Review Article

Nutrition and Epigenetics

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Abstract

Cardiovascular diseases (CVDs), diabetes, obesity and cancer are polygenic in nature and their prevalences and mortality vary depending upon genetic susceptibility and the presence of phenotype risk factors. The heritability of variant phenotypes may depend on the intake of proinflammatory or anti-inflammatory nutrients, for their expression as phenotypes or their suppression. Genes are important for the determination of enzymes, receptors, cofactors, structural components involved in the regulation of blood pressure, the metabolism of lipids, lipoproteins and inflammatory and coagulation factors that help discover individual risks for CVDs. An interaction of specific nutrients with the genetic code possessed by all nucleated cells can be recognized. Further studies indicate that methylation of the chromatin appears to be most important in epigenetics leading to an increased risk of obesity and metabolic syndrome. The new field of epigenetics has emerged with more impact on cellular transgenerational profiles primarily dealing with health perspectives. It is not a change in the sequence of the DNA itself, but in how genes are expressed because some of them may get 'shut off' as a result of environmental trauma and others are expressed due to a favorable environment.

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Introduction

Noncommunicable diseases (NCDs) are multifactorial, polygenic diseases including diabetes, hypertension, stroke, coronary artery disease (CAD) and cancer [1–8]. Epigenetics involves a process whereby changes in gene expression and, hence, function might occur even

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Table 1. Nutraceuticals with a possible influence on genes

Nutrient	Effect
Refined carbohydrates (sugar and refined starches)	adverse
Trans fatty acids	adverse
Excess of saturated fat	adverse
Excess of linoleic acid	adverse
ω -3 fatty acids	beneficial
Monounsaturated fatty acids	beneficial
Calcium, magnesium, potassium, iron	beneficial
Zinc, copper, selenium, chromium, manganese, molybdenum, cobalt	beneficial
Coenzyme Q10, L-carnitine	beneficial
Lead, mercury, arsenic, cadmium, fluoride	adverse
Excess of iron	adverse
Vitamin A, E, C, D, K and β -carotene and flavonoids	beneficial
Pyridoxine, thiamin, riboflavin, cyanocobalamin, nicotinic acid, folic acid	beneficial
Fiber (polysaccharides)	beneficial
Amino acids: arginine, taurine, cysteine	beneficial

when there are no alterations in the DNA sequence which can mediate the pathogenesis of NCDs.

It seems that wild foods and nutraceuticals, such as ω -3 fatty acids, antioxidants, essential and nonessential amino acids, vitamins and minerals, are important determinants of the enzyme function; hence, these foods and nutrients can suppress the expression of harmful genes [5–11]. The phenotypic expression for health or disease depends on the phenotype and the environment, as well as on the genotype and upon structural variations of genes. There is an interaction of specific nutrients with the genetic code possessed by all nucleated cells, which may cause nutritional modulation of the genetic expression for health or disease. Since nutrients and nutraceuticals interact with genes, a genetic cause may explain the continued appearance of nutritional disease in the population by nutritional silencing of phenotypic expression [2–9]. There are several nutrients and nutraceuticals which can influence genes (table 1). The following nutrients have been found to modulate gene expression in various experimental studies [4–18]: polyunsaturated fatty acids (ω -6 and ω -3), milk, calcium, vitamin, iron, ascorbate and saturated fat. It is possible that by changing the nutritional environment, the activity and conformation of chromatin may be altered, which may result in the genetic expression along with relaxation of chromatin (fig. 1, 2). The methylation of cytosine in the promoter region due to adverse effects of nutrients may repress gene expression by preventing the binding of specific transcription factors, such as early growth response protein 1 (Egr-1) and activating protein 1 (AP-1), or it may attract mediators of chromatin remodeling, such as histone-modifying enzymes or other repressors of gene expression.

Rapidly absorbed foods increase the generation of superoxide anion in the tissues as well as free fatty acids and glucose in the blood [3–9]. There is an increased amount and activity of nuclear factor- κ B (NF- κ B), a transcriptional factor regulating the activity of at least 125 genes, most of which are proinflammatory. A rapid rise in blood glucose may be associated with an increase in two other proinflammatory transcription factors: AP-1 and Egr-1 (fig. 1). AP-1 regulates the transcription of matrix metalloproteinases and Egr-1 modulates the transcription of tissue factor and plasminogen activator inhibitor 1. Excess intake of linoleic acid, saturated fat, trans fat and refined starches and sugars can increase the generation of superoxide anion and activate NF- κ B, leading to a rapid expression of proinflammatory genes. However, a low ω -6/ ω -3 ratio of foods with antioxidants, micronutrients, minerals, vitamins

Fig. 1. Egr-1 (blue), a proinflammatory transcription factor, increases due to superoxide anion, generated by glucose ingestion. Superoxide anion may cause methylation of cytosine in the promoter region and may repress gene expression by preventing the binding of Egr-1 and AP-1. The methylation process may attract mediators of chromatin remodeling, such as histone-modifying enzymes or other repressors of gene expression. Superoxide anion may also influence 3 zinc fingers in complex with DNA (orange). The middle zinc ion (green) modulates the transcription of the tissue factor and plasminogen activator inhibitor 1, also called Zif268, a mammalian transcription factor (modified from Mishra et al. [2]). For colors, see online version.

Color version available online

Fig. 2. A mechanism of genetic response to diet indicating directly inhibiting enzymes: histone acetylation complex that catalyzes DNA methylation or histone modifications, or by altering the availability of substrates necessary for those enzymatic reactions, such as activation of transcription.

Color version available online

and coenzyme Q10 may inhibit the generation of superoxide and suppress the proinflammatory transcription factors NF- κ B as well as AP-1 and Egr-1, which may inhibit phenotypic expressions (fig. 1). The phenotypic expressions and polymorphisms may be silenced by targeting simple sequence differences, known as single nucleotide polymorphisms (SNPs), by nutrients and slowly absorbed foods with a low ω -6/ ω -3 ratio. Recently, polymorphisms of the human Delta-5 (FADS1) and Delta-6 (FADS2) desaturase genes have been described to be associated with the level of several long-chain ω -3 and ω -6 polyunsaturated fatty acids in serum phospholipids, which may be important mediators of the risk of cardiovascular

diseases (CVDs) and other chronic diseases [1–5]. Further studies indicate that microRNAs regulate our genes but the microRNA sequence is not known. microRNA expression patterns have been identified that correlate with biological phenotypes of chronic diseases. Lipoprotein(a) gene expression is nutritional as well as genetic, and trans fat can increase its level as well as atherogenicity [8].

The Functions and Dysfunctions of Genes

The vital roles of genes in the life of a subject can best be understood through the total chromatin package rather than individual genes [2–4]. Chromatin is a DNA protein complex in higher organisms, which appears as a diffuse mass within the nucleus during the interphase of a cell. The region of the chromosome which possesses the compact chromatin is known as heterochromatin and the one including the relaxed chromatin as euchromatin. Epigenetics has emerged with a greater impact on cellular transgenerational profiles primarily dealing with health perspectives [4, 5]. There are tissue-specific genes responsible for cellular differentiation and organogenesis, whereas housekeeping genes maintain the basic requirements of the cells [2–5]. The designer genes dictate the barricading of cells and cellular recognition during development. In monozygotic twins, whose genomic DNA and chromatin complexes are indistinguishable, an influence of the environment and of the diet may be shown on gene function, independently of each other [3–9].

The epigenome diverges with diet, lifestyle and ageing, which refers to the heritable changes in the gene expression that occur without a change in the DNA sequence. Epigenesis implies a fundamental regulatory system beyond nucleotide sequence information of DNA, emphasizing that Mendel's alleles are not merely coding DNA portions [4, 5]. The genes are dormant when chromatin is condensed and they are expressed when chromatin is relaxed, indicating that genetic functions are dependent on the chromatin conformation.

Scientific Studies on Nutrition and Epigenetics

The health status of genes, copy number variations or SNPs, whether single or polymorphic, appears to be important in the manifestation of health or chronic diseases. A recent study has reported, for the first time, associations between obesity in parents and changes in DNA methylation at the insulin-like growth factor 2 (IGF-2) gene among offspring [18]. DNA methylation regulates the activity of certain genes, which reflect a higher risk for some diseases. Decreased DNA methylation at the IGF-2 gene has been associated with an increased risk of developing certain cancers, including colorectal and ovarian cancers. However, our genes are able to adapt to our environment, and we adjust in a way that may be problematic later by altering genetic expressions without changes in the DNA sequence. Some genes may get 'shut off' as a result of environmental trauma. The Newborn Epigenetics Study examined the influence of environmental exposures on genetic profiles in newborns. The DNA from the umbilical cords of 79 newborns was examined to determine potential associations between the offspring's DNA methylation patterns and parental obesity before conception. DNA methylation at the IGF-2 gene in the offspring of obese fathers was significantly lower than in the children of fathers who were not obese, which suggests that paternal obesity may be associated with an increased risk of children developing certain cancers.

An experimental study also indicated that paternal environmental factors, such as diet and weight, were important contributors to disease in the next generation [19]. Male rats were fed a high-fat diet to induce obesity and glucose intolerance and were then mated with

normal-weight females. The resulting female offspring exhibited impaired glucose tolerance and insulin secretion as young adults. This is the first report of the nongenetic, intergenerational transmission of metabolic consequences of a high-fat diet from father to offspring. A family history of diabetes is one of the strongest risk factors for the disease; however, until now, the extent of any influence of nongenetic paternal factors has been unclear. The study showed that overweight fathers can play a role in ‘programming’ epigenetic changes in their offspring, possibly through effects on their sperm caused by their consumption of high-fat food [19]. Nutrition might induce, at some loci, epigenetic or other changes that could be transmitted to the next generation impacting on health. In the study by Kaati et al. [20], the ancestors’ slow growth period was set at the ages of 8–12 years, and the availability of food during these years classified as good, intermediate or poor. The probands’ childhood circumstances were defined by the fathers’ ownership of land, the number of siblings and the order in the sibship, the death of parents and the parents’ level of literacy. An earlier finding of a sex-specific influence from the ancestors’ nutrition during the slow growth period, going from the paternal grandmother to the female proband and from the paternal grandfather to the male proband, was confirmed. A response from father to son also emerged when childhood social circumstances of the son were accounted for. Early social circumstances influenced longevity of the male proband. The transgenerational response to the ancestors’ nutrition prevailed as the main influence on longevity. The slow growth period before the prepubertal peak in growth velocity has emerged as a sensitive period where different food availability is followed by different transgenerational responses. The use of folic acid supplements by women in Norway in the period from 4 weeks before to 8 weeks after conception was associated with a reduced risk of the child having severe language delay at a particular age. A case study from Japan, comprising 383 consecutive patients with angiographically confirmed CAD and 368 non-CAD subjects adjusted for age and BMI, examined SNPs in the adiponectin gene [21]. Among SNPs, the frequency of the I164T mutation was significantly higher in CAD subjects (2.9%) than in the controls (0.8%, $p < 0.05$). The plasma adiponectin levels in subjects carrying the I164T mutation were significantly lower than in those without the mutation, and they were independent of the BMI. In contrast, SNP94 and SNP276, which are reported to be related with an increased risk of type 2 diabetes, were associated neither with CAD prevalence nor with the plasma adiponectin level. Subjects with the I164T mutation exhibited a clinical phenotype of the metabolic syndrome.

A study from West Africa reported that levels of DNA methylation were higher at regions of 5 genes in children conceived during the peak rainy season months of August and September, when food would typically have been less available to their mothers [22]. Two of the 5 genes in which elevated DNA methylation occurred warrant further study because they are associated with risk of disease [22]. Specifically, the *SLITRK1* gene is associated with Tourette’s syndrome, and the *PAX8* gene is linked to hypothyroidism. The researchers attributed the epigenetic variation to dramatic seasonal differences in the kinds and amounts of foods available in the 3 subsistence-farming villages that were the focus of the study. In a randomized, double-blind, single-center pilot study involving 8 healthy adults, supplementation with vitamin D₃ for a period of 2 months (during winter) was found to be associated with improvements in serum 25-hydroxyvitamin D concentrations and a 1.5-fold alteration in the expression of 291 genes [23]. Genes related to transcriptional regulation, immune function, response to stress and DNA repair were identified. The findings indicate that any improvement in vitamin D status will significantly affect the expression of genes that have a wide variety of biologic functions of more than 160 pathways linked to cancer, autoimmune disorders and CVDs, which have been associated with vitamin D deficiency. This study reveals for the first time molecular fingerprints that help explain the nonskeletal health benefits of vitamin D.

Epigenetic Mechanisms

Epigenetic mechanisms can impact, for example, the levels at which an everyday biochemical process, i.e. DNA methylation, occurs at regions of certain genes [22]. DNA methylation is essential for cell development and for stabilizing cell function. Nutrient deficiencies or excesses, pollutants and radiations can predispose cellular stress leading to DNA methylation [2–5]. Cellular stress during replication induces many small deletions and duplications in the genome, adding fuel for human diversity and disease. Replication stress is known to be hazardous for the cell and is thought to contribute to ageing and cancer.

How exactly stress causes DNA damage remains unclear. The patterns of DNA methylation differ in response to specific nutrients, inherited genetic polymorphisms and exposure to environmental factors. Nutrients and nutraceuticals provide the methyl group, which is added to DNA via folate and methionine pathways. It is not yet clear which methyl mark accounts for ageing or the development of diseases. The packaging and function of the human genome are controlled by epigenetic mechanisms.

The DNA sequence of humans appears to be under a strong influence of the genome and packed chromatin, which facilitates the differential expression of genes. The epigenome alters with ageing and may interact with nutrients and nutraceuticals, physical activity, mental stress, tobacco consumption and alcohol consumption and environmental pollutants. CVD, diabetes and cancer may involve proteins that interpret cytosine methylation signals, and epigenetic changes may precede genetic changes in the arterial and vascular cells due to different biochemical factors, such as glycemia, hyperinsulinemia and proinflammatory cytokines. The DNA hypomethylation activates the concerned genes, for example, oncogenes in cancer, and initiates chromosomal instability. However, DNA hypermethylation may also initiate silencing of protective genes resulting in cancers. These methylation patterns can develop molecular epigenetic markers for a variety of cancers.

An exception to this is seen when ω -3 polyunsaturated fatty acids activate peroxisome proliferator-activated receptor- α , leading to a sequestration of the retinoid X receptor. The peroxisome proliferator-activated receptor requires the formation of heterodimers with the retinoid X receptor and inhibition of gene transcription through the interference with T3 action at the thyroid hormone response elements [24]. It seems that several T3-regulated hepatic genes are suppressed by ω -3 fatty acids. NCX1.3 is more sensitive to inhibition by α -linolenic acid than NCX1.1 [25, 26]; NCX is a membrane protein (with isoforms) that is concerned with the transport of sodium and calcium ions into and out of cells. In cardiomyocytes, NCX maintains calcium homeostasis by controlling the movement of calcium across the cell membrane. In addition, only ω -3 fatty acids inhibit NCX1.1, but several classes of fatty acids inhibit NCX1.3. The differential sensitivity of NCX isoforms to fatty acids may have important implications as therapeutic approaches for hypertension, heart failure and arrhythmias [25]. A recent study showed that the risk of CAD associated with a variant of chromosome 9p21 is increased in the presence of poor glycemic control in patients with type 2 diabetes, indicating that not all diabetics may have a similar risk of vascular disease [26]. A new bioinformatics method for pinpointing an individual DNA profile within an aggregation of 1,000 or more DNA samples has been developed, which may revolutionize nutraceutical modulation of genetic expressions. The method uses SNPs, genetic irregularities routinely utilized to study human disease and genetic variation, as markers to probe a mixture of DNA for an individual's genetic signature. This finding and others may provide individual advice of a suitable nutraceutical or wild foods for the genetic modulation of the concerned risk factor related to CVDs [26–29].

Future Directions

It is possible that Mediterranean and Japanese foods and nutraceuticals, i.e. ω -3 fatty acids, antioxidants, flavonoids, essential and nonessential amino acids, vitamins and minerals, are important determinants of enzyme function; hence, these foods and nutrients can suppress the expression of genes responsible for phenotypes of NCDs. Further research is necessary to find out the effects of the high and low ω -6/ ω -3 fatty acids ratio on DNA and chromatin methylation. There is variability in the DNA and RNA functions according to the time structure, which may be further studied as a target for chronotherapy.

In conclusion, nutrient deficiencies or excesses, pollutants and radiation can predispose to cellular stress leading to DNA or chromatin methylation which is essential for cell development and for stabilizing cell function without alteration in DNA sequence. The genes are dormant when chromatin is condensed and they are expressed when chromatin is relaxed, indicating that genetic functions are dependent on chromatin conformation. The compact chromatin is known as heterochromatin and the relaxed one as euchromatin. The patterns of methylation are determined by specific nutrients, inherited genetic polymorphisms and exposure to environmental factors. Nutrients and nutraceuticals provide the methyl group, which is added to the DNA via folate and methionine pathways resulting in DNA methylation. DNA methylation regulates the activity of certain genes, which can reflect a modulation of health or risk for some diseases. It may be possible to find out the genetic signatures to provide individual advice of a suitable nutrient or food for genetic modulation.

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