Neutrigenomics: A New Frontier

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ABSTRACT

Nutrigenomics, which may be defined as the application of genomic tools to study the integrated effects of nutrients on the gene regulation, however, holds great promise in increasing the understanding of how nutrients affect molecular events in an organism for development and progression of various diseases. It provides a molecular and genetic understanding for how common dietary chemicals (i.e.: nutrition) affect health by altering the expression and/or structure of an individuals genetic makeup. The fundamental concept of the field are that the progression from a healthy phenotype to a chronic diseases phenotype must occur by change in gene expression or by differences in activities of proteins and enzymes and that dietary chemicals directly or indirectly regulate the expression of genomic information. One could focus on the effects of the nutrients of food bioactives on the regulation on gene expression (i.e.: nutrigenomics) or on the impact of variations in gene structure on one's response to nutrients or food bioactives (i.e.: nutrigenetics). The challenge of public health nutritionist will be to balance the needs of the community with those of the individual. In this regard, the excitement and promise of molecular nutrition should be tempered by the need to validate the scientific data emerging from the disciplines of nutrigenomics and nutrigeneties and the need to educate practitioners and communicate the value to consumers- and to do it all within a socially responsible bioethical framework.

INTRODUCTION

The success of human genome project and the powerful tools of the molecular biology have ushered in a new era of medicine and nutrition, nutrigenomics may be defined as the application of genomic tools to the study the integrated effect of nutrients on gene regulation and herds great promise, increasing the understanding of how nutrients affects molecular events in on organism.

Indeed this is a new validation of old Hippocrates philosophy "Let food be thy medicine and medicine thy food".

The working definition of nutrigenomics is that it seeks to provide a genetic and molecular understanding for how common dietary chemicals (i.e., nutrients) affect the balance between health and disease by altering the expression and/or structure of an individual's genetic makeup.[1] Dietary chemicals include nutrients and bioactive chemicals that do not directly produce energy but exclude man-made chemicals such as pesticides. This new branch of genomic and nutritional research can best be summarized with the following five tenets: and disease by altering the expression and/or structure of an individual's genetic makeup.[1]

1. Common dietary chemicals and nutrients directly or indirectly act on the human genome to alter gene expression or structure.
2. Under certain circumstances and in some individuals, diet can be a serious risk factor for a number of diseases.
3. Some diet-regulated genes (and their normal, common variants) are susceptibility genes and likely to play a role in the onset, incidence, progression, and/or severity of chronic diseases, (multifactorial disorder: polygenic).
4. The degree to which diet influences the balance between healthy and disease states may depend on an individual's genetic makeup. (e.g.: efficient genetic polymorphism and nutrient metabolism)
5. Dietary intervention based on knowledge of nutritional requirement, nutrition status, and genotype (i.e., "individualized nutrition") can be used to prevent, mitigate, or cure chronic disease.

This new area of molecular nutrition that is, nutrient–gene interaction can unfold dichotomous directions.

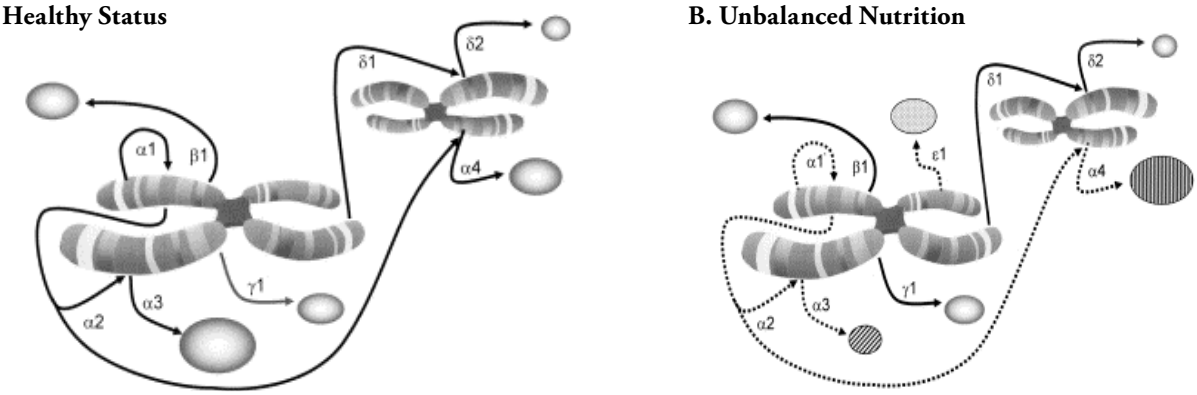

Fig. 1: Genotype × environment interactions. Example of gene expression in healthy (A) and unbalanced nutritional (B) states.

(A) Gene α1 is a global transcription factor regulating α2 (and other transcription) factors. In turn, α2 regulate α3 and α4 expression. Another transcriptional regulator, δ1, affects δ2 expression. Genes encoding β1, γ1, δ1, and δ2 are not regulated by transcription factors influenced by dietary chemicals.

(B) Unbalanced nutrition alters the expression (----) of α1, ultimately decreasing the amount of α3 but increasing the expression of α4 Gene ε1 is expressed in response to changes in metabolism or the altered concentration of a dietary ligand. Some transcription factors or expression of individual genes may not be affected directly or indirectly by dietary chemicals (e.g., β1, γ1 and δ1, δ2), whereas others (α1 through α4 and ε1) may be affected. The effects on transcription may result from increased concentrations of ligand derived from the diet acting on a reference or variant (single nucleotide polymorphism) gene sequence. Changing the concentration of effectors proteins (α3, α4, ε1) will alter metabolic flux and concentrations of metabolites, inducing further changes in cell physiology.

Table 1: Nutrients Deficiency and DNA damage

Nutrients	DNA damage	Health effect
Folic acid	Chromosome break and hampers DNA repair	Colon cancer, heart disease, brain dysfunction
Vitamin B_{12}	Unknown	Same as folic acid, memory loss
Vitamin B_6		Same as folic acid
Niacin	Hampers DNA repair	Nerve problem, memory loss
Vitamin	Mimics radiation damage	Cataract, cancer
Vitamin E	Mimics radiation damage	Colon cancer, heart disease, immune dysfunction
Vitamin D	Prevent gene variation	Colon, breast, prostate cancer
Zinc	Chromosome breaks	Brain and immune dysfunction

Adapted from UC Davis Center for Excellence Nutritional Genomics

We are reviewing key concepts that have emerged from epidemiologic, nutritional, molecular, and genetic experiments examining associations between genes and disease. The results and lessons from these different fields of research will affect the design, strategies, and approaches for nutritional genomic research and specifically for identifying diet-regulated and genotype- and diet-regulated genes involved in susceptibility, onset, incidence, progression, and/or severity of chronic diseases.

Although many chemicals in foods are nutrients, i.e. they are metabolized to energy or involved in key metabolic reactions (e.g. vitamins), some naturally occurring chemicals in foods are ligands for transcription factors and directly alter gene expression, whereas other dietary chemicals alter signal transduction pathways and chromatin structure to indirectly affect gene expression. Epidemiologic studies have repeatedly shown that intake of different diets are associated with the incidence and severity of chronic diseases.[2,3] Over consumption of energy,[3] proteins, types of fats or carbohydrates,[4-6] or lack of key micronutrients[7] are associated with obesity, T2DM, CVD, certain cancers, developmental defects, and neurological diseases such as Alzheimer's.

At the cellular level nutrient may:

1. Acts as a ligand for transcription factor receptor.
2. Be metabolized by primary, secondary pathways, thereby altering concentration of substrate or intermediates, or
3. Positively or negatively affects signal pathway.

Various studies are analyzing diet or responses to dietary changes with single nucleotide polymorphism (SNP) in candidate genes. Dietary chemicals may preferentially interact with one or more variants (i.e., susceptibility genes) to increase or decrease disease risk (Fig. 1).

GENOTYPE -AND- ENVIRONMENT INTERACTIONS

The concept of gene-and-environment interactions is not new to nutrigenomics,[8] but its definition and use are not always consistent. The precise, statistical definition of gene–environment interaction is "a different effect of an environmental exposure on disease risk in persons with different genotypes" or "a different effect of a genotype on disease risk in persons with different environmental exposures."[9]

In humans, many diseases are related to suboptimal nutrition in terms of deficits of essential nutrients, imbalance of macronutrients, micronutrients, or even toxic concentrations of certain food compounds. Biomedical research arena has unraveled a good number of molecular "disease mechanisms." Currently, the two disciplines are well on their way to closely interact.[10] Thus, we realize more and more that the nutrition

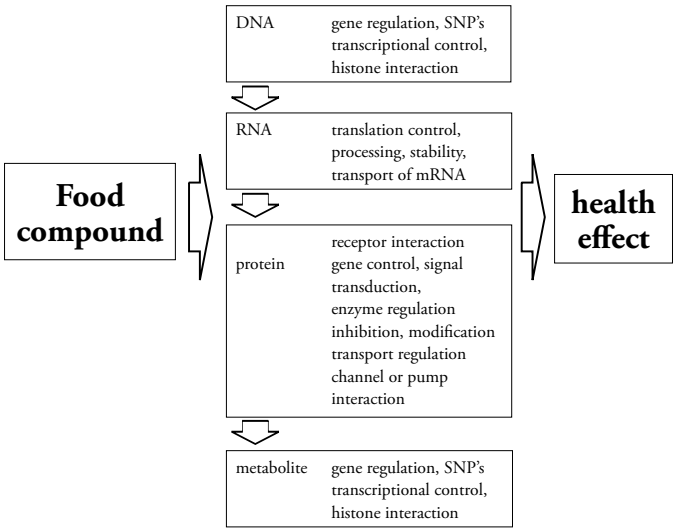

Fig. 2: Health effects of food compounds are related mostly to specific interactions on a molecular level. SNP, (single nucleotide polymorphism).

and health relationship is solidly anchored in interactions on the levels of DNA, RNA, protein, and metabolites. (Fig. 2)

Our diet consists of complex mixtures of many possible bioactive chemical compounds, chronically administered in different compositions, and with a multitude of biological effects. The vast majority of these biological responses are mediated through effector genes, effects on enzyme concentration or activity, and changes in metabolite concentration (Fig. 1).

Of course, not all individuals react identically to nutrition. If nutrigenomics describes changes in gene expression related to a specific nutritional intervention, deviations in genes will have an impact on these transcriptome changes and ultimately on the physiologic function. On average, each of our genes contains ten deviations in its code from the "standard gene." Of course, not all of these polymorphisms have a functional impact. A relatively small number of these polymorphisms have serious health implications and may even be lethal. This is the domain of clinical genetics. Many polymorphisms, however, have only a mild effect on the functionality of the resulting protein. It is here that, within certain limits of "health," a large variety in response to nutrition is observed.

Paradoxically, food itself may contribute to this diversity, because there are many examples in which nutritional compounds directly cause DNA damage or modulate susceptibility (in the positive and negative sense) against DNA damage through regulation of specific pathways involved in many processes involved in these events.

MICRONUTRIENTS, MACRONUTRIENTS: EFFECTS ON GENE

Approximately 40 micronutrients are required in human diet. Suboptimal intakes of specific micronutrients have been associated with CVD (B vitamins, vitamin E, carotinoids), cancer (folate, carotinoids) neural tube defect (folate) and bone mass.[11] Deficiency of vitamin B_{12}/B_6/folic acid/niacin/, vitamin C and E or iron and zinc appear to mimic radiation in damaging DNA

Table 2 : Some important Single Nucleotide Polymorphism (SNP) of B-vitamins genes associated with various clinical conditions:

C677 T variant of 5,10 methylene tetrahydrofolate reductase gene	• Colon cancer • Spina bifida • Down syndrome • Leukemia • Oral cleft • Risk of vascular diseases due to elevated homocysteine • Complication of pregnancy (pre-eclampsia, recurrent pregnancy loss, fetal growth retardation)
A 1298C variant of 5,10 methyle netetrahydrofolate reductase gene	• Spina bifida • Leukemia
A 2756G variant of methionine synthase gene	• Thromboembolic diseases
A66G variant of methionine synthesis gene	• Spina bifida • Down syndrome
C1420T variant of serine hydroxy-methyletransferase gene	• Leukemia
2R3R variant of thymidylate synthatase gene	• Leukemia
C1561T variant of glutamate carboxypeptidase gene	• May affect cardiovascular diseases

by causing single and double strand breaks, oxidative lesion or both.[12] A number of other degenerative diseases of aging are also associated with low fruit and vegetable intake. Progress is also being made in determining specific mechanisms for the role of certain minerals (calcium, magnesium, manganese, copper and selenium) and vitamins in heart disease from work in humans in cell culture systems.[13] Unbalanced intake of any of the three major macronutrients, fats, carbohydrates, proteins, contributes to the initiation, development progression, and/or severity of chronic disease.

The hunt for a single macronutrient or micronutrient that will prevent chronic diseases is destined to fail. It is more likely that dietary imbalances, from micronutrients deficiencies to overconsumption of macronutrients of dietary supplements, are the modifiers of metabolism and potentiates of chronic diseases. Although the complexity of food and genotypic variations appears daunting, molecular and genetic technologies may provide the means for identifying causative genes (or their variants) and the nutrients that regulate them.

Based on the interindividual differences, it is tempting to speculate on "personalized nutrition" based on genotyping differences. Of course, without stressing the genetic background of variation of nutritional response, specific subgroups are already targeted with "subgroup nutrition" (e.g., cholesterol-lowering margarines). A debate is arising as to whether nutrition should enter into the area of linking genetic differences with tailor-made nutrition. Apart from the social, ethical, and communication issues involved, from a scientific point of view, a big challenge is ahead of us in validating the combined action of these minor-

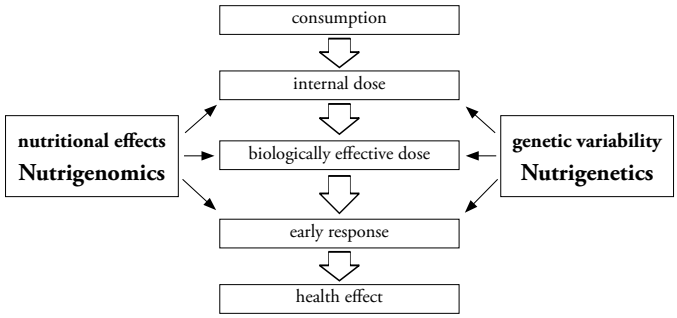

Fig. 3 : Nutritional efficacy is subject to external and internal variability. The human genome affects the relation between nutrition and health in two ways: 1) genetic variation, resulting in interindividual differences in response, with implications toward susceptible subgroups in the population (nutrigenetics), and 2) the effect of the many bioactive compounds in our nutrition on gene expression and the resulting changes in physiology (nutrigenomics).

impact polymorphisms and their practical effect on the relation between nutrition and health (Fig. 3).

ROLE OF FOLIC ACID IN NEUTRIGENOMICS

Preface to folic acid

Dietary phytochemicals, e.g. quercetin-a flavinoid can modulate gene expression related to oxidative stress and anti-oxidant defense system. Riboflavin, folic acid, cobalamin may improve the picture of cystathiol deficiency.

Folic acid is the mostly studied and clinically utilized, hence we take these opportunities to unfold the story of folate.

Human genomic project revolutionized the process of localizing and identifying the genes that involved in the diseases.

To date 1000 human diseases gene identified; 97% of which causing monogenic diseases,[14] however most of the chronic diseases (obesity, diabetes, cardiovascular diseases, cancer) are due to complex interaction between several genes and environmental factors.

More complete single nucleotide polymorphism (SNP) and halo type maps are helpful in identifying the genes involve in the diseases.

Deficiency of folic acid and other macro and micronutrients appear to mimic radiation in damaging DNA by causing single and double strand breaks, oxidative lesion or both. Nutrient deficiencies are orders of magnitude more important than radiation because of constancy of exposure to milieu promoting DNA damage.[15-17] Folate deficiency breaks chromosome due to substantial incorporation of uracil in human DNA. Single strand break in DNA are subsequently formed during base excision repair, with two nearby single-strand breaks on opposite DNA strands leading to chromosomal fragmentation.

In humans folate level and variation of different genes that code the folate-dependent enzymes are linked to many diseases like cancer, vascular diseases, birth defects and complications of pregnancy.[18] In humans the genomic machinery is very much sensitive to folate and vitamin B status and responsible to interaction between folate nutrition and folate-dependent enzyme polymorphism (folate neutrigenomics).

Mechanisms that may affect include:

1. Maintenance of genomic CpG methylation pattern (which regulate gene expression).
2. Synthesis of nucleotide to prevent DNA damage.
3. Influence plasma homocysteine status, thus risk of vascular diseases.

This complex relationship is shown in Fig. 4.

Currently, worldwide interest in folate research due to discovery of several single nucleotide polymorphism (SNP) which modulate risk of several diseases[19,20] (Table 2).

Dietary folate interacts with proteins that are encoded by various genes and reduces the risk to development of various diseases, and gives overt protection against the diseases.

Table 2 : shows the consequence of SNP of 5, 10 methylenetetra hydrofolate reductase in terms of dTMP nucleotide biosynthesis, DNA methylation, homocysteine metabolism; all these are related with pathology of cancer and vascular and developmental diseases.

Direct biochemical effects

Folate stabilizes the polymorphic enzyme, encoded by C677 T variant gene, by preventing it from relinquishing its flavin cofactors.

Several studies suggest that as 5,10 methylenetetrahydrofolate reductase is a flavin protein, people with TT recessive genotype may respond more rapidly to riboflavin (vitamin B) supplements as well as folate to lower homocysteine.

Nucleotide biosynthesis

dTMP synthesized by thymidylate synthatase from dUMP and requires the one carbon unit of 5, 10 methylenetetrahydrofola te. dTMP is used by DNA. If there is low level of folate, uracil misincorporation occurs, leading to breakage of DNA strand, which predisposes to cancer.

The polymorphic enzyme coded by C677 T variant genes can enhance the synthesis of dTMP nucleotide if folate status is good, and this is thought to afford protection against colon cancer and leukemia.

Polymorphism in gene for MTHFR

A common functional polymorphism in the gene for methylenet etrahydrofolate reductase (MTHFR, a major enzyme involved in folate metabolism) is associated with an increased risk for colorectal cancer. Dietary folate and methionine intake modify colorectal cancer risk in people with MTHFR polymorphism. A recent report from the National Health and Nutrition Examination Survey (NHANES I) found a statistically significant 60% risk reduction in colon cancer in men and a similar nonsignificant effect in women.[21]

Nurses Health Study showed that folate in women who used alcohol had a 25% reduction in breast cancer risk.

Recently a team of American and Chinese researchers showed that folic acid have protective effect against breast cancer, it's effect pronounced when taken with other vitamins especially B_6, B_{12} and methonine. Researchers believe that folic acid exerts it's protective effect by preventing errors in DNA replication and by

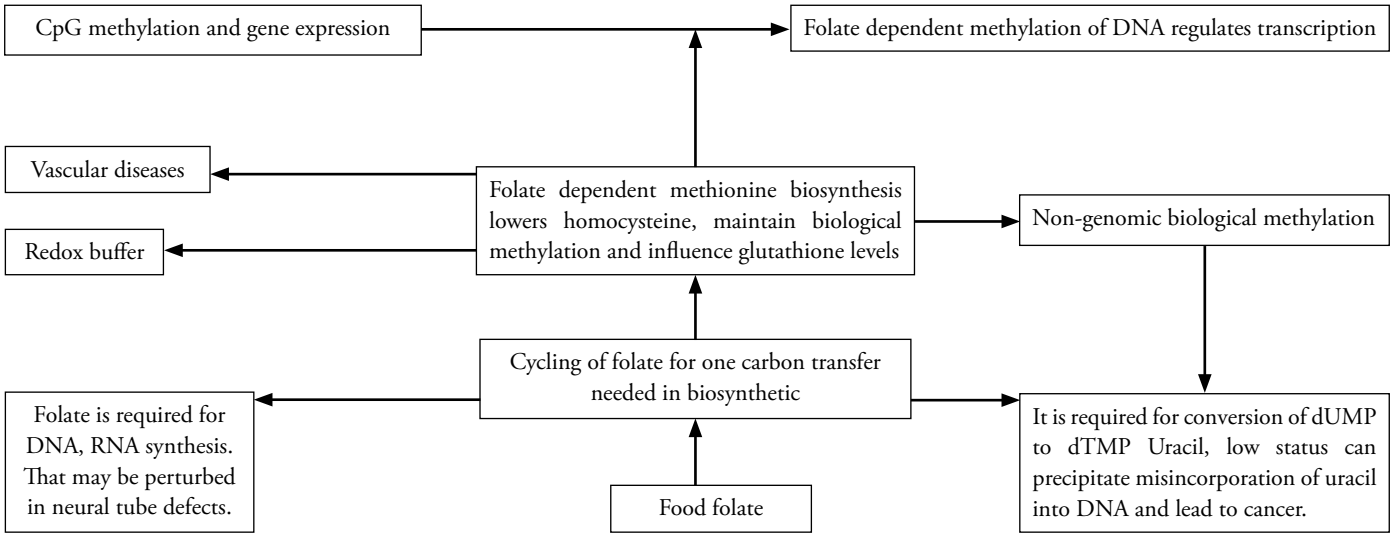

Fig. 4 : Molecular mechanism affected by dietary folate.

helping to regenerate methionine, a vital component of DNA synthesis, vitamin B_6, B_{12} and act as cofactors required for folic acid to "do it's job".[22]

If folate status is poor, the single nucleotide polymorphism may confer risk rather than protection.

Biological Methylation

As dietary methionine cannot provide all methyl groups for cellular methylation reaction, there is requirement of de novo synthesis of methionine from folate one carbon pool. S-adenosylmethionine (AdoMet) regulates protein, biogenic amine, lipid, and DNA methylation. AdoMet dependent DNA methylation of specific CpG site regulates gene expression and play critical role in the developmental process.

Methylation of cluster of CpG sites associated with promoter regions tends to silence gene expression.

A deficiency of methyl group may therefore alter the normal control of proto-oncogene expression. The polymorphic enzyme encoded by the C677T variant gene may reduce availability of de novo methyl groups for this important reaction.

As folate is necessary in embryogenesis its supplementation reduces the risk of neural tube defects. Various studies proved that folate supplementation decreases the risk of first occurrence of neural tube defect and recurrent defects in women with a previously affected pregnancy.[23,24]

Homocysteine Metabolism

Polymorphic 5, 10 methylenetetrahydrofolate reductase reduces one carbon flux to methylfolate, the donor molecule for conversion of homocysteine into methionine. This single nucleotide polymorphism may thus elevate homocysteine which is the independent risk factor for the cardiovascular diseases.

Homocysteine is atherogenic and undergoes redox cycling in the presence of transition metal ions, forming radical that causes oxidative damage to low density lipoprotein. It also reacts with cysteine SH groups and modifies apolipoprotein. It is also a hypertensive compound, reacts with endothelium-derived relaxation factor to form S- nitrosohomocysteine and superoxide. This leads to loss of vasodilatation action. Several studies concluded that as homocysteine promote atherosclerosis through oxidative stress and by encouraging endothelial dysfunction, hyperhomocysteinemia associated with coronary artery diseases. It is generally accepted that folic acid supplementation reduce the risk of CAD, recently researchers at the Queen Elizabeth II Health Sciences Center reported that supplementation with 5 mg folic acid /day significantly decreases the endothelial dysfunction.[25]

It also inhibits and downregulates anticoagulants, including prostacycline synthesis, activation of protein C, thrombomodulin expression, heparin sulphate expression and fibrinolysis. People with inflammatory bowel diseases (ulcerative colitis, Crohn's disease) have high risk of thromboembolic events such as stroke and peripheral venous thrombosis. Researchers point out that the patients with Crohn's disease may benefit from supplementation of folic acid.[26]

In addition, it activates procoagulant such as factor V and tissue clotting factor.

Other effects include proliferation of vascular smooth muscles and increased platelet coagulability. Its final effects are to chelate copper and inhibit lysyl oxidase which impairs cross-linking of collagen and elastin and leads to connective tissue abnormalities.

CONCLUSION

Although relatively new technology, the various genomic applications searching for new receptors and pathways already have found their way to many nutritional applications. Moreover, the new science of nutritional systems biology is emerging, taking up the challenge of exploiting all available data generated by genomics technology in a complete description of a biological system. As a consequence, this new paradigm is ideally fit for the evaluation of many subtle changes in biological activity as triggered by nutrition. In this case, a multitude of bioactive

compounds act simultaneously and chronically in constantly changing combinations.

Propelled by recent unrevealing of human genomic and the coinciding technological developments, genotyping, transcriptomics, proteomics and metabolomics are now available to nutritional research. In future we are likely to see new screening tools for the selection of bioactive nutrients, new biomarkers for the in vivo efficacy of nutrients, and better insight into the influence of genetic polymorphisms on nutrient metabolism. However, are these promise just based on biotechnological hype or is it a real fundamental change in human nutritional science at hand?

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