Effects of Nutraceuticals on Genetic Expressions

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Abstract: The prevalence and mortality due to multifactorial polygenic diseases; hypertension, coronary artery disease (CAD), diabetes and cancer vary depending upon genetic susceptibility and environmental precursors because they have identifiable mendelian subsets. Rapid changes in diet and lifestyle, may influence heritability of the variant phenotypes that are dependent on the nutraceutical or functional food supplementation for their expression. It is possible to recognize the interaction of specific nutraceuticals, with the genetic code possessed by all nucleated cells. There is evidence that South Asians have an increased susceptibility to CAD, diabetes mellitus, central obesity and insulin resistance at younger age, which may be due to interaction of gene and nutraceutical environment. These populations appear to have enherited predisposition and may have interaction of internal nutritional status and environmental factors. Higher intake of refined starches and sugar increases generation of super oxide anion in the leucocytes and mononuclear cells, and free fatty acids (FFA), as well as higher amount and activity of nuclear factor-kB (NF-kB), a transcriptional factor regulating the activity of at least 125 genes, most of which are pro-inflammatory. Glucose intake also causes an increase in two other pro-inflammatory transcription factors; activating protein-1 (AP-1) and early growth response protein-1 (Egr-1), the first regulating the transcription of matrix metallo-proteinases and the second modulating the transcription of tissue factor and plasminogen activator inhibitor-1. Refined food, mixed meal induces activation of NF-kB associated with free radicals generation by mononuclear cells. The super oxide anion is an activator of at least two major pro-inflammatory transcription factors, NF-kB and AP-1. Increased intake of linoleic acid, saturated fat, trans fat and refined starches and sugars can increase the generation of free radicals and activate the NF-kB, leading to rapid expression of proinflammatory genes. It is possible that nutraceuticals; antioxidants, micronutrients, minerals, vitamins, coenzyme Q10 and w-3 fatty acids may inhibit the generation of super oxide and suppress NF-kB as well as AP-1, and Egr-1 leading to suppression of phenotypic expressions. It is known that genes are important in determining enzymes, receptors, cofactors, structural components involved in regulation of blood pressure, the metabolism of lipids, lipoproteins and inflammatory and coagulation factors that are involved in determining individual risk for vascular diseases and diabetes. It seems that these phenotypic expressions may be silenced by targeting simple sequence differences known as single nucleotide polymorphisms by nutraceuticals and slowly absorbed wild foods rich in micronutrients and antioxidants.

Key Words: Single nucleotide polymorphism, chromosome variant, proteome, transcription factor, epigenetics.

INTRODUCTION

It seems that supplementation of nutraceuticals and wild foods as well as wild lifestyle may be protective, whereas western diet and lifestyle may enhance the expression of genes related to chronic diseases. Our genes or pathways are most likely regulated by microRNA [1-4]. It is difficult to tell which miRNA sequences might be responsible? It has been possible now to apply a simple and accurate real-time PCR technique to identify miRNA expression patterns that correlate with biological phenotypes of the disease. Cardiovascular diseases (CVD), diabetes, obesity and cancer are polygenic in nature and their prevalences and mortality vary depending upon genetic susceptibility and presence of risk factor (1-6). It is believed that most humans are deviate, and may inherit risk and may have interaction of nature and nurture [1-6].

Rapid changes in diet and lifestyle may rapidly enhance the expression of harmful genes, which manifest in a sequence. There is a sequence in the emergence of chronic diseases as the diet of the developing countries, becomes more westernized [1]. Overweight and central obesity come first in conjunction with deficiency of angiotensin, and adiponectin, hyperinsulinemia, increase in interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-alpha), followed by pre-metabolic syndrome, hyperlipidemia, diabetes and insulin resistance, hypertension, and gall stones. Coronary artery disease (CAD), and cancer come later and finally there is emergence of dental caries, gastro-intestinal diseases, and bone and joint diseases (Fig. **1**). Rapid changes in nutritional environment can influence the heritability of the variant phenotypes that are dependent on the nutrient environment for their expression. Poor nutrition in fetus may create an adverse nutritional environment, decreasing the possibility of

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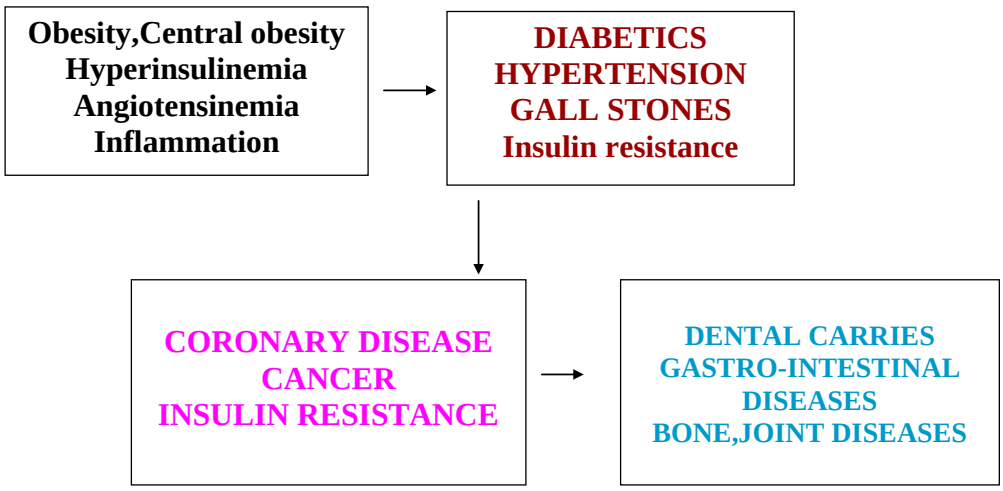

Fig. (1). Emergence of chronic diseases due to interaction of gene and environment (Singh *et al*., 1999, modified from Burkitt and Trowell).

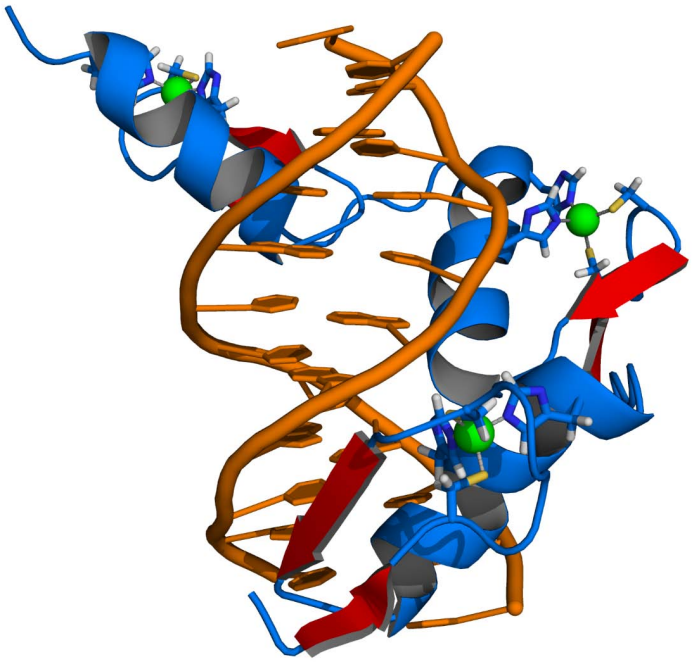

Fig. (2). Early growth response protein 1 (blue color), a proinflammatory transcription factor increases due to glucose ingestion. Contain three zinc fingers in complex with DNA (orange)and middle zinc ion in green. Modulates the transcription of tissue factor and plasminogen activator inhibitor-1. Also called Zif268, a mammalian transcription factor. (modified from ref. [65]).

explaining the cause by a single gene variant, because of adaptations. However, single gene variants may be useful models to measure the other determinants of genetic diseases [4-8]. It is not possible to exactly estimate the nutritional or genetic variance.

There is evidence regarding the role of nutritional and genetic variance in the occurrence of chronic diseases. The genetic variance in cancer appears to have greater genetic component than it is in CAD, hypertension, obesity and diabetes [4-8]. In the primary or secondary prevention of chronic diseases, every effort should be made to prevent the expressions of genotype resulting into phenotype risk factor. These preventive efforts should begin in pregnancy and infancy because there is evidence that the lipoprotein (a) phenotype can change during childhood and possibly also during pregnancy. Lack of energy and iron during pregnancy may cause development of conserving mechanisms in the mother and the fetus, which may be harmful during infancy on modest increase in these nutrients. While iron conservation may increase free radical generation, and can damage the genes, energy conservation may result in to central obesity, on modest intake in food intakes, due to interaction of gene and environmental factors. It is also possible that time structure may also influence the functioning of genes [9-11]. While nutraceuticals and micronutrient rich foods, consumed in the morning may prevent the expression of harmful genes, increased intake of refined foods may cause increased generation of super oxide which may damage the genes, resulting into further increase in the adverse biological environment in the second quarter of 24 hours, in our body.

THE BIOLOGY AND FUNCTIONS OF GENES

The life of an individual, may have formative phase, growth phase, maturation phase and senescent phase and each one of them is being characterized by specific heritable genetic information [2-5].

Designer genes dictate the barricading of cells and cellular recognition during development. There are tissue specific genes responsible for cellular differentiation and organogenesis. House keeping genes maintain the basic requirements of the cells. Isogenic individuals; such as monozygotic twins whose genomic DNA and chromatin complexes are indistinguishable, can show the influence of environment and diet on gene function, independent of each other is not clear. Their epigenone diverges with diet and lifestyle and ageing. The vital roles of genes in the life of a subject can be best understood through the total package of chromatin rather than individual gene [2-4].

Chromatin

Chromatin is a DNA-protein complex in higher organisms. Its diameter is 30nm, whereas the diameter of DNA is 2nm. The chromatin appears as diffuse mass within the nucleus during the interphase of a cell. The new field of epigenetics has emerged with a more impact on cellular transgeneration profiles primarily dealing with the health perspectives. This refers to the heritable changes in the gene expression that occur without a change in 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. The human genome contains nearly 40,000 genes and they tend to express in specific cells at precise times. The region of the genomic DNA comprising of a function specific nucleotide sequence makes up a gene. Each unit of histone, (nuclear proteins) octamer is wraped by genomic DNA, either as compact or relaxed conformation. These units are called nucleosomes. The region of the chromosome which possess the compact chromatin is known as heterochromatin and the relaxed one euchromatin. The temporal status of the gene in either of these conformations appears to be important because environmental modulation of genes is quite possible. The genes are dormant when chromatin is condensed and they are expressed if chromatin is relaxed. Therefore, it seems that genetic functions are dependent on the chromatin conformation. It is possible that by altering nutritional environment, the activity and conformation of the chromatin may be altered, which may result in to genetic expression along with relaxation of chromatin.

It seems that wild foods and nutraceuticals; w-3 fatty acids, antioxidants, vitamins and minerals are important determinent of enzymes, hence these foods and nutrients can suppress the expression of harmful genes.

Several enzymatic machineries; such as methyltransferases, histone deacetylases, histone acetylases, histone methyltransferases and methyl binding chromatin protein are under influence of chromatin complex. In cellular function, a gene is made either awake or silent depending upon specific post translational modifications of histones on one side and methylation of cytosine of phenotype guanine (CpG) islands in the promoter region of a gene on the other side. This results in to a distinct trait for example CpG island methylator phenotype, which are nucleotides in DNA. The unmethylated clusters of CpG pairs may be seen in tissue specific genes and house keeping genes and are foot-prints for transcription factors. The DNA methylation patterns reprogramme cells and tissues in the overall context of individuals life. The epigenetic mechanisms regulate gene accessibility and expressivity depending upon environmental factors. There is evidence that chromatin is a physiological template and modifies histones by covalent coupling with methyl or acetyl groups, resulting into dysregulation or commitment for cellular differentiation. It can establish, maintain and propagate patterns of gene expression, by organizing epigenetic marks. There is strong correlation between tissue specific expression and non methylation of non CpG islands; maspin gene, a tumor suppressor gene. Several chromatin regulatory proteins are dynamic and are continuously recruited, bound and ejected which may be due to environmental factors like dietary proteins, antioxidants and vitamins.

It is possible that cytosine methylation, histone modifications, and nucleosomal remodeling are closely related and under influence of nutrient and nutraceutical environment in the body. Each nucleosome contains characteristic histone octamer constituted by histones dimer proteins of chromatin; H1, H2A, H2B, H3 and H4. The amino acids lysine and arginine residues that are involved in chromatin modification are relatively in higher proportion among histones. Lysine is important for establishing an epigenetic programme and its residues (K) on H3 and H4 are prone for post translational alterations. Methylation of K9, which means lysine, is at 9^{th} position in the histone protein molecule, and K27 in H3 are the epigenetic marks for silenced chromatin. Loss of acetylation at K16 and trimethylation at K20 in H4 are the epigenetic marks for cancer. It is possible that lysine and arginine containing foods or supplementation of these nutraceuticals can influence epigenetic marks and methylation of chromatin, resulting in to protection of genes. Most of the methyl mark on histone has some biological message, called epigenetic information that is maintained through cell cycle. Methylated lysine residues of histones appear to be important epigenetic markers which may be modulated by nutrients and the nutraceuticals.

THE SEQUENCING OF HUMAN GENOME

The genetic variation between people was largely pinned on simple sequence differences known as single-nucleotide polymorphisms (SNPs). This leads to large-scale SNP-mapping ventures, such as the International HapMap Project, to identify regions of the genome underlying phenotypic variation and disease susceptibility [12-18]. However, SNPs are only part of the picture, because most scientists understand that structural differences - including deletions, duplications, inversions, and copy-number variants - encompass millions of bases of DNA, and are at least, as important as SNPs in contributing to genomic variation in humans. We know that gains or losses of large swaths of DNA - known as copy number variants (CNVs) - are common features of the

human genome. Recently, genome-wide studies identified a few hundred CNVs, but because of the techniques used, researchers could detect only large-scale differences of roughly 50 kb and greater [12-14]. The interaction of environmental factors such as radiation, pollutants, magnetic forces and nutritional factors or dietary modulators with SNPs and CNVs need to be studied more clearly [19].

Recent studies, [12, 13] discovered close to 700 finer-scale CNVs within the human genome. These researchers looked for odd patterns in the existing HapMap SNP data, to uncover deletion "footprints. " McCarooll *et al.*, 2006 [12] discovered apparent violations of Mendelian inheritance, while Conrad *et al.*, [13] inspected clusters of SNPs that are out of expected equilibrium frequencies, and other genotyping errors. These experts also showed that deletions and their neighboring SNPs are tightly linked, indicating that most polymorphic deletions have ancient origins. The role of w-3 fatty acids or w-6: w-3 on deletion SNPs and CNVs need further studies. The large number of segregating deletions indicates stability of the genome and extent of genomic dynamism and to the wave of structural variations. It seems that deletion polymorphisms are a "binary CNVs," because only two possible states exist in an individual. The genomic region is either there or it's not. Deletions, however, make up only a small subset of a much larger number of CNVs and structural variants in general.

Feuk helps to maintain the online database of Genomic Variants (http://projects. tcag. ca/variation), which, as of April, contained 9,735 individual variants greater than 100 bp. It is possible that the patterns observed were true for unique regions of the genome, but they're not necessarily true for complex regions where deletions reoccur with high frequencies. Therefore, scientists are using different methods to find structural variants today. This approach helped to construct an unbiased genome-wide CNV map, and discovered around 1,500 CNVs greater than 1 kb covering 12% of the genome [14]. The effect of environmental factors; such as in-utero nutrition on mutations causing structural variance need further clarifications. Further analysis of data showed more than 400,000 deletion and insertion polymorphisms ranging from 1 bp up to 10 kb [15]. He has unpublished data revealing 1. 5 million more deletions, over 99% of which are less than 100 bp. "Add up the bases, and it's almost as many as the known SNPs. Many researchers are also turning to resequencing techniques. A recent comparison of Craig Venter's diploid genome with the human genome reference sequence found close to a million structural variants encompassing around 10 Mb of DNA [16]. New approaches such as high-throughput sequencing and older-generation technologies, are also probing genome-wide variation. Eichler and his colleagues used fosmid clone-based sequencing of eight genomes to identify close to 1,700 CNVs greater than 8 kb, around a third of which were not present in the human reference genome sequence [17].

NUTRIENT, NUTRACEUTICALS AND GENE INTERACTIONS

Wild foods or functional foods are rich in protective nutrients and nutraceuticals. Since nutrient and nutraceuticals have interactions with genes, it poses the possibility that a genetic cause may explain the continued appearance of nutritional disease in the population by nutritional silencing of phenotype expression [2-9]. Nutraceuticals which can influence genes are given in (Table **1**). Nutrients, which can modulate concerned genes or genetic determinants are given in (Table **2**). Polyunsaturated fatty acids (w-6 and w-3), milk, calcium, vitamin, iron, ascorbate and saturated fat have been found to modulate gene expression in various experimental studies [4-8].

Table 1. Nutraceuticals Having Possible Influence on Genes

Nutrient	Effects
Refined carbohydrates (sugar and refined starches)	Adverse
Trans fatty acids	Adverse
Excess of saturated fat	Adverse
Excess of linoleic acid	Adverse
Omega-3 fatty acids	Beneficial
Monounsaturated fatty acids	Beneficial
Calcium, magnesium, potassium,iron	Benefcial
Zinc, copper, selenium, chromium Manganese, molybdenum, cobalt	Beneficial
Coenzyme Q10,carnitine	Beneficial
Lead, mercury, arsenic, cadmium, fluoride	Adverse
Excess of iron	Adverse
Vitamin A and beta-carotine	Beneficial
Pyridoxine, thiamin, riboflavin, cynacobalamin, nicotinic acid, folic acid	Beneficial
Vitamin E	Beneficial
Vitamin C	Beneficial
Vitamin D	Beneficial
Vitamin K	Beneficial
Fibre, (polysaccharides)	Beneficial
Amino acids; arginine, taurine, cystein	Beneficial

Table 2. Dietry Modulators of Genes and Genetic Determinants

Genes and Genetic Determinants	Nutrients
1. Hepetic gene expression	Polyunsaturated fatty acids [PUFA]
2. Hormonal regulating gene encoding enzyme	Fat synthesis
3. Lactose intolerance and lactase	Milk
4. Gastrointestinal lipase gene expression	Fat
5. Gastrointestinal hormone gene expression, mRNA-translation in cells.	Calcium
6. Adipocyte gene expression	Vitamin A
7. Ferritin synthesis	Iron
8. Apolipoprotein B mRNA editing	PUFA, insulin, T3

Many apparently healthy subjects may be walking around with broken copies of genes because several insertion and deletion polymorphisms land in the coding regions of genes. Some individuals homozygous for one of the most commonly deleted genes; *UGT2B17*, may have lower levels of urinary testosterone, suggesting that steroid users might often pass undetected in current athletic doping tests simply based on their DNA [18]. There is only a limited information of a subset of the complete view of structural variations. Many current hybridization probes can reliably detect some CNVs, and two newly developed genotyping platforms from Affymetrix and Illumina include CNV probes in combination with SNP probes. There is a need to design more comprehensive microarray chips dedicated to genome-wide structural variation. This goal might not be far off, because by next year, we may see the first arrays targeted specifically toward structural variation, which may tell us more specifically the role of drugs, wild foods or western foods on genetic variations.

The phenotypic expression for health or disease would depend on phenotype and environment, as well as on genotype and upon structural variations of genes. There is interaction of specific nutrient with the genetic code possessed by all nucleated cells which may cause nutritional modulation of genetic expression for health or disease. There is a limited food supply such as in the rural population of developing countries and lower social classes in urban areas, which also have greater physical activity due to physically demanding occupations [1, 2]. There is also in-utro undernutrition due to wide spread malnutrition during pregnancy common in developing countries [9]. These interactions predispose the biological mechanisms to adapt and develop survival gene which may modulate genotype for increased survival. In urban population of developing countries and immigrants from developing to developed countries, better food supply, usually western diet, may be associated with phenotypic expression for disease [5, 8]. The thrifty gene utilizes the energy with a better capability resulting into obesity on modest increase in energy intake and sedentary behavior. Fatty acids are metabolized more efficiently and misdirected to arterial wall for cell membranes and there is better storage of iron resulting into free radical stress which may damage the genetic code (Table **2**).

The health status of gene, CNVs or SNPs, whether single or polymorphic appear to be important in the manifestation of health or CAD, hypertension or diabetes and obesity (Table **3**). Increased intake of energy, may cause obesity due to expression of obesity genes, which is major cause of cardiovascular disease. In one study [20], subjects were 383 consecutive patients with angiographically confirmed CAD and 368 non-CAD subjects adjusted for age and BMI in the Japanese population. Single nucleotide polymorphisms (SNPs) in the adiponectin gene were determined by Taqman polymerase chain reaction (PCR) method or a PCR-based assay for the analysis of restriction fragment length polymorphism. The plasma adiponectin concentration was measured by enzyme-linked immunosorbent assay. Among SNPs, the frequency of I164T mutation was significantly higher in CAD subjects (2. 9%) than in the control (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 were independent of BMI. In contrast, SNP94 and SNP276, which are reported to be associated with an increased risk of type 2 diabetes, were associated neither with CAD prevalence nor with plasma adiponectin level. Subjects with I164T mutation exhibited a clinical phenotype of the metabolic syndrome. Genetic and environmental risks factors of CAD and the dietary modulators are are given in Table **4**.

Table 4. Gene Status and Phenotypic Expression for Health or Disease

1. Expressed at birth eg. phenylketonuria.
2. Nonevident clinically but expressed e. g. glucose 6-phosophate deficiency evident on fava bean intake
3. Expressed with change in diet and lifestyle
a. Obesity and central obesity on increase in energy
b. Noninsulin dependent diabetes mellitus-energy
c. Hypercholestrolemias and LDL receptors-SF,TF
d. Lipoprotein a,coenzyme Q10,trans fatty acids
e. Homocystenemia,pyridoxine,folic acid,B12
f. Iron storage- free radical stress
g. ACE gene-coenzyme Q10
4. Non expressed

Table 3. Survival Gene and Development of Genotype

Gene Expression	Environmental Factor	Phenotypic Expression
1. Thrifty gene	Excess of food supply	Obesity
2. Conservation of iron during anemia		
3. Lipoprotein transport	Low cholesterol	Atherosclerosis
4. In utero	Under nutrition	
5. Early childhood nutrition		
6. Growth spurt	Rapid changes in lifestyle	

Table 5. Genetic and Environmental Risk Factors of Coronary Disease

Genetic Determinants	Environmental Risk Factors
1. Family history at<50 years of age	1. Smoking
2. Total and LDL cholesterol and Apo A 1 and APO A II levels	2. Sedentary life-style
3. HDL cholesterol, Apo A 1 and Apo,A II levels	3. Diet
4. Apo A-IV-1/1	a. Excess
5. Apo E polymorphism	b. High total and saturated fat
6. Lipoprotein a	c. High trans fatty acids
7. LDL receptor activity	d. High n-6 fatty acids
8. Thrombosis,coagulation parameters	e. High sucrose
9. Triglycerides and HDL levels	f. Low n-3 fatty acids
10. RFL is in DNA at the Apo A-1/Apo C-III and Apo B loci and other DNA markers	g. Low antioxidant
11. Hypertension	h. Low coenzyme Q10 levels
12. Noninsulin dependent diabetes mellitus	i. Low folic acid
13. Obesity and central obesity	j. Low pyridoxine and B12
14. Insulin levels and response	
15. Heterozygosity for homocytinuria	
	4. Type A behavior
	5. Stress
	6. Depression
	7. Anxiety
	8. Social class

MECHANISMS

Cellular stress can occur due to nutrient deficiencies or excesses, pollutants and radiations**.** The patterns of DNA methylation differ in response to specific nutrients, enherited genetic polymorphisms and exposure to environmental factors. Nutrients and nutraceuticals provide the methyl group, which are added to DNA *via* folate and methionine pathways. It is not yet clear which methyl mark accounts for ageing or development of diseases. The packaging and function of human genome are controlled by epigenetic mechanisms. The DNA sequence of humans appears to be under strong influence of genome and packed chromatin which facilitate for 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. Cardiovascular disease, 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 like glycemia, hyperinsulinemia and proinflammatory cytokines. The DNA hypomethylation activates the concerned genes for example, oncogenes in cancer and initiate chromosomal instability. However, DNA hypermethylation may also initiate silencing of protective genes resulting in to cancers. These methylation patterns can develop molecular epigenetic markers for variety of cancers.

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. But exactly how stress causes DNA damage has remained unclear. Human-mouse hybrid cells exposed to an antibiotic that inhibits DNA polymerase and induces mitotic stress -- lead to a high frequency of submicroscopic deletions at a particular genomic site with elevated susceptibility to DNA damage. Exposition of human fibroblast to the same stressful conditions, compared the stressed out cells with their normal counterparts appears to be interesting. It has revealed a suite of sequence copy number changes -- deletions and duplications -- between the stressed sample and control DNA. The deletions varied from 25 to around 1,300 kilobases, while the duplications were slightly larger, ranging from 143 to around 2,800 kilobases. In one instance, the researchers observed the same deletion in two independent cell lines, indicating that there might be a predictable pathway to stress-induced DNA damage. Sequencing of the deletions' breakpoint junctions, revealed that they were all characterized by short equivalent DNA sequences called microhomologies. This pattern is consistent with a particular form of DNA repair that uses microhomologies to mend DNA damage, known as nonhomologous end joining rather than other genetic fix-it methods that rely on matching DNA templates. Since the observed deletions and duplications closely resembled CNVs seen in screens of human

diversity, as well as spontaneous DNA changes implicated in diseases such as cancer, stress during cell division is likely a major contributor to both normal and aberrant genomic copy number changes.

REFINED FOODS, CLOCK GENES, AND CARDIAC EVENTS, IN THE MORNING?

There is a need to identify genes that express according to time structure, because more than half of the cardiovascular events (stroke, sudden cardiac death and heart attacks), other vascular variability disorders (VVDs), including blood pressure variability, coronary constriction, endothelial dysfunction, rise in blood pressure and heart rate occur between 8. 00AM to 11. 00AM. There is evidence of increased oxidative stress and decrease in plasma levels of antioxidants; coenzyme Q10, vitamin A, E and C, during this period, characterized with increased catecholamines and cortisol levels in the plasma. These dietary deficiencies in conjunction with refined foods may have adverse effects on healthy cholesterol making it atherogenic. There is evidence that increased intake of meals rich in refined carbohydrates, w-6 fatty acids, saturated fat and trans fat and decreased consumption of w-3 and phytochemicals can cause increased generation of super oxide anion and free fatty acids, resulting in to endothelial dysfunction. The super oxide anion can damage the nutrophils and liver cells which may be associated with increased concentration of TNF-alpha, interleukin-6 and interleukin-18, which are proinflammatory cytokines known to predispose atherothrombosis resulting in to heart attack, sudden cardiac death and stroke in the morning.

It is known that the signature of a circadian gene is characterized in such a way, that its expression levels oscillate once each day according to time structure [10, 11]. Some genes peak in the evening, others in the daytime, but all such genes cycle on a 24-hour period consistent with sleep and awakening. Dietary intakes of wild foods rich in antioxidants and w-3 fatty acids appear to have important role in the pathogenesis and prevention of cardiovascular disease [19-32]. We understand, that at the heart of the fly's circadian rhythm, lies a core set of "pacemaker" genes, which control the fly's sleep-wake cycle and its daily rhythms in temperature and hormones. Much mapping of clock genes was done under constant conditions in so far as light or darkness are concerned and free-running rhythms under these conditions do not allow you to extrapolate to clock hour and there is the added problem that mapping under conditions of alternating light and darkness is done often on a usually nocturnally active organism and you deal with diurnally active humans.

In earlier studies, a total of 458 unique genes were identified. They all used essentially the same fly stocks, the same lab protocols for isolating RNA, and the same Affymetrix GeneChip technologies. Seven genes were found in common among all five studies, and these included most, but not all, of the known pacemaker genes. We still need to find out about the hundreds of other genes, which may be responsible for coronary thrombosis, that occurs in the second quarter of 24 hours and which may be related to sleep and wake cycle. Keegan and Allada who discovered, a list of 214 genes, more than half of which were not found in the earlier studies, are important [10, 11]. The types of genes they found also ran the gambit of functions, from protein kinases to ion channels. Further studies are needed to find out, how many of these genes could be responsible for increased concentration of super oxide anion and pro-inflammatory cytokines; IL-6, IL-18, IL-1,2 and TNF-alpha that may be determinent of a rupture of hot coronary plaque, resulting in to heart attack, sudden death and stroke in the morning. Because western foods may enhance the expression of these genes and wild foods may be protective.

It is clear that Keegan and Allada's technique added a key element: an ANOVA test that screened the list of candidate genes and tossed out those whose expression levels did not significantly peak and trough over 24 hours. It seems that this method drastically reduced the number of genes, and when they combined all five data sets, they uncovered a suite of new genes whose activity levels cycled on a daily basis. It is possible, that a cosinar analysis of various genes, according to time structure, may through further light on the charaterization of clock genes discovered by these investigators. Such analysis appears to be quite important because increased consumption of refined starches and sugar in the breakfast, increases generation of super oxide anion in the leucocytes and mononuclear cells, FFA, as well as higher amount and activity of nuclear factor-kB (NF-kB), a transcriptional factor regulating the activity of at least 125 genes, most of which are pro-inflammatory [24]. Ingestion of glucose also causes an increase in two other pro-inflammatory transcription factors; activating protein-1 (AP-1) and Egr-1, the first regulating the transcription of matrix metalloproteinases and the second modulating the transcription of tissue factor and plasminogen activator inhibitor-1. Since Americans consume a heavy breakfast rich in pro-inflammatory foods; refined carbohydrates, trans fatty acids, saturated fat and w-6 fat, this poses the possibility that these foods act as a trigger for the peaking of some of the clock genes in the morning after 8. 00AM, causing excess of circadian rhythm of cardiovascular events in the second quarter of 24 hours of the day. Taking Wild food breakfast; Columbus eggs, almonds, walnuts, Columbus soup, Columbus oil, fruits, vegetables, whole grains may inhibit the expression of these genes and may be protective.

NUTRACEUTICALS AND GENETIC MODULATION: THE COLUMBUS CONCEPT

Omega 3 fatty acid is an important nutraceutical. Since w-6 fatty acid is proinflammatory, excess of w-3 fatty acid up to a ratio of 1:1 is being advised for suppression of adverse effects of w-6 fatty acids and prevention of chronic diseases according to Columbus concept (www. columbus-concept. com). Proper function of blood cholesterol is related to the presence of essential nutrients characteristic of wild food, including omega-6/3 fatty acids, antioxidant vitamins and minerals, which prevent the atherogenecity of blood cholesterol and supported mankind evolution. Wild foods whether of animal or plant origin, are rich sources of w-3 fatty acids. Foods that comply with the Columbus Concept promote a balance ratio of omega-6 and omega-3 fatty acids (ω6:ω3-PUFAs = 1:1) in plasma total lipids and, in turn, protect mankind against modern chronic degenerative

diseases. Modern chronic degenerative diseases have their roots deeply intertwined with those of the inherited fight-or-flight phenotype of human's hunter-gatherer ancestor. Inflammatory processes that are at the basis of such phenotype have on the long run deleterious effects on homeostasis and health. Those genetically predisposed develop the disease with age and - under certain conditions - transfer the disease to their offspring, constitutionally. In South Asians, there is marked increase in w-6/w-3 fatty acids ratio to 50 which may be a cause of their increased susceptibility to CAD and diabetes, because of the interaction of gene and environment. Fatty acid metabolism appears to be important in the pathogenesis, progression and prevention of cardiovascular disease [19-32]. The functions of EPA, DHA and AA appear to have competition in metabolism. Increased intake of fish or fish oil can antagonise AA from membrane phospholipids in practically all cells, platelets, erythrocytes, nutrophils, monocytes, endothelial, arterial smooth muscle and liver cells, which is protective [4-7]. Diets in both, developing and industrialized countries have become rich in w-6 fatty acids, which enhances the production of eicosanoid metabolic products whereas w-3 fatty acids are known to have least of these adverse effects. These eicosanoids are biologically active and may cause thrombosis, atherosclerosis as well as allergies and inflammatory disorders. However, w-3 fatty acids lead to a state of increased production of prostanoids, anti-vasoconstrictive and anti-inflammatory products which may have beneficial effects *via* genetic modulations. The genetic and environmental risk factors common in others are also common in south Asians, increasing their predisposition to CAD.

Several candidate proteins have been identified in the search for the signaling pathway involved to study the effects of specific nutrients on gene transcription. The metabolic path-ways and cellular growth are under continuous influence of dietary w-6 and PUFA as well as highly USFA [33]. While supplementation of long chain PUFA (e. g., EPA) enhances mitochondrial and peroxisomal fatty acid oxidation [34, 35]; linoleate consumption suppresses the hyperproliferation of keratinocytes associated with essential fatty acid deficiency [36], arachidonate promotes cellular growth in chemically induced mammary cancer [37] and stimulates *in vitro* the conversion of preadipocytes to adipocytes [38]. Omega-3 fatty acid rich diets can modulate mRNAs, encoding several lipogenic enzymes within hours of feeding of the animals [39, 40]. If w-3 PUFA remains in the diet, these effects are sustained. The fatty acids act like a hormone to control the activity or abundance of key transcription factors. It seems that some fatty acids can act as hormones that control the activity of transcription factors. It is possible that fatty acids are passive energy-providing molecules as well as are also metabolic regulators.

Application of molecular biology techniques indicated that PUFA elicit changes in gene expression that precede changes in membrane composition by directly governing the activity of nuclear transcription factors [17, 41]. PUFA regulation of gene transcription occurred within a matter of minutes: such a time frame was too rapid to be explained simply by changes in membrane composition and altered hormone release or signaling, but is most consistent with a ligand mediated event [17]. Recent research indicates novel perspectives for deeper understanding of energy metabolism and therapeutic interventions. Peroxisome proliferater -activated receptor- α [PPAR- α] was the first transcription factor identified as a prospective fatty acid receptor [42]. PPAR- α plays a role in the regulation of an extensive network of genes involved in glucose and lipid metabolism. In animal models, w-6 and w-3 fatty acids are potent inducers of fatty acid oxidation and potent suppressors of fatty acid and triacylglycerol synthesis [34, 35, 41, 42]. It is clear that PUFA are potent PPAR activators.

Experimental studies revealed that, in animals fed with a diet rich in 20-carbon and 22-carbon PUFA, the expression of the genes may be associated with high rates of fat oxidation and reduced body fat deposition [44-46]. It has been established that the 5'flanking regions of genes encoding carnitine palmitoytransferase, acyl-CoA oxidase, mitochondrial hydroxymethylglutaryl-CoA synthase, fatty acyl-CoA synthetase and mitochondrial uncoupling proteins all contain DNA recognition sequences for PPAR [43-46]. However, studies with the PPAR- α null mouse have shown that PPAR- α is not the sole transcription factor involved in mediating fatty acid effects on gene transcription.

Desvergne and Wahli, reported that activated PPAR-y induces lipoprotein lipase and fatty acid transporters and enhances adipocyte differentiation as well as inhibits the function of the transcription factor NF-kB and cytokines, and therefore COX-2 expression [42]. PPAR- γ also binds 20:5 n-3. In experimental studies, drug induced activation of PPAR- α and PPAR-γ, reduces lipid levels in muscle and adipose tissue and improves insulin sensitivity [47, 48]. Omega-3 PUFA are weak agonists of PPAR as compared with drug agonists (e. g. thiazolidinediones), these fatty acids have significant effects on insulin sensitivity in various tissues, particularly skeletal muscle [49].

PPAR requires the formation of heterodimers with retinoid X receptors (RXR), in order to bind with DNA and activate transcription. Apart from PPAR family (PPAR- α, β, -y1, and -y2) several other transcriiption factors have been indentified as targets for fatty acid regulation. These are hepatic nuclear factor-4α (HNF- 4α), sterol regulatory element-binding protein (SREBP), liver X receptors (LXR- α and -β), retinoid X receptors (RXR-α), and NF-kB [17, 50-53]. PUFA antagonize oxysterol activation by liver X receptors (LXR)- α in HEX 293 and hepatoma cell lines by interfering with oxysterol binding. The targets for fatty acid regulation may be; the liver X receptors (LXR- α and LXR-β) [8, 53]. LXRs bind oxysterols and regulate the expression of genes involved in hepatic bile acid synthesis [54]. It plays an important role in lipogenesis, *via* regulation of transcription of the gene, encoding the SREBP-1c isoform [55]. This is a transcription factor required for the insulin-mediated induction of hepatic fatty acid and triglyceride synthesis [56]. PUFA also suppresses the nuclear content of SREBP -1c [17]. The hierarchy for fatty acid regulation of mRNA SREBP-1c levels is 20:5n-3=20:4n-6>18:2n-6>18:1n-9.

Mediterranean or Indo-Mediterranean diets or any other diet supplemented with olive, corn, soybean or walnut oil at<20% of total calories suppress hepatic lipogenic gene expression by suppressing the transcription of many genes involved in *de novo* lipogenesis including fatty acid synthase,

stearoyl -CoA desaturase -1, L-Pyruvate kinase, and S14 protein [40, 57-60]. It is possible that fatty acid regulation of hepatic *de novo* lipogenesis and fatty acid oxidation was not mediated through a common factor, i. e. PPAR- α. coupling this action with the PUFA-mediated induction of PPAR- α-regulated genes shifts hepatic metabolism away from lipid synthesis and storage toward lipid oxidation [57, 60]. This mechanism prevents lipotoxicity associated with lipid overload. All glycolytic and lipogenic genes that are suppressed by dietary PUFA do not contain recognition sites for SREBP-1c. It seems that PUFA regulation of the SREBP-1c isoform appears to be a key player in PUFA suppression of lipogenic genes. The nucleus of the liver cells may have a second PUFA-regulated transcription factor.

Hepatic nuclear factor -4 (HNF-4) may be most suitable to complete above role [61]. HNF-4 is also a member of the steroid receptor super family. Like PPARs, HNF-4 appears to enhance the promoter activity of selected genes such as fatty acid synthase. This enhancer activity is suppressed when PUFA esters bind to the ligand domain of the HNF-4. A sequence is also a component of the PUFA response region of the pyruvate kinase gene, apart from, an HNF-4 recognition [62]. Thyroid hormone (TRs) play an important role in metabolism, growth and differentiation. One study found that PUFA inhibited binding of T3 to TR- α and TR-β [63]. Transfection studies using primary hepatocytes have failed to show that TR or thyroid hormone response elements are major targets for PUFA regulation of these genes [63-69]. An exception to this is seen when n-3 PUFA activate PPAR-α, leading to a sequestration of RXR. PPAR requires the formation of heterodimers with RXR and inhibition of gene transcription through the interference with T3 action at the thyroid hormone response elements [64]. It seems that several T3-regulated hepatic genes are suppressed by PUFA particularly w-3 fatty acids. NCX1. 3 is more sensitive to inhibition by ALA than NCX1. 1. In addition, only w-3 PUFA inhibits 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 [70]. 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 all diabetics may not have similar risk of vascular disease [71]. 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 single nucleotide polymorphisms, genetic irregularities regularly used to study human disease and genetic variation, as markers to probe a mixture of DNA for an individual's genetic signature. This finding poses the possibility of providing individual advice of a suitable nutraceutical or wild foods for genetic modulation of the concerned problem.

In brief, these studies indicate that nutraceuticals and wild foods that are rich sources of various nutraceuticals; w-3 fatty acids and antioxidants, can modulate genetic function and gene expression and may be important in the pathogenesis and prevention of chronic diseases of affluence. Further studies are required to demonstrate that a ratio of w-6/w-3 of 1:1 in the blood by nutraceutical supplementation can modulate the genes and provide further protection against CVD, diabetes and cancer. These manipulations according to time structure or as chronotherapy may be highly rewarding.

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