REVIEW

The Genome Health Clinic and Genome Health Nutrigenomics concepts: diagnosis and nutritional treatment of genome and epigenome damage on an individual basis

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The evidence of a direct link between increased genome/epigenome damage and elevated risk for adverse health outcomes during the various stages of life, such as infertility, foetal development and cancer is becoming increasingly stronger. The latter is briefly reviewed against a background of evidence indicating that genome and epigenome damage biomarkers, in the absence of overt exposure of genotoxins, are themselves sensitive indicators of deficiency in micronutrients required as cofactors or as components of DNA repair enzymes, for maintenance methylation of CpG sequences and prevention of DNA oxidation and/or uracil incorporation into DNA. The latter is illustrated with cross-sectional and dietary intervention data obtained using the micronucleus assay and other efficient biomarkers for diagnosing genome and/or epigenome instability. The concept of recommended dietary allowances for genome stability and how this could be achieved is discussed. The ‘Genome Health Nutrigenomics’ concept is also introduced to define and focus attention on the specialized research area of how diet impacts on genome stability and how genotype determines nutritional requirements for genome health maintenance. The review concludes with a vision for a paradigm shift in disease prevention strategy based on the diagnosis and nutritional treatment of genome/epigenome damage on an individual basis, i.e. The Genome Health Clinic.

Introduction

The central role of the genetic code in determining health outcomes such as developmental defects and degenerative diseases such as cancer is well established. In addition, it is evident that DNA metabolism and repair is dependent on a wide variety of dietary factors that act as cofactors or substrates in these fundamental metabolic pathways (1–3). The DNA inherited from our parents is continuously under threat of major mutations, from conception onwards, by a variety of mechanisms which include point mutation, base modification owing 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 owing to reduced methylation of CpG as well as accelerated telomere shortening (4–6). It is true to say that all of the above mechanisms of genome damage occur spontaneously because of the effects of endogenously generated mutagens and/or because of deficiency in cofactors required for DNA metabolism and repair and/or exposure to environmental genotoxins. However, it is also true that genetic defects in DNA metabolism and repair, the latter involving more than 100 genes in humans (7,8), are also a key factor. Although 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 that cause increased risk for breast cancer (8,9), much less is known of the impact of cofactor and/or micronutrient deficiency on DNA repair. In simple words, a deficiency in a micronutrient required as a cofactor 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 the removal of oxidized guanine or Mg as a cofactor for several DNA polymerases) could mimic the effect of a genetic polymorphism that reduces the activity of that enzyme (1,10).

Therefore, nutrition has a critical role in DNA metabolism and repair and this awareness is leading to the development of the new field of Genome Health Nutrigenomics (3,11). The purpose of this paper is to:

(i) briefly review the link between genome instability and adverse health outcomes during the various stages of life;
(ii) examine the evidence for genome instability as a marker of nutritional deficiency;
(iii) explain the application of the micronucleus (MN) assay as an efficient biomarker for diagnosing genome instability and nutritional deficiency;
(iv) briefly discuss dietary and genetic factors that cause epigenetic change and the use of the MN assay as a biomarker of altered CpG methylation;
(v) introduce the concept of recommended dietary allowances (RDAs) for genome stability and how this could be achieved;
(vi) provide some insight into the importance of the emerging field of genome health nutrigenomics;
(vii) propose and introduce the framework for a disease prevention strategy based on the diagnosis and nutritional treatment of genome and epigenome damage, i.e. the Genome Health Clinic concept.

The evidence linking genome damage with adverse health outcomes during the various stages of life

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 (12), when their chromosomal stability is measured in lymphocytes using the MN assay (13). The 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 eliminated (14–16). When the latter mechanism fails, reproductive cells with genomic abnormalities may survive leading to serious

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developmental defects (17, 18). There is ample evidence to show that many genotoxic carcinogens are also teratogens (19, 20). Specific micronutrient deficiencies that cause genome damage may themselves cause developmental defects in the foetus or increased risk of cancer in the child. Specific examples, include (i) increased oxidation of sperm DNA in humans with inadequate vitamin C intake (21) and the aggravating effect of vitamin C deficiency on diabetes-induced teratogenesis (22); (ii) neural tube defects in folate-deficient human foetuses at deficiency levels that coincide with increased genome damage rate (23–25) and the increased risk of childhood leukaemia in children of mothers who did not take folic acid supplements during pregnancy (26); (iii) the observation that zinc deficiency, which induces oxidative damage to DNA and impairs DNA repair, is itself teratogenic (27, 28); and (iv) increasing rates of human male infertility and testicular cancer may be linked via a common mechanism, i.e. increased genome damage events in spermatogonial stem cells owing to environmental genotoxins and/or micronutrient deficiency (29).

The probability of mutations is relatively high during early development and childhood, because a much larger proportion of cells are in DNA synthesis phase during which cells may be more prone to insult by genotoxins or genome damage by insufficiency of micronutrients required for the synthesis of nucleotides (e.g. folate and vitamin B12) needed for DNA replication, DNA repair and maintenance of DNA methylation patterns (30–32). That an elevated rate of chromosomal damage is a cause of cancer has been proven by ongoing prospective cohort studies in Italy and the Scandinavian countries which demonstrated a 2- to 3-fold increased risk of cancer in those whose chromosomal damage rate in lymphocytes was shown to be in the highest tertile, when measured 10–20 years before cancer incidence was measured (33).

Chromosomal damage is also associated with accelerated ageing and neurodegenerative diseases. Several studies have shown that chromosomal abnormalities, including MN frequency, increase progressively with age in somatic cells (34, 35). Accelerated ageing and cancer-prone syndromes, such as progeria, Bloom’s syndrome, Fanconi’s anaemia and Werner’s syndrome, exhibit increased chromosomal instability and/or accelerated telomere shortening because of defects in a variety of genes essential for DNA repair and telomere maintenance, such as ATM, PARP, BRCA1, BRCA2 and DNA helicases (8, 36–38). Equally interesting is the observation that neurodegenerative diseases, such as Alzheimer’s disease and Parkinson’s disease, exhibit much higher rates of MN frequency in human peripheral blood lymphocytes (39, 40).

In the case of Alzheimer’s disease, there is also evidence that the frequency of cells exhibiting trisomy 21 is elevated, which leads to the hypothesis that these individuals may be mosaics for the Down’s syndrome phenotype which is associated with accelerated ageing and increased risk of neurodegenerative disease (39).

Increased chromosomal DNA damage may be partly due to inefficient or incorrect DNA repair, which increases the sensitivity of an individual’s cells to normal genotoxic stresses. A typical example are cells from individuals with truncation mutations in the BRCA1 and BRCA2 genes, which result in a highly penetrant condition for increased breast cancer risk (41). These genes are required for the error free homologous recombinational repair of double-stranded breaks in DNA (8). In the absence of normal function of these genes, non-homologous end-joining repair occurs and leads to exchanges between chromosomes and the formation of abnormal chromosomal structures such as dicentric chromosomes and chromosome fragments (41), which can be measured as increased nucleoplasmic bridge (NBP) and MN formation in interphase (5, 42–45). These abnormal chromosomes lead to a chromosomal instability phenotype because of the difficulty in segregating dicentric chromosomes equally between cells leading to the formation of the so-called breakage-fusion-bridge (BFB) cycles which, in turn, lead to gene amplification and altered gene dosage [for detailed account of these mechanisms see Fenech (5)]. This genome instability phenotype, involving BFB cycles, is typical of most cancer cells (46, 47).

Apart from faulty DNA repair, other defects in mechanisms involved in chromosome segregation may lead to abnormal chromosome number or aneuploidy, an event that is increasingly being considered as potentially cancer-initiating and a definite cause of developmental abnormalities. Important mechanisms include defects in assembly of the spindle, inadequate mitotic cycle checkpoints and abnormal replication of the centrosome that coordinates the assembly of the spindle (5, 6). Duesberg and co-workers (48, 49) have suggested that induction of aneuploidy (abnormal number of chromosomes) either by a chemical agent or by other means such as a genetic abnormality in the mechanism of chromosome segregation, or a defect in microtubule polymerization owing to a deficiency in an essential cofactor, such as magnesium (50), may cause altered dosage of oncogenes and tumour suppressor genes. The role of centrosome abnormalities in cancer first proposed by Boveri (51) is now increasingly being confirmed for a variety of cancers, such as prostate cancer, in which the level of these abnormalities has been shown to accumulate with progression to a more malignant state (52, 53). Abnormal centrosome replication leads to multi-polar mitoses and aneuploidy (54, 55). The micronutrients required for proper centrosome replication and function remain to be unknown and uninvestigated.

The concept of genome damage as a marker of nutritional deficiency

There is overwhelming evidence that a large number of 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 reviewed extensively (2,3). Examples of micronutrients involved in various genome stability processes are given in Table I. The main point is that micronutrient deficiency can cause genome damage and, as explained below, the increments in genome damage caused by micronutrient deficiency are of the same order of magnitude, if not greater, than the genome damage levels caused by exposure to significant doses of environmental genotoxins such as chemical carcinogens, ultraviolet radiation and ionizing radiation. An example from our laboratory is the observation that the chromosomal damage in cultured human lymphocytes caused by reducing folate concentration from 120 to 12 nmol/l is equivalent to that induced by an acute exposure to 0.2 Gy of low-linear energy transfer (LET) ionizing radiation (e.g. X-rays)—a dose of radiation which is ~10 times greater than the annual allowed safety limit of exposure for the general
micronutrients (such as Zn, vitamins C and E) or folate, methionine and vitamin B12 are deficient (7,62).

The MN assay as a biomarker for diagnosing genome damage and nutritional deficiency

Micronuclei (MNi) originate from chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division (Figure 2) (5,63). The MN index in rodent and/or human cells has become one of the standard cytogenetic measurements used in genetic toxicology testing of chemicals and radiation in vivo or ex vivo. MNi can be easily measured in erythrocytes, buccal cells or lymphocytes to obtain a measure of genome damage induced in vivo. MNi in erythrocytes originate from genome damage events in their precursors (normoblasts) in the bone marrow (for more detailed explanation see Figure 3). MNi in buccal cells originate from genome damage events in the basal layer of the oral mucosa (for more detailed explanation see Figure 4). In the case of lymphocytes it is possible to score MNi expressed in vivo directly (64); however, a more comprehensive approach is to culture the lymphocytes because this allows a measure of genome damage that is accumulated while lymphocytes circulate around the body in the quiescent phase (5,65). Lymphocytes have a half-life of 3–6 months and travel throughout the body, integrating genotoxic events across body tissues (56). In comparison, buccal cells and erythrocytes turn over every 21 and 120 days, respectively (66,67).

The earliest studies on the relationship between MN induction and micronutrient deficiency were performed using the erythrocyte MN assay. In fact MNi were first noted by haematologists, and they are still referred to as Howell–Jolly bodies in recognition of the scientists who first described the relationship between megaloblastic anaemia in humans and the prevalence of MNi in erythrocytes or in their immature stage, i.e. reticulocytes (68). This was the first evidence that folate and/or vitamin B12 deficiency induces chromosomal

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### Table I. Examples of the role and the effect of deficiency of specific micronutrients on genomic stability

<table>
<thead>
<tr>
<th>Micronutrients</th>
<th>Role in genomic stability</th>
<th>Consequence of deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C, vitamin E</td>
<td>Prevention of oxidation to DNA and lipid oxidation (157,158)</td>
<td>Increased baseline level of DNA strand breaks, chromosome breaks and oxidative DNA lesions and lipid peroxide adducts on DNA (157,158)</td>
</tr>
<tr>
<td>Folate and vitamins B2, B6 and B12</td>
<td>Maintenance methylation of DNA; synthesis of dTMP from dUMP and efficient recycling of folate (24)</td>
<td>Uracil misincorporation in DNA, increased chromosome breaks and DNA hypomethylation (24)</td>
</tr>
<tr>
<td>Niacin</td>
<td>Required as substrate for poly(ADP-ribose) polymerase (PARP) which is involved in cleavage and rejoining of DNA and telomere length maintenance (61,159)</td>
<td>Increased level of unrepaired nicks in DNA, increased chromosome breaks and rearrangements, and sensitivity to mutagens (61,159)</td>
</tr>
<tr>
<td>Zinc</td>
<td>Required as a cofactor for Cu/Zn superoxide dismutase, endonuclease IV, function of p53, Fapy glycosylase and in Zn finger proteins such as PARP (27,28)</td>
<td>Increased DNA oxidation, DNA breaks and elevated chromosome damage rate (27,28)</td>
</tr>
<tr>
<td>Iron</td>
<td>Required as component of ribonucleotide reductase and mitochondrial cytochromes (160)</td>
<td>Reduced DNA repair capacity and increased propensity for oxidative damage to mitochondrial DNA (160)</td>
</tr>
<tr>
<td>Magnesium</td>
<td>Required as cofactor for a variety of DNA polymerases, in nucleotide excision repair, base excision repair and mismatch repair. Essential for microtubule polymerization and chromosome segregation (50)</td>
<td>Reduced fidelity of DNA replication. Reduced DNA repair capacity. Chromosome segregation errors (50)</td>
</tr>
<tr>
<td>Manganese</td>
<td>Required as a cofactor to assist in mitochondrial DNA (152,161)</td>
<td>Increase susceptibility to superoxide damage to mitochondrial DNA and reduced resistance to radiation-induced damage to nuclear DNA (152,161)</td>
</tr>
</tbody>
</table>

For information on other micronutrients (e.g. carotenoids, vitamin D, polyphenols, selenium and copper) refer to other papers in Ref. (2).
Micronucleus formation - Chromosome breakage or loss

Nucleoplasmic bridge - Chromosome translocation

Fig. 2. Expression of MNi and NPBs during nuclear division. MNi originate from either (i) lagging whole chromosomes (upper panel) that are unable to engage with the mitotic spindle owing to a defect in the spindle, or a defect in the centromere–kinetochore complex required to engage with the spindle or (ii) an acentric chromosome fragment originating from a chromosome break (upper and lower panels), which lags behind at anaphase because it lacks a centromere–kinetochore complex. Misrepair of two chromosome breaks may lead to an asymmetrical chromosome rearrangement producing a dicentric (i.e. two centromeres) chromosome and an acentric fragment (lower panel)—frequently the centromeres of the dicentric chromosome are pulled to opposite poles of the cell at anaphase resulting in the formation of a NPB between the daughter nuclei. NPBs are frequently accompanied by an MN originating from the associated acentric chromosome fragment. Since 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 Thomas et al. (91) and Fenech (5)).

Fig. 3. MN formation in erythrocytes. Genome instability events in the normoblasts in the bone marrow lead to chromosome breakage/loss and MN formation in the precursor cells of erythrocytes, the reticulocytes. During the maturation process to the reticulocyte stage, nuclei are excluded but MNs remain in the reticulocyte. The reticulocyte (with or without an MN) eventually enters the peripheral blood and becomes a fully mature erythrocyte (with or without an MN). Erythrocytes containing an MN are eventually eliminated by the spleen; therefore, the assay is best performed in individuals that have been splenectomized or by using a method that allows the isolation of nascent reticulocytes identified by the presence of the transferrin receptor (CD71) which is absent in mature erythrocytes. The reticulocyte is also distinguishable from the erythrocyte by its larger size and much higher RNA content. MN frequencies in reticulocytes and erythrocytes are usually recorded separately. For a more detailed explanation refer to MacGregor et al. (71), Abramsson-Zetterberg et al. (72), Dertinger et al. (73) and Offer et al. (74).

Fig. 4. MN formation in buccal cell mucosa. Genome instability in the basal cells leads to chromosome breakage/loss and MN formation. Some cells with genome damage may be eliminated via the apoptotic process. The daughter cells from the basal layer differentiate into ‘prickle cells’ which are eventually differentiated into the flattened and keratinized surface mucosal cells. Each of these cell types may contain an MN. The frequency of MNi observed may depend on the proportion of the various cell types scored. For a more detailed account of mucosal cell biology and MN expression refer to Wertz and Squier (156), Rosin and German (75) and Titenko-Holland et al. (80).

instability. It eventually became evident that the sensitivity to dietary deficiency and inter-individual variability of the erythrocyte MN assay could be improved if the subject being investigated happened to be splenectomized because, in humans, the spleen removes micronucleated erythrocytes from circulation (69). It was shown that supplementation of humans who lacked a spleen, with folic acid and the vitamins A, C and E, caused a reduction in MN frequency in peripheral blood erythrocytes and, that drinking more than five cups of coffee or tea per day and consumption of calcium supplements were associated with an increase in MN frequency in erythrocytes (69,70). Using the same model in mice, MacGregor and co-workers (67,71) showed that MN induction by folate deficiency was aggravated by increased caffeine intake and, that moderate magnesium deficiency doubled the MN frequency in foetal and maternal blood. Recently, flow cytometric methods for scoring MNi in nascent erythrocytes (immature CD71-positive) have been described, making these methods practical for dietary studies in which small alterations in genome damage rate may be expected (72–74). In the study of Abramsson-Zetterberg et al. (72), it was observed that MN frequency in erythrocytes was significantly and negatively correlated with vitamin B12 status.

Although the buccal cell MN assay has been successfully applied to demonstrate elevated spontaneous genome damage rate in individuals with inherited genome instability syndromes such as Bloom’s syndrome (75,76) and in those exposed to chemical genotoxins (66) or ionizing radiation (77), much less is known about the impact of dietary deficiency on this index, with only three studies reporting on the impact of diet. The first by Piyathilake et al. (79),
a cross-sectional study on smokers and non-smokers, showed a 3-fold increment in MN frequency in smokers who also had lower buccal mucosal folate and B12 when compared with non-smokers. The third by Titenko-Holland et al. (80), a depletion–repletion study of nine post-menopausal women, showed a reduction in MN frequency in the buccal exfoliated cells after dietary supplementation with 516 µg/day folate. None of these studies made allowance for the possible effects of supplements on cell division kinetics that may influence MN expression. For an extensive review of the application of the MN assay in buccal cells as well as other exfoliated cells (e.g. cervical epithelium) refer to Majer et al. (81).

The cytokinesis-block MN (CBMN) assay is the preferred method for measuring MNi in cultured human lymphocytes because scoring is specifically restricted to once-divided cells. These cells are recognized by their binucleated (BN) appearance after the inhibition of cytokinesis by cytochalasin-B (5,65). Restricting scoring of MN in BN cells prevents confounding effects caused by suboptimal cell division kinetics, which is a major variable in this ex vivo assay. Over the past 20 years the cytokinesis-block MN assay has evolved into a comprehensive method for measuring chromosome breakage, chromosome loss, non-disjunction, gene amplification, necrosis, apoptosis and cytostasis (Figure 5). This assay also has the added advantage that mitogenic response, a biomarker of immune responsiveness (82) can be measured by the proportion of cytokinesis-blocked BN and multinucleated cells or the estimated nuclear division index (13).

The results of cross-sectional studies of vegetarians and non-vegetarians (83), older men (84–86) and young adults (87,88) indicated that MN frequency in lymphocytes was negatively correlated with plasma folate and vitamin B12, positively correlated with homocysteine and vitamin C and unrelated to vitamin E status. Placebo-controlled dietary interventions have shown that supplementation with 700 µg folic acid and 7 µg vitamin B12 (87) reduced MN frequency in lymphocytes by 25%, which is in accordance with the predictions from the cross-sectional data (83). Supplementation with vitamin E (84–86) or vitamin C (89) did not decrease MN frequency in lymphocytes. A depletion–repletion study in nine post-menopausal women showed a decrease in lymphocyte MN frequency following repletion with 516 µg/day folic acid (80). Daily intake over a 4 month period of a multivitamin, containing 15 mg β-carotene, 75 mg rutin, 3 mg retinol acetate, 30 mg, α-tocopherol, 150 mg ascorbic acid and 0.2 mg folic acid, resulted in a significant reduction of baseline MN frequency in lymphocytes from older subjects (56–80 years) and increased the resistance of lymphocytes to radiation-induced MNi in both younger and older adults (90). These results suggest that MN frequency can be ‘normalized’ or reduced on supplementation with specific micronutrients and that there is an optimal level of micronutrient intake for minimizing genome damage rate that may, in some instances, exceed intake levels from normal diet.

More recently, we have proposed that NPBs between nuclei in BN cells should also be scored in the CBMN assay because they provide a measure of asymmetrical chromosome rearrangement (owing to misrepair of DNA strand breaks or possibly telomere end-joining), which is otherwise not measured in this assay if only MNi are scored (91). The NPBs are assumed to occur when the centromeres of dicentric (abnormal, rearranged) chromosomes are pulled to opposite poles of the cell at anaphase. It is rarely possible to observe dicentric anaphase bridges before the nuclear membrane is formed, because cells proceed through anaphase and telophase rapidly, completing cytokinesis and ultimately end in breakage of the

**Fig. 5.** The various possible fates of cultured cytokinesis-blocked lymphocytes following exposure to cytotoxic/genotoxic agents or micronutrient deficiency. Using these biomarkers within the CBMN assay it is possible to measure the frequency of chromosome breakage (MN), chromosome loss (MN), chromosome rearrangement, e.g. dicentric chromosomes (NPB), gene amplification (nuclear buds), necrosis and apoptosis. In addition, cytostatic effects are readily estimated from the ratio of mono-, bi- and multinucleated cells. Chromosome loss can be distinguished from chromosome breakage using pancentromeric probes or anti-kinetochore antibodies. In addition, non-disjunction (malsegregation of chromosomes) can also be measured in BN cells using chromosome-specific centromeric probes. In the example shown, non-disjunction results (asterisk) in one nucleus containing only one chromosome (i.e. one less than normal diploid state—monosomy) and the other nucleus containing three homologous chromosomes (i.e. one more than the normal diploid state—trisomy). Both monosomy and trisomy of specific chromosomes are associated with developmental defects and cancer risk (5).
NPB when the daughter cells separate. However in the CBMN assay, BN cells with NPBs are allowed to accumulate because cytokinesis is inhibited and the nuclear membrane is eventually formed around the chromosomes allowing an NPB to be observed.

Over the past decade another unique mechanism of MN formation, known as nuclear budding, has emerged. This process has been observed in cultures grown under strong selective conditions (92–94) which induce gene amplification. Shimizu et al. (95,96) showed that amplified DNA is selectively localized to specific sites at the periphery of the nucleus and eliminated via nuclear budding to form MNi during S-phase of mitosis and, eventually, excluded from the cell altogether by extrusion of the MN from the cytoplasm leading to the formation of a ‘mini-cell’.

In a recent study on folic acid deficiency in long-term primary human lymphocyte cultures, we carefully quantified the inter-relationship among MN, NPBs and nuclear buds in an attempt to validate the use of these biomarkers, and to determine more comprehensively the impact of folic acid deficiency on various aspects of genomic stability (58,97). The data from this study verified that folic acid concentration within the physiological range (12, 24, 60 and 120 nM) correlated significantly ($P < 0.0001$) and negatively ($r = -0.63$ to $-0.74$) with all these markers of chromosome damage, which were minimized at 60–120 nM folic acid, the latter being greater than the concentration of folate normally observed in plasma (10–30 nM) (Figure 6). However, even more interestingly, we observed that the frequency of NPBs and nuclear buds correlated significantly and negatively with folic acid dose, suggesting that asymmetrical chromosome rearrangement and gene amplification are induced by folic acid deficiency. The strong cross-correlation among MN, NPB and nuclear bud frequency ($r = 0.75–0.77, P < 0.001$) suggests a common mechanism initiated by folic acid deficiency-induced DNA breaks, although coincidence of effects with other DNA damage events (e.g. folic acid deficiency-induced CpG hypomethylation) cannot be excluded.

Since folic acid deficiency is known to cause gene amplification and chromosome damage such as double-stranded breaks (24,30,98,99), it is likely that these events contribute to the formation of MN, NPBs and nuclear buds in this system. Gene amplification is thought to be a key event in cellular resistance to drugs like methotrexate (100) and in tumour progression (101). Several plausible models of gene amplification have been proposed (102,103); however, the presence of NPBs in the cytokinesis-blocked cells provides support for the BFB cycle model described in the seminal work of McClintock (104) in maize. According to this theory, sister chromatids that have both undergone double-stranded breakage fuse at a distal position (possibly telomeric) forming a dicentric chromosome which has two copies of homologous genes positioned between the two centromeres. During anaphase these dicentric chromosomes are drawn towards both poles and form NPBs. During cytokinesis these dicentric chromosomes, which span both daughter nuclei, are thought to break unevenly and may form a chromosome with two copies of one or more genes and a chromosome (fragment) with no copies of these genes. The chromatids with multiple copy number of these genes may fuse again during interphase forming a dicentric chromosome (doubling again the gene copy number within the chromosome), which is then replicated during the next nuclear division leading to the next bridge-breakage-fusion cycle and further gene amplification (105).

Folic acid deficiency-induced fragile site expression and DNA hypomethylation may also have contributed to

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**Fig. 6.** The effect of medium folic acid concentration on the induction of MNed BN cells, nuclear buds, NPBs and uracil in primary human lymphocytes *in vitro*. $N = 30$. Data = mean ± SEM. *$P < 0.01$ versus 24, 60 and 120 nM. **$P < 0.01$ versus 60 and 120 nM (Tukey’s post test). Data from Crott et al. (58,97). MNed BN cells, micronucleated binucleated cells.
the promotion of gene amplification and resulted in the elimination of this DNA by nuclear budding in our system. For example, amplification of the multidrug resistance 1 gene in Chinese hamster cells occurs through the induction of fragile sites which determined the initiation and size of amplicons (106); and, the induction of hypomethylation by 5-aza-2′-deoxycytidine has been reported to enhance N-(phosphorylacetetyl)-L-aspartate-induced amplification of the CAD gene in Syrian hamster kidney cells (107).

In summary, the genomic instability phenotype can be readily recognized simply by examining cells for abnormal nuclear morphology indicative of BFB cycles, i.e. MNi, NBPs and nuclear buds. In addition, genomic instability can also be manifested by a high rate of aneuploidy and multipolar mitoses that are detectable, respectively, by fluorescence in situ hybridization with chromosome-specific centromere probes or cytologically (108). One of the better methods for measuring/observing BFB cycles and non-disjunction/chromosome loss is the cytokinesis-block MN assay. The results with folic acid show quite clearly that micronutrient deficiencies can, on their own, cause the type of genomic instability observed in cancer. In fact, these observations have (i) provided further impetus for the concept that RDAs should be based on the prevention of genomic instability and (ii) highlight the potential importance of micronutrient concentration as an important modifier, not only spontaneous chromosome abnormality but also chemical- or radiation-induced genome damage. These points have important implications in the relative risk assessment of chemical/radiation exposure, depending on micronutrient status and the need to determine nutritional status when studies of the genotoxic effects of occupational exposure to mutagens and carcinogens are conducted.

**Dietary and genetic factors that can modify genome methylation**

Methylation of cytosine in CpG sequences plays an important role in the suppression of expression of parasitic DNA and certain housekeeping genes (4). In cancer and ageing, global DNA methylation is usually reduced; however, methylation of specific CpG islands may be increased and could lead to unwanted silencing of housekeeping or tumor suppressor genes (109). Other studies have shown that global DNA methylation in lymphocytes or colonic tissue is influenced by the extent of folate intake. For example, the depletion–repletion study performed by Jacob et al. (110) with post-menopausal women in a metabolic unit showed >100% increase in DNA hypomethylation after 9 weeks on low folate (56–111 μg/day) and a subsequent increase in DNA methylation after a further 3 weeks on a high folate diet (286–516 μg/day). Fowler et al. (111) and Cravo and co-workers (59) showed, using the Sss1 methylase assay (which measures the quantity of unmethylated CpG), that cervical and gastric/colonic/rectal epithelium DNA methylation is significantly correlated to serum and tissue folate concentration, respectively. Furthermore, it was shown that intrinsic methylation of DNA was lower in the normal colorectal mucosa of adenoma and carcinoma patients; however, supplementation with 10 mg folic acid/day for 6 months increased methylation 15-fold \((P < 0.0002)\), and 3 months after cessation of therapy methylation decreased 4-fold (59).

Another important possibility of prevention of genomic instability could be the prevention of integration of oncogenic virus DNA. Prevention of hypomethylation may enable a better surveillance of foreign DNA integration into human DNA, because DNA methylation appears to have evolved partly for this purpose (112). It is interesting to note in this regard that HPV virus tends to integrate in fragile sites that may be folate-dependent (113), which raises the hypothesis that viral integration into DNA \textit{in vivo} may be facilitated when folate status is low enough to cause fragile site expression. It is also important to note that transcription of retroviral or parasitic DNA sequences integrated into mammalian DNA is inhibited by cytosine methylation, and conversely demethylation may activate transcription of endogenous retroviruses. The significance of these observations is underscored by the fact that the large majority of 5-methylcytosine in the genome actually lies within parasitic, retroviral or transposon DNA (114,115) and that gene silencing by methylation may be the only mechanism available to neutralize parasitic DNA that cannot be readily ejected from the cell, once incorporated. Whether folate deficiency can activate transcription of retroviral DNA remains untested. Vitamin B12 may also play a direct role in the prevention of integration of oncogenic viruses, because it has been shown that increasing the concentration of cobalamin inhibits HIV integrase and the integration of HIV-DNA into nuclear DNA (116). On the basis of these results, combination treatment with folic acid and vitamin B12 supplements have been used in the treatment of AIDS patients with apparent success (117).

Combined deficiency in folic acid and vitamin B12 was associated (i) with transient 7q- in one patient (118) and (ii) in a series of patients produced a persistent abnormal deoxyuridine suppression test result (which is indicative of inadequate capacity to generate dTMP) and increased frequency of chromosomes showing despiralization and chromosomal breaks (119). The latter studies showed that it took up to 84 days after supplementation with folic acid and vitamin B12, before the deoxyuridine suppression and the chromosomal morphology tests returned to normal. With respect to the question of chromosome despiralization it may be important to note that the DNA methylation inibitor, 5-azacytidine, induces distinct undercondensation of the heterochromatin regions of chromosomes 1, 9, 15, 16 and Y, and the specific loss of these chromosomes as MNi in human lymphocytes \textit{in vitro} (120). Similarly, it has been shown that 5-azacytidine causes decondensation in the homogenously staining region in the highly methylated heteromorphic chromosome 15 and loss of this genetic material as MNi in human diploid lung fibroblast cell line (TIG-7) (121). The ICF immunodeficiency syndrome, which is caused by mutation in the DNA methyl transferase gene, is characterized by despiralization of heterochromatin of chromosomes 1, 9 and 16 and loss of this chromatin into MNi and nuclear blebs (122,123). These events are likely to be relevant to the ageing process because Suzuki et al. (124) demonstrated that \textit{in vitro} ageing of normal human fibroblasts results in concomitant demethylation of satellite 2 and satellite 3 DNA which is abundant in the juxta-centromeric DNA of chromosomes 1, 9 and 16, and the increasing frequency of MNi that specifically contained these sequences. We have also recently demonstrated that folate deficiency caused a significant 26–35% increase in frequency of aneuploidy of chromosome 17 \((P = 0.0017)\) and aneuploidy of chromosome 21 \((P = 0.0008)\) relative to 120 nM 5-methyltetrahydrofolate or folic acid. The pattern of aneuploidy in BN cells was significantly correlated with that observed in mononucleated cells \((R = 0.51–0.75, P < 0.0004)\), and was consistent with a model...
based on chromosome loss or partial aneusomy rescue as the cause rather than non-disjunction, although the latter mechanism could not be excluded (108).

It is evident that MN expression could be increased as a result of hypomethylation of satellite DNA. However, it is also possible that increased genome damage may be caused by hypermethylation of CpG islands within or adjacent to the promoter regions of housekeeping genes involved in cell cycle check points and DNA repair. For example, CpG island hypermethylation of the mitotic spindle check-point genes, such as APC, BUB1 and HCDC4, could reduce their expression and, therefore, increase the possibility of chromosome malsegregation leading to MN formation (125–128).

There is some concern that excess folate and methionine intake may, in fact, cause hypermethylation of CpG islands and silencing of housekeeping genes. Recent experiments in yellow agouti [A(vy)] mice, which harbour a transposable element in the agouti gene, suggest that dietary methyl supplementation of dams with extra folic acid, vitamin B12, choline and betaine alter the phenotype of their A(vy)/a offspring via increased CpG methylation at the A(vy) locus, and that the epigenetic metastability which confers this lability is due to the A(vy) transposable element (129,130). This suggests that excessive efficiency in silencing of transposable elements that have located in housekeeping genes could inadvertently lead to the silencing of those genes. These results have important implications for humans, because transposable elements constitute over 35% of the human genome and are found within ~4% of human genes, and many human genes are transcribed from a cryptic promoter within the L1 retrotransposon (4,131–133).

It is plausible that the increase in CpG island methylation and gene silencing in ageing and in cancer may be due to the relocation of transposable elements owing to hypomethylation in the early stages of life and their subsequent hypermethylation when relocated within coding sequences of housekeeping genes. Therefore, in aged individuals and in cancer, the problem to solve is the reversal of hypermethylation of CpG island and activation of silenced housekeeping genes. It is therefore interesting, from the nutritional aspect, to note that the green tea polyphenol epigallocatechin-3-gallate, which is associated with reduction in cancer risk and rate of ageing in animals and humans, is also a potent inhibitor of 5-cytosine DNA methyltransferase at physiological concentration, and that it caused the reversal of hypermethylation of p16(INK4a), retinoic acid receptor β (RARβ), O(6)-methylguanine methyltransferase (MGMT) and human mutL homologue 1 (hMLH1) genes in a cancer cell line (134). These observations are important as they underscore the role of diet for increasing or decreasing DNA methylation. Transcriptional silencing of genes involved in chromosome segregation (e.g. BUB1 and HCDC4) and DNA repair (e.g. BRCA1, BRCA2 and ATM) are expected to increase the MN index, and reversal would be expected to reduce the frequency of this biomarker. Recently, it has also been shown that oxidation of guanine in promoter CpG sequences in brain tissue increases with age and is correlated with reduced gene expression (135). Therefore, oxidative damage to guanine in CpG islands could also be considered an important toxic event with epigenetic consequences. Figure 7 illustrates how both an excess and a deficiency of genome methylation can lead to MN formation in proliferating tissues.

**Important dietary sources of folate, vitamin B12, choline and methionine**

Folate, vitamin B12, choline and methionine are the major dietary sources required for genome methylation maintenance (60). It is becoming increasingly evident that large proportions (10–70%) of populations in developed countries are not meeting the current RDA of folate and vitamin B12 (60,136–138). The extent of choline and methionine deficiency remains unknown because RDAs for these nutrients have not been set. These dietary deficiencies could be due to insufficient knowledge of the micronutrient content of foods and/or poor choice of foods. It is, therefore, useful to consider important dietary sources of folate, vitamin B12, choline and methionine (60,139).

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**Fig. 7.** Schematic diagram illustrating the possible effects of dietary factors on methylation of CpG islands, parasitic DNA, gene expression, centromere function and MN formation. EGCG, epi-gallo catechin gallate. For further explanation refer to Fenech (24,60) and Kimura et al. (146).
This knowledge is essential for anyone who intends to achieve the RDA of folate (400 µg/day) and vitamin B12 (2.4 µg/day), and have an adequate intake of choline and methionine via foods rather than supplements. Recent data suggest that dietary choline intake should exceed 250 mg/day to maintain adequate plasma homocysteine levels for genomic stability, which is essential for the prevention of diseases of deficiency, such as scurvy in the case of vitamin C, anaemia in the case of folic acid and pellagra in the case of niacin. However, these diseases of deficiency are rare in the developed world but degenerative disease and developmental disease are very important and common. Recently, the dietary allowance for folic acid for the prevention of neural tube defects has been revised to more than double the original RDA (140). There is a strong international awareness that it is also necessary to redefine RDAs for the prevention of degenerative disease (such as cancer, cardiovascular disease and Alzheimer’s disease) and compression of the morbidity phase during old age. Since diseases of development, degenerative disease and ageing itself are partly caused by damage to mitochondrial DNA. To date, our knowledge on optimal micronutrient levels for genomic stability is scanty and disorganized.

Table II lists some examples of important and less important dietary sources of folate, vitamin B12, methionine and choline. It is evident that one of the richest sources of folate is liver. Although broccoli is among the vegetables with the highest folate content one would have to consume ~600 g of cooked broccoli, but only 30 g of fried chicken liver, to achieve the RDA for folate. Alerone flour, made from the aleurone layer of wheat grain is one of the richest plant sources of fibre and B vitamins, such as folate, with the additional benefit of high bioavailability in humans (63,64). Liver is also an excellent source of vitamin B12 such that 10–30 g provides above RDA level of this vitamin and, in contrast, plant foods are devoid of this critical vitamin. Meat, liver, fish, cheese and nuts have the highest content of methionine, exceeding the concentration in fruits and vegetables by 5- to 10-fold. Eggs and liver are the best known sources of choline such that 50–100 g of these foods is sufficient to meet the recommended adequate intake of choline. Therefore, making careful food choices can have an important impact on an individual’s success in achieving the correct intake of key micronutrients required for maintaining methylation of DNA.

The concept of RDAs for genome stability

Current RDAs for vitamins and minerals are based largely on the prevention of diseases of deficiency, such as scurvy in the case of vitamin C, anaemia in the case of folic acid and pellagra in the case of niacin. However, these diseases of deficiency are rare in the developed world but degenerative disease and developmental disease are very important and common. Recently, the dietary allowance for folic acid for the prevention of neural tube defects has been revised to more than double the original RDA (140). There is a strong international awareness that it is also necessary to redefine RDAs for the prevention of degenerative disease (such as cancer, cardiovascular disease and Alzheimer’s disease) and compression of the morbidity phase during old age. Since diseases of development, degenerative disease and ageing itself are partly caused by damage to mitochondrial DNA. To date, our knowledge on optimal micronutrient levels for genomic stability is scanty and disorganized.

Both in vitro and in vivo studies with human cells clearly show that folate deficiency, vitamin B12 deficiency and elevated plasma homocysteine are associated with the expression of chromosomal fragile sites, chromosome breaks, excessive uracil in DNA, MN formation and DNA hypomethylation (30,58,59,80,87,97,98,110,144–146). In vitro experiments indicate that DNA breaks in human cells are minimized when folic acid concentration in culture medium is >180 nmol/l (80 ng/ml) (144,145). Recently, we have shown that uracil incorporation in human lymphocytes cultured for 8 days is minimized at a folic acid concentration of 120 nmol/l (58,97,146). The latter in vitro data may not predict precisely in vivo folic acid requirements; however, they provide a useful guide of optimal concentration range for genome health. In addition, in vitro data are directly relevant to genome health maintenance in ex vivo culture of human cells, such as stem cells, that need to be maintained and expanded under conditions that prevent malignant genotypic changes prior to therapeutic use as suggested by Rubio et al. (147).

<table>
<thead>
<tr>
<th>Folate (µg/100 g)</th>
<th>Vitamin B12 (µg/100 g)a</th>
<th>Methionine (mg/100 g)</th>
<th>Choline (mg/100 g)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meats</td>
<td>Ox liver (stewed)—110</td>
<td>Meats</td>
<td>Meats</td>
</tr>
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<td>Lambda liver (fried)—81</td>
<td>Beef steak (fried)—840</td>
<td>Beef liver (cooked)—711</td>
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<td>Beef (boiled)—750</td>
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<td>Lamb kidney (fried)—79</td>
<td>Pig liver (fried)—500</td>
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<td>Beef steak (fried)—2</td>
<td>Fish</td>
<td>Cauliflower (cooked)—59</td>
</tr>
<tr>
<td>Beef steak (fried)—15</td>
<td>Beef mince (fried)—2</td>
<td>Salmon (steamed)—620</td>
<td>Potato (cooked)—12</td>
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<tr>
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<td>Orange—10</td>
</tr>
<tr>
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<td></td>
<td>Other</td>
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<td>Cauliflower (boiled)—31</td>
<td>Egg (cooked)—754</td>
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<td>Orange—18</td>
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<tr>
<td>Lettuce (fresh)—58</td>
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aPlant foods such as vegetables, fruit, cereals and nuts do not contain vitamin B12.
bThe figures shown for total choline represent choline content plus choline equivalents from phosphatidylcholine and sphingomyelin.
DEFINING OPTIMAL MICRONUTRIENT CONCENTRATION FOR GENOME STABILITY – IN VITRO

LYMPHOCYTES, FIBROBLASTS, STEM CELLS

DOSE-RESPONSE FOR EACH MICRONUTRIENT USING COMPREHENSIVE CBMN ASSAY TO MEASURE BASE-LINE GENOME STABILITY AND DNA REPAIR EFFICIENCY

OPTIMAL CONCENTRATION OF MICRONUTRIENTS FOR GENOME STABILITY

Fig. 8. Schematic diagram of in vitro studies that should be performed to determine optimal micronutrient concentration for genome stability. The cells should be cultured for a minimum of 8 days in chemically defined culture medium (ideally without serum which varies greatly in composition and may contain traces of the micronutrient under investigation even after dialysis or chelation). A culture time greater than 6 days is required to observe the effects of micronutrient deficiency in vitro. A dose–response study is essential to define not only the optimal concentration range for genome stability but also to determine the concentration when excess of the micronutrient induces cytotoxicity. CBMN assay, cytokinesis-blocked MN assay.

Intervention studies in humans taking folate and/or vitamin B12 supplements show that DNA hypomethylation, chromosome breaks, uracil misincorporation and MN formation are minimized when plasma concentration of vitamin B12 is >300 pmol/l, plasma folate concentration is >34 nmol/l, red cell folate concentration is >700 nmol/l folate and plasma homocysteine is <7.5 μmol/l (30,59,69,80,87,98,110). These concentrations are best achieved at intake levels in excess of current RDAs, i.e. >400 μg folic acid per day and >2 μg vitamin B12 per day. It is relevant to point out that epidemiological studies on diet and colorectal or breast cancer suggest that intake >400 μg folate per day may be required to minimize cancer risk (148,149), yet recent intake data indicate that <25% Americans met this intake level before 1998, when folate fortification became mandatory in the United States (3). The most recent data (analysed from CSIRO 1999 National Nutrition Survey, unpublished data) for Australians indicates that >85% had intake levels <400 μg folate per day in 1998 and, in Holland >50% of the population were below this optimum intake before 1998 (150). Dietary intakes above the current RDA may be particularly important in those with extreme defects in the absorption and metabolism of these vitamins, for which ageing is a contributing factor. The above suggests that both, controlled in vitro experiments and placebo-controlled in vivo interventions are informative in determining optimal micronutrient intake for optimal genome health.

Our current stage of knowledge on the role of micronutrients in the maintenance of genomic stability has been recently reviewed in a special issue of Mutation Research (2). These reviews identify the current gaps in our knowledge and provide the basic information for appropriate design of both, in vitro studies with normal human cells (Figure 8) and placebo-controlled intervention trials (Figure 9). These studies are needed to define optimal tissue concentration and determine RDAs for genomic stability. In the future, clinical trials with a wide array of complementary DNA damage end-points would be necessary (e.g. point mutations, telomere shortening, balanced chromosomal translocations, chromosome non-disjunction or aneuploidy, MN formation, single and double strand breaks in DNA and DNA adducts). It is clear that this objective requires multiple expertise. That there is a need for an international collaborative effort to establish RDAs for genomic stability, as well as intake levels that may be genotoxic, is evident.

DEFINING RDAs FOR GENOME STABILITY – IN VIVO

1. Identify high risk population (i.e. individuals with a high DNA damage rate and/or are DNA repair deficient)

2. Placebo-controlled intervention with increasing doses over an extended period of time depending on the turn-over rate of the tissue examined (e.g. 3 weeks for buccal cells).

3. Assay genome damage using complementary DNA damage biomarkers to identify dose of micronutrient at which optimal genome stability is achieved.

Fig. 9. Schematic diagram for intervention studies required to define the optimal dietary intake of a particular micronutrient for genome stability. Ideally, more than one cell type is sampled for analysis to verify that an optimum is achieved for more than one cell type. A combination of the MN assay with other molecular/cytogenetic biomarkers of genome stability, such as telomere shortening, DNA oxidation, DNA hypomethylation and aneuploidy of specific chromosomes (e.g. chromosomes 17 and 21) and/or chromosomal regions (e.g. loss of the p-arm of chromosome 17 which includes the p53 gene) associated with increased cancer risk are recommended.

Genome health nutrigenomics

One of the most important emerging areas of nutrition science is the field of nutrigenomics, i.e. the effect of diet on gene expression and chromosomal structure, and the extent to which genetic differences between individuals influence response to a specific dietary pattern, functional food or supplement in terms of a specific health outcome. The specific field of Genome Health Nutrigenomics (11) is proposed on the premise that a more useful approach for the prevention of diseases caused by genome damage is to take into consideration the genotype of individuals with a focus on common genetic polymorphisms that alter the bioavailability of micronutrients and their metabolism and/or the affinity of key enzymes involved in DNA metabolism for their micronutrient cofactor. Supplementation of diet with appropriate minerals and vitamins could, in some cases, help to overcome inherited metabolic blocks in key DNA maintenance pathways (10,31). 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 with respect to 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 developmental defects and cancer risk (23,151,152). The product of the MTHFR gene determines the availability of folate for the
synthesis of thymidylate acid (TMP) from deoxyuridylate acid (dUMP). Polymorphisms in the MTHFR gene, such as the C677T mutation, that reduce activity of the MTHFR enzyme are predicted to minimize uracil misincorporation into DNA while making less methylfolate available for the synthesis of S-adenosyl methionine, the common methyl donor (24,142). Epidemiological studies have suggested that individuals homozygous for the C677T polymorphism (i.e. TT genotype) may be protected against colorectal cancer and acute lymphocytic leukae mia relative to those with the wild-type CC genotype (151,153). 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 (146). This is illustrated by (i) the reduction in nuclear bud frequency (a biomarker of gene amplification) in TT homozygotes relative to CC homozygotes for the MTHFR C677T mutation and (ii) the observation that high riboflavin concentration increases nuclear bud frequency under low folic acid conditions, probably by increasing MTHFR activity that diverts folate away from thymidylate synthesis and, therefore, cause uracil incorporation into DNA which leads to chromosome breakage and gene amplification by the BFB cycle mechanism (5). LFLR, 12 nmol/l folic acid and 0 nmol/l riboflavin; LFHR, 12 nmol/l folic acid and 500 nmol/l riboflavin; HFLR, 120 nmol/l folic acid and 0 nmol/l riboflavin; HHFR, 120 nmol/l folic acid and 500 nmol/l riboflavin. For a more detailed explanation of these results refer to Kimura et al. (146).

SIGNSIFICANT INTERACTIONS BETWEEN FOLIC ACID, RIBOFLAVIN AND MTHFR GENOTYPE

![Diagram](http://image.oxfordjournals.org/downloaded_from/.../S0141022909002762.pdf)

**Fig. 10.** Nutritional genomics of genome stability illustrated by results from a recent experiment from our laboratory using long-term cultured lymphocytes from individuals with either the C677C or the T677T genotype for the methylenetetrahydrofolate reductase (MTHFR) gene. (A) The results of this study show a significant reduction of nuclear bud formation under both low folate (LF, 12 nmol/l folic acid) and high folate (HF, 120 nmol/l folic acid) conditions for the T677T genotype relative to C677C and a much reduced nuclear bud frequency in the HF medium relative to the LF medium. (B) Interaction of folic acid and riboflavin in determining genome stability—specifically, the results show that when folic concentration is low (12 nmol/l), high concentration of riboflavin (500 nmol/l), an essential cofactor for MTHFR, increases genome instability (in this case gene amplification). This effect could be due to an increase in MTHFR activity which may divert folate away from thymidylate synthesis and, therefore, cause uracil incorporation into DNA which leads to chromosome breakage and gene amplification by the BFB cycle mechanism (5). LFLR, 12 nmol/l folic acid and 0 nmol/l riboflavin; LFHR, 12 nmol/l folic acid and 500 nmol/l riboflavin; HFLR, 120 nmol/l folic acid and 0 nmol/l riboflavin; HHFR, 120 nmol/l folic acid and 500 nmol/l riboflavin. For a more detailed explanation of these results refer to Kimura et al. (146).

The genome health clinic concept—a paradigm shift in disease prevention based on the diagnosis and nutritional treatment of genome and epigenome damage

The advances in our knowledge described above have opened up a new opportunity in disease prevention based on the concepts that (i) excessive genome damage is the most fundamental cause of developmental and degenerative disease, (ii) genome damage caused by micronutrient deficiency is preventable, (iii) accurate diagnosis of genome instability using DNA damage biomarkers that are sensitive to micronutrient deficiency is technically feasible and (iv) it is possible to optimize nutritional status and verify efficacy by diagnosis of a reduction in genome and epigenome damage rate after intervention. Given the emerging evidence that dietary requirement of an individual may depend on their inherited genes, we can anticipate (i) important scientific developments in the understanding of the relationships between dietary requirement and genetic background to optimize genome stability and (ii) 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 and/or epigenome damage, health/medical practitioners will be trained, in the near future, to diagnose and nutritionally prevent a most fundamental initiating cause of developmental and degenerative disease, i.e. genome and...
epigenome damage. This novel approach also opens up the possibility for the massive 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. The conceptual framework of the diagnostics and databases required to implement this complementary preventive medicine approach is illustrated in Figure 11.

Acknowledgements

The contribution of numerous volunteers, students, post-doctoral fellows, visiting scientists and technical staff to the research performed in our laboratory over many years is gratefully appreciated. Sally Record is gratefully acknowledged for her analysis of folate intake data from the CSIRO National Nutrition Survey. A special acknowledgement is in order for the late Dr Ludmila Mikhailichev because as a result of our collaborative study on genome damage in children exposed to the Chernobyl disaster (84–86) it became evident that although individuals could do little to minimize environmental contamination, much could be achieved by optimizing dietary status to prevent genome damage caused by micronutrient deficiency. I also would like to acknowledge the foresight of the pioneers in the field of anti-mutagenesis and anti-carcinogenesis whose initial vision that damage to the genome is preventable by nutritional or pharmaceutical means is now being realized (155).

Fig. 11. Conceptual framework of the diagnostics and databases that will be required to implement the novel complementary preventive medicine discipline of nutritional treatment of genome instability, i.e. ‘The Genome Health Clinic’. (i) Genome health diagnostics which would include generic chromosomal damage biomarkers (e.g. MN assay, telomere shortening) as well as genetic anomalies specific to unique disease states (e.g. chromosome 17 and 21 aneuploidy or deletions of unique genes such as p53). (ii) Genotype diagnostics which would include single nucleotide polymorphisms with reduced affinity of cofactors involved in genome stability maintenance as well as assessment of gene silencing/epression. (iii) Micronutrients essential for genome stability would be measured (e.g. folate, zinc, etc.). (iv) The Genome Health Nutrigenomics database would include ‘normal range’ and ‘optimal range’ values and be used to determine whether an individual’s genotype and nutritional status to prevent genome damage caused by micronutrient deficiency. I also would like to acknowledge the foresight of the pioneers in the field of anti-mutagenesis and anti-carcinogenesis whose initial vision that damage to the genome is preventable by nutritional or pharmaceutical means is now being realized (155).

References


