Genome health nutrigenomics and nutrigenetics – diagnosis and nutritional treatment of genome damage on an individual basis

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Abstract

The term nutrigenomics refers to the effect of diet on gene expression. The term nutrigenetics refers to the impact of inherited traits on the response to a specific dietary pattern, functional food or supplement on a specific health outcome. The specific fields of genome health nutrigenomics and genome health nutrigenetics are emerging as important new research areas because it is becoming increasingly evident that (a) risk for developmental and degenerative disease increases with DNA damage which in turn is dependent on nutritional status and (b) optimal concentration of micronutrients for prevention of genome damage is also dependent on genetic polymorphisms that alter function of genes involved directly or indirectly in uptake and metabolism of micronutrients required for DNA repair and DNA replication. Development of dietary patterns, functional foods and supplements that are designed to improve genome health maintenance in humans with specific genetic backgrounds may provide an important contribution to a new optimum health strategy based on the diagnosis and individualised nutritional treatment of genome instability i.e. Genome Health Clinics.

Keywords: Genome health; Nutrigenomics; Nutrigenetics; DNA damage; Micronutrients; Vitamins; Minerals; Polymorphisms

1. Introduction

The central role of the genetic code in determining genome stability and related health outcomes such as developmental defects and degenerative diseases such as cancer is well established (Ames, 2006; Ames and Wakimoto, 2002; Ames, 2003; Fenech and Ferguson, 2001; Fenech, 2005; Egger et al., 2004; Fenech, 2002; Rajagopalan and Lengauer, 2004; Nathanson et al., 2001; Thompson and Schild, 2002). In addition it is evident that DNA metabolism and repair is dependent on a wide variety of dietary factors that act as co-factors or substrates in these fundamental metabolic pathways (Ames, 2006; Ames and Wakimoto, 2002; Ames, 2003; Fenech and Ferguson, 2001; Fenech, 2005). DNA is continuously under threat of major mutations from conception onwards by a variety of mechanisms which include point mutation, base modification due to reactive molecules such as the hydroxyl radical, chromosome breakage and rearrangement, chromosome loss or gain, gene silencing due to inappropriate methylation of CpG at promoter sequences, activation of parasitic DNA expression due to reduced methylation of CpG as well as accelerated telomere shortening (Egger et al., 2004; Fenech, 2002; Rajagopalan and Lengauer, 2004). The main challenge to a healthy and long life is the ability to continue to replace senescent cells in the body with fresh new cells with normal genotypes and gene expression patterns that are tissue-appropriate. Understanding the nutritional requirements for genome health maintenance of stem cells is essential in this regard but has so far not been adequately explored.

While much has been learnt of the genes involved in DNA metabolism and repair and their role in a variety of pathologies, such as defects in BRCA1 and BRCA2

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genes that cause increased risk for breast cancer (Nathanson et al., 2001; Thompson and Schild, 2002), much less is known of the impact of cofactor and/or micronutrient deficiency on DNA repair. Put simply, a deficiency in a micronutrient required as a co-factor or as an integral part of the structure of a DNA repair gene (e.g. Zn as a component of the DNA repair glycosylase OGG1 involved in removal of oxidised guanine or Mg as a co-factor for several DNA polymerases) could mimic the effect of a genetic polymorphism that reduces the activity of that enzyme (Ames, 2006; Ames and Wakimoto, 2002; Ames, 2003). Therefore nutrition has a critical role in DNA metabolism and repair and this awareness is leading to the development of the new fields of genome health nutrigenomics and genome health nutrigenetics (Fenech, 2005). The critical aim of these fields is to define optimal dietary intakes for prevention of DNA damage and aberrant gene expression for genetic sub-groups and ultimately for each individual.

2. Evidence linking genome damage with adverse health outcomes

Genome damage impacts on all stages of life. There is good evidence to show that infertile couples exhibit a higher rate of genome damage than fertile couples (Trkova et al., 2000) when their chromosomal stability is measured in lymphocytes using the cytokinesis-block micronucleus (CBMN) assay (Fenech, 2000, 2007) (Fig. 1). Infertility may be due to a reduced production of germ cells because genome damage effectively causes programmed cell death or apoptosis which is one of the mechanisms by which grossly mutated cells are normally eliminated (Narula et al., 2002; Ng et al., 2002; Hsia et al., 2003). When the latter mechanism fails reproductive cells with genomic abnormalities may survive leading to serious developmental defects (Liu et al., 2002; Vinson and Hales, 2002). That an elevated rate of chromosomal damage is a cause of cancer has been demonstrated by ongoing prospective cohort studies in Italy and the Scandinavian countries which showed a 2 to 3-fold increased risk of cancer in those whose chromosomal damage rate in lymphocytes was in the highest tertile when measured 10–20 years before cancer incidence was measured (Bonassi et al., 2000). It has also been shown that an elevated micronucleus frequency in lymphocytes predicts cancer risk in humans (Bonassi et al., 2007). Chromosomal damage is also associated with accelerated ageing and neurodegenerative diseases (Thompson and Schild, 2002; Fenech, 1998; Bonassi et al., 2001; Joenje and Patel, 2001; Shen and Loeb, 2001; Lansdorp, 2000; Migliore et al., 1999, 2001). Those individuals with accelerated ageing syndromes (e.g. Down syndrome) and sub-optimal DNA repair (e.g. carriers of deleterious mutations in the ATM or BRCA1 genes) may be particularly susceptible to the genome damaging effects of sub-optimal micronutrient intake.

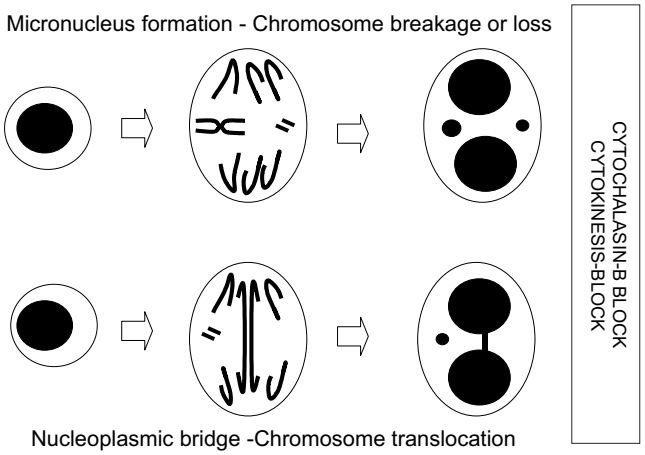

Fig. 1. Expression of micronuclei (MNi) and nucleoplasmic bridges (NPBs) during nuclear division. MNi originate from either (a) lagging whole chromosomes (top panel) that are unable to engage with the mitotic spindle due to a defect in the spindle, or a defect in the centromere/kinetochore complex required to engage with the spindle or (b) an acentric chromosome fragment originating from a chromosome break (top and bottom panel) which lags behind at anaphase because it lacks a centromere/kinetochore complex. Mis-repair of two chromosome breaks may lead to an asymmetrical chromosome rearrangement producing a dicentric (i.e. two centromeres) chromosome and an acentric fragment (bottom panel) – frequently the centromeres of the dicentric chromosome are pulled to opposite poles of the cell at anaphase resulting in the formation of a nucleoplasmic bridge (NPB) between the daughter nuclei. NPBs are frequently accompanied by a micronucleus originating from the associated acentric chromosome fragment. Because MNi and NPBs are only expressed in cells that have completed nuclear division it is necessary to score these genome instability biomarkers specifically in once-divided cells. This is readily accomplished by blocking cytokinesis using cytochalasin-B (for more detailed explanation refer to Fenech (2002, 2000, 2007)).

3. The concept of genome damage as a marker of nutritional deficiency

There is overwhelming evidence that several micronutrients (vitamins and minerals) are required as cofactors for enzymes or as part of the structure of proteins (metalloenzymes) involved in DNA synthesis and repair, prevention of oxidative damage to DNA as well as maintenance methylation of DNA. The role of micronutrients in maintenance of genome stability has recently been extensively reviewed (Ames, 2006; Ames and Wakimoto, 2002; Fenech and Ferguson, 2001; Fenech, 2003). The main point is that genome damage caused by moderate micronutrient deficiency is of the same order of magnitude as the genome damage levels caused by exposure to significant doses of environmental genotoxins such as chemical carcinogens, ultra-violet radiation and ionising radiation. An example from our laboratory is the observation that chromosomal damage in cultured human lymphocytes caused by reducing folate concentration (within the normal physiological range) from 120 nmol/L to 12 nmol/L is equivalent to that induced by an acute exposure to 0.2 Gy of low linear energy transfer (LET) ionising radiation (e.g. X-rays), a dose of radiation which is approximately ten times greater than the annual allowed safety limit of exposure for radiation workers

(IAEA, 1986) (Fenech, 2005). If moderate deficiency in just one micronutrient can cause significant DNA damage it is reasonable to be concerned about the possibility of additive or synergistic effects of multiple moderate deficiencies on genome stability. Clearly there is a need to start exploring the genotoxic effects of multiple micronutrient deficiencies, as well as excesses, which are prevalent in human populations. This aspect is analogous to genetic studies that explore, for example, the combined effects of polymorphisms in DNA repair genes on DNA damage.

4. Results from a recent epidemiological study suggest that at least nine micronutrients affect genome stability in humans in vivo

We recently reported the results of an epidemiological study on 190 healthy individuals (mean age 47.8 years, 46% males) designed to determine the association between dietary intake, measured using a food frequency questionnaire and genome damage in lymphocytes (Fenech et al., 2005a) measured using the CBMN assay (Fig. 1). Multivariate analysis of base-line data showed that (a) highest tertile of intake of vitamin E, retinol, folic acid, nicotinic acid (preformed) and calcium is associated with significant reductions in MN frequency, i.e. −28%, −31%, −33%, −46%, and −49%, respectively, (all $P < 0.005$) relative to lowest tertile of intake and (b) highest tertile of intake of riboflavin, pantothenic acid and biotin was associated with significant increases in MN frequency, i.e. +36% ($P = 0.054$), +51% ($P = 0.021$), and +65% ($P = 0.001$), respectively, relative to lowest tertile of intake (Fig. 2). Mid-tertile β-carotene intake was associated with an 18% reduction in MN frequency ($P = 0.038$), however, the highest tertile of intake (>6400 μg/d) resulted in an 18% increment in MN frequency. We were interested in investigating the combined effects of calcium or riboflavin with folate consumption because epidemiological evidence suggests that these dietary factors tend to interact in modifying the risk of cancer (Lamprecht and Lipkin, 2003; Willett, 2001; Xu et al., 2003) and they are also associated with reduced risk of osteoporosis and hip fracture (Cagnacci et al., 2003; Sato et al., 2005; Macdonald et al., 2004). Interactive additive effects were observed such as the protective effect of increased calcium intake (−46%) and the exacerbating effect of riboflavin (+42%) on increased genome damage caused by low folate intake. The results from this study illustrate the strong impact of a wide variety of micronutrients and their interactions on genome health depending on level of intake.

As shown in Fig. 3, the amount of micronutrients that appear to be protective against genome damage vary greatly between foods and careful choice is needed to design dietary patterns optimised for genome health maintenance. Because dietary choices vary between individuals, due to taste preferences which may be genetically determined or cultural or religious constraints, several options

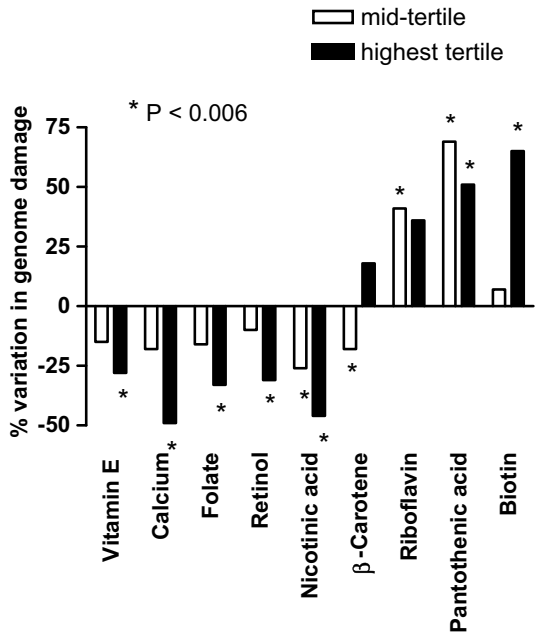

Fig. 2. Percentage variation in genome damage rate for mid- and highest tertile of intake of vitamin E, calcium, folate, retinol, nicotinic acid, beta-carotene, riboflavin, pantothenic acid and biotin relative to the lowest tertile of intake. Genome damage rate was measured in peripheral blood lymphocytes using the cytokinesis-block micronucleus assay. For more information refer to Fenech et al. (2005a).

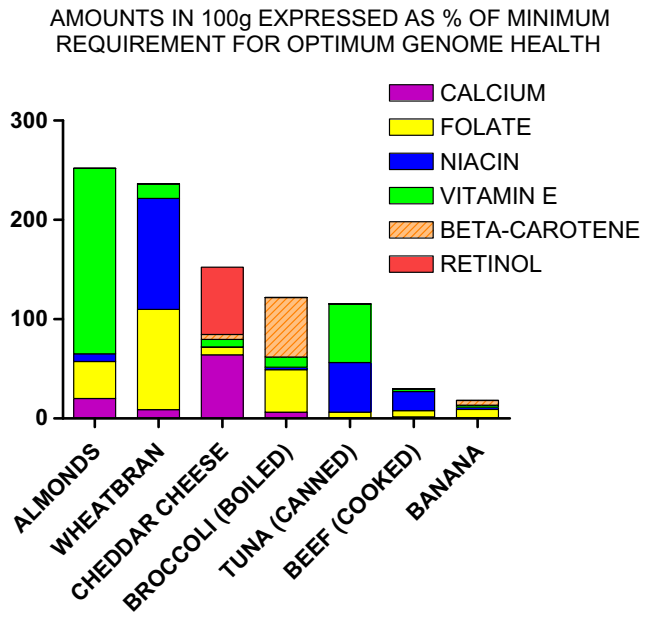

Fig. 3. Content of micronutrients associated with reduced DNA damage in selected common foods. The height of each bar for each micronutrient within the separate foods corresponds to the amount of the micronutrient expressed as the percentage of the minimum daily intake associated with a reduced micronucleus frequency index in lymphocytes as determined in the study of Fenech et al. (2005a). The relative contribution of each of the micronutrients (if present) is indicated by the height of each specifically coloured bar. The nutrient content of the foods was determined using published food content tables (Paul and Southgate, 1978).

are required and supplements may be needed to cover gaps in micronutrient requirements. Clearly the development of nutrient dense foods and ingredients, such as aleurone flour which is rich in bioavailable folate as well as other micronutrients (Fenech et al., 2005b; Beetstra et al., 2006), is essential in making it feasible for individuals to achieve their daily nutrient requirements for genome health maintenance without intake of excess calories.

An important consequence of these considerations is also the need to start defining recommended dietary allowances for all nutrients based on prevention or minimisation of genome damage.

5. Genome health nutrigenomics and genome health nutrigenetics

Two of the important emerging areas of nutrition science are the fields of nutrigenomics and nutrigenetics. The term nutrigenomics refers to the effect of diet on gene expression. The term nutrigenetics refers to the impact of genetic differences between individuals on the response to a specific dietary pattern, functional food or supplement on a specific health outcome. The specific fields of genome health nutrigenomics (Fenech, 2005) and genome health nutrigenetics (Fig. 4) are proposed on the premise that a more useful approach to prevention of diseases caused by genome damage is to take into consideration that (a) inappropriate nutrient supply can cause significant levels of genome mutation and alter expression of genes required for genome maintenance and (b) common genetic polymorphisms may alter the activity of genes that affect bioavailability of micronutrients and/or the affinity for micronutrient cofactor in key enzymes involved in DNA metabolism or repair. Supplementation of diet with appropriate minerals and vitamins could, in some cases, help overcome inherited metabolic blocks in key DNA maintenance pathways (Ames, 2003, 2004). Increasing concentration of a cofactor by supplementation is expected to be particularly effective when a mutation (polymorphism) in a gene decreases the binding affinity for its cofactor resulting in a lower reaction rate. The interaction between genotype and diet in modulating risk is emerging as an exciting area of research as regards micronutrient effects on DNA. This is illustrated by recent research on the common mutations in the methylene-tetrahydrofolate-reductase (MTHFR) gene and other genes in the folate/methionine cycle with regard to their modulating affect on risk of developmental defects and cancer (Skibola et al., 1999; Chen et al., 1999; Fenech, 2001). Recent results from our laboratory have shown that there are important significant interactions between the MTHFR C677T polymorphism, its cofactor riboflavin and folic acid with respect to chromosomal instability (Kimura et al., 2004). This is illustrated by (a) the reduction in nuclear bud frequency (a biomarker of gene amplification) in TT homozygotes relative to CC homozygotes for the MTHFR C677T mutation and (b) the observation that high riboflavin concentration increases nuclear bud frequency under low folic acid conditions (12 nM folic acid) probably by increasing MTHFR activity which diverts folate away from dTTP synthesis, increasing the odds for uracil incorporation into DNA, the generation of breakage-fusion-bridge cycles and subsequent gene amplification and nuclear bud formation. Clearly the relative impact of genetic factors and nutrients on genome maintenance and their interactions needs better understanding so that appropriate knowledge on the most critical factors is developed. Our in vitro studies on the interactive effects of folic acid deficiency and inherited mutations in the MTHFR, BRCA1 and BRCA2 genes indicated that moderate deficiencies in folic acid have a stronger impact on genome instability measured by the cytokinesis-block micronucleus assay than these important inherited mutations (Kimura et al., 2004; Fenech et al., 1999) which again emphasises the magnitude of the impact of diet on genome maintenance.

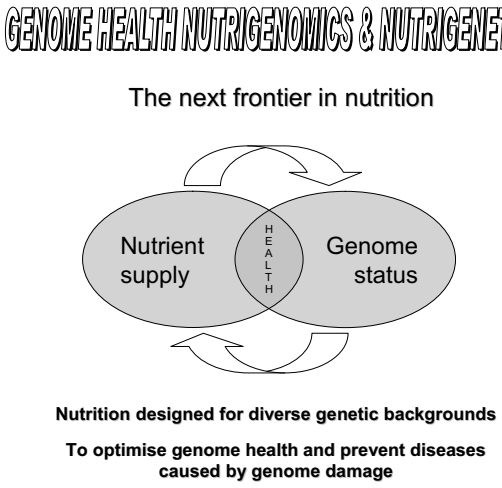

Fig. 4. Concept diagram showing the interdependence of the genome and nutrient supply with respect to genome health maintenance. Genome health nutrigenomics is the science of how nutrient intake or supply affects genome stability and gene expression which ultimately affects and modifies genome status which can vary with time. Genome health nutrigenomics is the science of how inherited characteristics determine bioavailability and bioefficacy of micronutrients required for genome health maintenance. It is essential to appreciate that this is a dynamic process because nutrient status can effectively alter genotype depending on mutations induced by inappropriate nutrition. A possible extreme example is a cancer cell that has a very different genotype to the host cells from which it originated which leads to a more complicated scenario in which nutritional intake needs to be modified in a way that optimises genome health of host cells whilst suppressing growth of cancer cells.

6. The Genome Health Clinic concept – a paradigm shift in disease prevention based on the diagnosis and nutritional treatment of genome damage on an individual basis

The advances in our knowledge described above have opened up a new opportunity in disease prevention based on the concepts that (a) excessive genome damage is the

most fundamental cause of developmental and degenerative disease, (b) genome damage caused by micronutrient deficiency is preventable, (c) accurate diagnosis of genome instability using DNA damage biomarkers that are sensitive to micronutrient deficiency is technically feasible and (d) it is possible to optimise nutritional status and verify efficacy by diagnosis of a reduction in genome damage rate after intervention. Given the emerging evidence that dietary requirement of an individual may depend on their inherited genes, we can anticipate (a) important scientific developments in the understanding of the relationships between dietary requirement and genetic background to optimise genome stability and (b) that the accumulated knowledge on dietary requirements for specific genetic subgroups will be used to guide decisions by the practitioners of this novel preventive medicine in what might be called "Genome Health Clinics". In other words, one can envisage that instead of diagnosing and treating diseases caused by genome damage, health/medical practitioners will be trained, in the near future, to diagnose and nutritionally prevent the most fundamental initiating cause of developmental and degenerative disease i.e. genome damage itself. The feasibility that it is possible to reduce DNA damage in placebo-controlled trials using either a folic acid with vitamin B12 combination or a mixture of antioxidant micronutrients has already been demonstrated (Fenech et al., 2005a, 1998). However, the real challenge is to tailor the doses to individuals so that benefit is maximised and any potential harm from excess supplementation is eliminated in those who do not require supplementation. This novel approach also opens up the possibility for the large numbers of health-conscious consumers to be able to assess directly the effect of their dietary and nutritional supplement choices on their genome and that of their children. In addition there will be scope to develop new dietary patterns, functional foods and supplements for genome health that can be mixed and matched so that they are appropriately tailored to an individual's genotype and genome status. It is evident that knowledge on the genetic factors and metabolome parameters that predict who is more likely or least likely to benefit from specific micronutrient supplementation is required to maximise the potential beneficial impact of this strategy. An important aspect of this strategy is also to minimise exposure to genotoxic dietary factors (e.g. carcinogens generated by high temperature cooking and excessive alcohol consumption) particularly in those with susceptible genotypes.

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