical, legal and social issues particularly with respect to how the public may access nutrigenetic tests and associated nutritional and lifestyle advice [59].

Five areas have been identified by international experts [60] in the context of both basic nutrigenomics research and its clinical and commercial uses: (i) health claims benefits arising from nutrigenomics, (ii) managing nutrigenomic information, (iii) delivery methods of nutrigenomics services, (iv) nutrigenomics products, and (v) equitable accessibility to nutrigenomics. Hence it is important to elevate the depth of depate to understand and manage all these area.

7. Conclusion

Nutrigenomics offers the potential of important health benefits for some individuals. Primary care physicians have minimal training in nutrition and genetics, and medical geneticists are in high demand and short supply [59]. Dietetic practitioners are experts in nutrition science and interest in nutrigenomics is growing among members of this professional group. However, as with physicians, dietetics practitioners would require considerable training to bring nutrigenomics into their practice capacity [59].

In recent years, a high-resolution recombination map of the human genome has provided and increased the information on the genetic order of polymorphic markers and the SNP map of the human genome [61]. It is hoped that the map of SNPs in the human genome will provide powerful molecular tools to decipher the role of nutrition in human health and disease and help defining optimal diets [10]. Advanced genetic analysis in combination with twin studies may provide opportunities to understand the basis of complex traits and the role of individual genotypes on the development of polygenic diet-related diseases such as cancer and CVS [62].

Thus nutrigenomics treats food as a major environmental factor in the gene–environment interaction, with the final aim to personalize food and nutrition and ultimately individualize strategies to preserve health, by tailoring the food to individual genotype [22].

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