Problems and approaches in investigating the role of micronutrients in the aetiology of cancer in humans

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Observational studies have provided leads regarding a number of micronutrients which may account for the apparent protective effects of high intakes of vegetables and fruit against many types of cancer. In general, these leads have not been confirmed by randomised controlled trials. This apparent conflict raises issues about the timing and duration of a critical period or periods during which micronutrient intake may influence the development of cancer, the dose, possible interaction between high doses of micronutrients and exposures conferring a high risk of cancer and gene-micronutrient interactions. When gene-environmental interaction exists, failure to take both of these sets of factors into account leads to bias in the estimation of disease risk. As a result of recent advances, it is now possible to take measures of genetic susceptibility into account. Therefore, in future studies, the opportunity should be taken to obtain DNA samples to determine genotypes for polymorphisms potentially affecting micronutrient metabolism.

It is estimated that, world-wide, 7.8 million cases of cancer were newly diagnosed in 1990. This represents an increase of almost 1.5 million cases since 1980. The absolute numbers of cases are expected to increase as the generation born after the Second World War attain ages where the age-specific rates of cancer start to increase. This has important public health and health service implications, and cancer control activities will need to be expanded to reduce the burden of morbidity and mortality which is otherwise likely to occur.

One potential cancer control activity is dietary change. About one-third of the total world-wide number of cases of cancer may be attributable to dietary factors, obesity or a sedentary lifestyle. High intakes of vegetables and fruit show a consistent inverse relationship with cancers of the larynx, lung, oesophagus and stomach, and there is weaker evidence that this is also the case for cancers of the mouth and
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Pharynx, pancreas and cervix. High levels of vegetable consumption are associated with a reduced risk of colon cancer. Current dietary recommendations from many sources include consumption of a variety of vegetables and fruits. However, because of the difficulties of changing the dietary patterns of populations, a great deal of research has been carried out in an attempt to identify the specific nutrients which account for the observed effects of vegetables and fruit. This is relevant both to the specification of more precise dietary recommendations, and to the possible use of supplemental nutrients. As vegetables and fruits are relatively rich in micronutrients, that is vitamins, minerals and trace elements, compared with other food groups, research has focussed on micronutrients. In this chapter, methodological problems in investigating the role of micronutrients in the aetiology of cancer in humans are discussed, and the possible value of investigating genetic factors affecting micronutrient metabolism is considered.

Observational studies

The most widely used epidemiological study designs in the investigation of the possible roles of specific micronutrients in the aetiology of cancer have been case-control and cohort studies. These have typically involved the use of a self or interviewer-administered food frequency questionnaire, and/or, to a lesser extent, measurement of micronutrient status in blood or other tissues. In the more recent studies, investigators have addressed methodological limitations of earlier work by use of validated instruments to assess dietary intake, adjusting for total energy intake in the analysis and including sufficient numbers of subjects to have adequate statistical power to detect a trend in the risk of cancer associated with an increase in intake of the specific micronutrient. However, because vegetables and fruits contain many micronutrients, intakes of these are correlated and these observational studies suffer from the fundamental difficulty of identifying the effects of specific micronutrients. This problem also occurs with biomarkers of dietary micronutrient intake. Also, for some other micronutrients, blood levels may not be a good marker of dietary intake; for example, blood retinol levels do not reflect dietary intake either in the short or in the longer term and plasma levels of 25-hydroxyvitamin D may only be a good marker of dietary intake in subjects with low sun exposure. For these reasons, investigators have sought to determine possible cancer-protective effects of specific micronutrients by means of randomised controlled trials.
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Randomised controlled trials

Randomised controlled trials are the definitive test of an intervention because, if they are of adequate size, the distribution of both known and unknown potential confounding factors should be equal between the intervention and control groups. A series of trials of micronutrient supplements has been reported, either with specific types of cancer or with precursor lesions such as colorectal adenomatous polyps or oral leukoplakia as end-points. The trials which have provoked the greatest debate are those which included β-carotene supplementation in subjects at high risk of developing lung cancer. An unexpected increase in the incidence of lung cancer was observed in those assigned to take β-carotene in the Alpha-Tocopherol Beta Carotene Lung Cancer Prevention Trial (henceforth ATBC trial), and in those who received a combination of β-carotene and retinol in the Beta-Carotene and Retinol Efficacy Trial (henceforth CARET). Moreover, other trials have not suggested beneficial effects of β-carotene supplementation on the prevention of either cancer or the recurrence of colorectal adenomas.

There has been considerable discussion of the results of the β-carotene trials. Possible explanations for the apparent lack of beneficial effects include the timing of starting supplements in relation to the chain of events that leads to clinical lung cancer, the use of doses exceeding the dietary intakes associated with reduced risk in the observational studies, and the possibility that the apparent effect of β-carotene in the observational studies was secondary to another micronutrient or to a combination of micronutrients. Huttunen attributed the increased incidence of lung cancer and higher mortality from ischaemic heart disease in those who received β-carotene (20 mg/day) in the ATBC trial to chance as no such effects had been observed in two other trials. One of these was an intervention trial in the Linxian area of China in which 15 mg β-carotene per day had been given in combination with vitamin E and selenium, and the other was a preliminary report of a trial of 50 mg β-carotene every other day in men with chronic stable angina. However, the statistical power of the Linxian trial to detect a change in lung cancer risk of the magnitude observed in the ATBC trial was low. In a subsequent report of the trial in men with angina, a non-significant increase in the risk of death from cardiovascular disease was observed. Moreover, an increased risk of lung cancer in those who received β-carotene and retinol was observed in the CARET. Therefore, chance may not account for the finding of an increased risk of lung cancer. If β-carotene were toxic at high dose, the excess lung cancer cases would have been expected to occur in subjects with the highest serum β-carotene values after a sustained period of treatment.
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However, there was no excess in subjects in the highest tertile of serum β-carotene after three years of treatment in the ATBC trial. Huttunen commented on the absence of prior data suggesting β-carotene toxicity in humans or animals, and this has also been noted by an IARC Working Group. In the ATBC trial, the effect appeared to be confined to men smoking at least 20 cigarettes per day, while in CARET, the increased risk was observed among current smokers and asbestos-exposed workers, but not among former smokers. These observations suggest that β-carotene supplementation accelerates the appearance of clinical lung cancer among current heavy smokers. In both trials there was some evidence that the effect of β-carotene was strongest in those with the highest alcohol intake, but it was difficult to separate the effects of correlated behaviours of smoking and drinking.

**Types of data needed before embarking on future trials**

At the time these trials were being planned, the available data suggested that β-carotene might be protective and there was no known toxic effect. It was recognised that a protective effect could not be confirmed without trials, and the unexpected findings of the trials confirm the wisdom of this approach to testing hypotheses. The apparent conflict between the observational and trial evidence raises issues to be considered before embarking on future trials. One issue is possible interaction between a supplemental micronutrient and an exposure conferring a high risk of cancer. However, it is possible that this might only become apparent at pharmacological doses, and not at the upper levels of dietary intake, as appears to have been the case in the ATBC trial and CARET. Another issue relates to the choice of dose. In chemoprevention trials in general, the dose is chosen either to be near to the recommended daily dose or to be the highest dose thought not to be toxic. The trials in which β-carotene was assessed were initiated at a time when there was a rapid increase in supplementation with micronutrients and this seemed likely to increase further, despite of a lack of evidence from randomised trials on side effects or health benefits. A further issue is the value of investigation of intermediate end-points for cancer; more work is required on validation of the predictive value of these. In addition, there is a need to consider when the critical period (or periods) in life during which micronutrient intake may influence the development of cancer. As somatic genetic alterations appear to occur in particular sequences in specific types of tumour, investigation of the relationship between measures of micronutrient intake or status and specific molecular changes in tumours might identify the critical period when micronutrients influence cancer development. However, even for the
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most established sequence – that for colon cancer – it is known that there are many cases in which the number or the order of the genetic alterations is altered.

There remains the fundamental difficulty of the correlation between intakes of specific micronutrients. The apparent conflict between the results of observational studies suggesting a possible protective effect of β-carotene and the lack of evidence of benefit in randomised trials raises the question as to whether epidemiology is ‘facing its limits’. One of the most important aspects of causal inference is the integration of diverse sources of evidence. An important source of evidence is data from experiments in animals. β-Carotene was ineffective in inhibiting chemically induced respiratory tract carcinogens in mice and hamsters. Moreover, in two of the studies in hamsters, there was evidence that there were enhanced carcinogenic effects in animals fed a β-carotene supplemented diet. However, a limitation of data on experimental animals results from inter-species differences in metabolism. For example, in contrast to humans and other primates, most common laboratory animal species efficiently convert β-carotene to vitamin A, and very little is absorbed intact. As there are inter-species differences in micronutrient metabolism, might there also be intra-species variation, attributable to genetic factors? If so, then if a micronutrient were of aetiological importance, it would be expected that the different alleles of the genes coding for the enzymes responsible for its metabolism would be associated with different risks of cancer.

Genetic susceptibility

A number of ‘ecogenetic’ polymorphisms have been characterised which are associated with inter-individual differences in response to environmental agents. The enzymes involved in the metabolism of these agents, and the receptors regulating these enzymes, have evolved because of the interaction between animals and plants. For example, gene-duplication events in the cytochrome P450 family appear to have occurred when animals first came onto land and began to exploit plants. It is thought that mutations in plants which made them less palatable or more toxic conferred a selective advantage, and mutations in animals which enabled them to detoxify the toxic metabolites in plants in turn enabled them to adapt. In the course of evolution, the diversity of secondary metabolites in plants and enzyme systems for metabolising these in animals is thought to have increased. The available evidence suggests that in the course of human evolution, the usual diet was rich in leafy vegetables and fruit and, therefore, hominids have been part of this process of co-evolution.
As yet, relatively few polymorphisms affecting the metabolism of specific micronutrients have been identified. The inter-relationships between micronutrients for which metabolic polymorphisms have been identified, the polymorphisms and cancer are now discussed.

**Iron and hereditary haemochromatosis**

There has been considerable interest in the role of DNA-damaging free oxygen radicals in the aetiology of cancer. Iron can catalyse the production of oxygen radicals. As large quantities of unabsorbed iron reach the lumen of the colon, and it has been estimated that faeces contain levels of iron 10-fold higher than other tissues, it might be expected that any pro-oxidant effect of iron might be particularly apparent for colorectal cancer. Bile pigments in faeces, and also haem if bleeding is present, keep iron soluble at near neutral pH values and, thereby, promote the catalysis of hydroxyl radical formation. A positive association between colorectal cancer and body iron stores, as assessed by transferrin saturation and total iron-binding capacity, determined prior to disease development, was found in cohort studies in the US and in Finland. After extended follow-up of the US cohort, cases of colon cancer were shown to have had higher reported iron intake, serum iron levels and transferrin saturation at enrolment than other subjects. A positive association between reported dietary intake of iron and rectal cancer has been reported in one case-control study, but in other case-control studies no clear association between colorectal cancer and dietary iron has been observed. A positive association between serum ferritin levels and colorectal adenomas has been found in the one study in which this has been investigated, but no positive association between reported dietary intake of iron and colorectal adenomas has been found. Thus, while the data on dietary iron and colorectal neoplasia are inconsistent, a positive association with biomarkers of body iron stores has been observed in the few studies in which this has been investigated.

Heterozygotes for the mutation associated with hereditary haemochromatosis, an autosomal recessive disease of iron overload, have higher serum ferritin and transferrin saturation than normal homozygotes. Therefore, if high levels of iron intake were involved in the aetiology of colorectal cancer, it would be expected that heterozygotes for hereditary haemochromatosis would be at increased risk of colorectal cancer. In a study in the US of parents of homozygotes for hereditary haemochromatosis, who are predominantly heterozygotes, there was an increased risk for colorectal, haematological and gastric cancers in the parents of homozygotes as compared to that in the parents of spouses of
The age-adjusted relative risk of colon cancer in fathers was 1.3 (95% CI 1.07–1.53), and in mothers 1.1 (95% CI 0.87–1.34). The relative risk of colon polyps in fathers was 1.2 (95% CI 1.05–1.46) and in mothers 1.3 (95% CI 1.08–1.53). The possibility of an interaction between haemochromatosis genotype and dietary iron intake does not appear to have been investigated.

**Folate and the 5,10 methylenetetrahydrofolate reductase and N-acetyltransferase 1 polymorphisms**

**Colorectal cancer**

Investigations on the possible effect of folate intake on colorectal neoplasia have been stimulated by the observation that hypomethylation of DNA appears to be an early step in colorectal carcinogenesis, by the widespread observation that low intakes of vegetables are positively associated with cancer of the large bowel and by evidence of a positive association between alcohol intake and colorectal cancer. Fresh green leafy vegetables are major sources of folate, while alcohol is known to have an adverse effect on folate metabolism. Folate has an important influence on methyl availability and it has been postulated that a methyl deficient diet may influence the accumulation of DNA methylation abnormalities observed during the progression of human colorectal neoplasia.

Inverse associations between reported dietary folate intake and both colorectal cancer and colorectal adenomatous polyps have been observed, although the available studies have not been entirely consistent. In male smokers who participated in the ATBC trial, serum folate levels on enrolment were not associated with the subsequent risk of colorectal cancer. However, serum folate is a poor indicator of long-term folate status. Inverse associations between red blood cell folate, considered to be a better marker of long-term folate intake and adenomas have been found. In a small double-blind randomised controlled trial of 60 consecutive patients from whom colonic adenomas had been removed by polypectomy, carried out in Greece, the recurrence rate at 12 months in the group assigned to receive 1 mg/day folate was 23% (7/31), compared with 38% (11/29) in the group assigned to receive the placebo. The recurrence rates at 24 months were 13% (4/31) and 28% (8/29), respectively.

The gene coding for the enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR), which is involved in the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, is polymorphic, with the 677C-T and the 1298A-C mutations associated with decreased activity. At first sight, it might be expected that these...
mutations would be associated with an increased risk of colorectal cancer. However, in two recent studies of male health professionals in the US, individuals homozygous for the 677C-T mutation were found to have a lower risk for colorectal cancer than heterozygotes or those homozygous for the wild-type allele. Chen et al. observed a statistically significant interaction between MTHFR genotype and alcohol consumption, but not methionine or folate intake. Compared with those who consumed one alcoholic drink or less per week and who were not homozygous for the mutation, the relative risk of colorectal cancer in those who consumed a similar amount of alcohol and were homozygous for the mutation was 0.1 (95% CI 0.01–0.85, based on one case in the latter group). Ma et al. found that the inverse association with homozygosity for the MTHFR mutation was not apparent in those with a plasma folate level of less than 3.0 ng/ml. As in the study of Chen et al., the risk of colorectal cancer in those who were homozygous for the mutation and who drank little or no alcohol was substantially lower than in normal homozygotes with a similar intake of alcohol (RR 0.1, 95% CI 0.03–0.57).

A catabolite of folate, p-aminobenzoyl-L-glutamate is a substrate for NAT1. Polymorphism for the gene coding for this enzyme is associated with differences in enzyme activity in human colon tissues. The association between NAT1 genotype and colorectal neoplasia has as yet been only investigated in a few studies, in the context of the role of NAT enzymes in the activation of carcinogetic heterocyclic amines. The results of the studies of colorectal cancer are inconsistent. A larger study of colorectal adenomas showed a 2-fold increased risk in fast, compared to slow, acetylators. None of these analyses has examined the possible interaction of NAT1 genotype and measures of folate intake or status.

The mechanisms by which folate status may affect the risk of colorectal cancer are unclear. There are conflicting data as to whether blood markers of folate status reflect colonic mucosal folate concentrations. Folic acid is crucial in both methyl metabolism and in DNA synthesis and repair and it seems likely that these are involved.

Folic acid, in the form 5-methyltetrahydrofolate, is important for the production of S-adenosylmethionine (SAM), the primary methyl donor for DNA methylation. Decreased DNA methylation, (hypomethylation) appears to be an early step in colorectal carcinogenesis. Folate deficiency may deplete cellular SAM levels, causing DNA hypomethylation and inappropriate activation of proto-oncogenes. Folate deficiency increases DNA hypomethylation in human lymphocytes. In a small study of the effect of folic acid supplementation on the degree of DNA methylation in the normal colonic mucosa from patients with colonic neoplasms, DNA methylation was increased in patients treated with
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Folate for 6 months and was unchanged in the placebo group. Moreover, 3 months after cessation of treatment with folic acid, there was a significant decrease in DNA methylation.

Folic acid is crucial for DNA synthesis and repair. The conversion of deoxyuridinemonophosphate (dUMP) to thymidinemonophosphate (TMP) requires folic acid in the form 5,10-methylenetetrahydrofolate as methyl donor. Under conditions of folate depletion, a block in methylation of dUMP to TMP may cause uracil misincorporation into DNA in place of thymine. Normal DNA repair processes remove the uracil. However, if conversion of dUMP to TMP is continually limited because of low availability of folate, uracil is misincorporated and removed in a cycle which may ultimately cause malignant transformation. Uracil misincorporation may also impair the ability of the cell to repair other potentially mutagenic lesions. Therefore, a possible explanation for the observation that MTHFR mutation carriers have a reduced risk of colorectal cancer is that they may have increased cellular levels of 5,10-methylenetetrahydrofolate relative to 5, methyltetrahydrofolate, thereby reducing uracil misincorporation.

Brain tumours in children
In a study of primitive neuroectodermal brain tumours (PNET) diagnosed at ages 5 years and under, there was an inverse association with reported maternal dietary folate intake in pregnancy. There was no association between astrocytomas, the most common type of brain tumours in children, and folate intake. An important limitation of the study, acknowledged by the authors, is that the food frequency questionnaire was not designed to assess folate intake, and assessed only about 55% of intake. In an international case-control study, use of vitamin supplements during pregnancy was associated with a decreased risk of brain tumours in the offspring, with a trend of decreasing risk with increasing duration of reported use. This effect did not vary by histology. It was not possible to distinguish the effect of folate from that of other micronutrients.

Folate supplementation has been shown to prevent the recurrence of neural tube defects and is likely to prevent the first occurrence of these defects. In addition, spina bifida has been associated with the MTHFR 677C-T mutation, albeit inconsistently.

If it were true that a factor related to neural tube closure also affected the risk of PNET, it would be expected that children with spina bifida might be at increased risk of developing a PNET later in life. While an association between brain tumours and spina bifida has been reported, the possibility that this was a spurious association cannot be excluded, and the association was not specific to PNET.
Trends in the incidence of medulloblastoma, the most common type of PNET, in parts of the UK were assessed for the period 1976–1991. In the south-western and northern regions combined, the incidence declined from 5.5 cases per million childhood-years in the period 1976–84 to 2.8 in the period 1985–91. In one county in which the trend was more marked (incidence rates 9.4 and 1.6 per million respectively), the pathological findings of all posterior fossa tumours were reviewed for changes in diagnostic criteria; none was found. The authors considered that this change may be compatible with increased use of multivitamin supplementation to prevent neural tube defects, although this appears to have been used primarily to prevent recurrence rather than first occurrence.

Other types of cancer
A possible role of folate has also been investigated in a number of studies of cancer and precancerous lesions of the cervix, but these have been inconclusive. A few studies have been carried out in relation to cancers of other sites, but these have been insufficiently investigated to draw clear conclusions.

Vitamin D and the vitamin D receptor polymorphism

Breast cancer
On the basis of two ecological studies which suggested an inverse association between breast cancer and sunlight exposure, it has been postulated that lack of exposure to sunlight, a source of UV radiation which stimulates vitamin D synthesis, may be a risk factor. Hydroxylation of vitamin D produces 1,25-dihydroxyvitamin D (1,25-D), the physiologically active hormone. 1,25-D binds to intracellular vitamin D receptors (VDR) which are present in breast and other tissues. Several polymorphisms in the VDR have been described which, although they do not change the coding sequence, are assumed to mark functional sequence elements which lie nearby and have been shown to be associated with varying levels of circulating 1,25-D. The association between VDR genotype and breast cancer risk has been investigated in two case-control studies. First, in African-American and Hispanic subjects in the US, the FokI polymorphism was associated with breast cancer in African-Americans only with a relative risk of 0.4 (95% CI 0.24–0.68) for women with the FF genotype compared to Ff and ff, while in both ethnic groups, the relative risk for those homo- or heterozygous for the long poly-A allele was 0.5 (95% CI 0.24–0.95) compared to those homozygous for the short form of the allele. Secondly, in a study in Japan, women who were homozygous for the presence of the
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**BsmI** site (bb) had a relative risk of 3.9 (95% CI 1.63–9.30) compared to women who were heterozygous (Bb) or homozygous for the absence of the **BsmI** site (BB). Less direct evidence for a possible association between VDR and breast cancer risk is provided by the observation that women in the highest quartile of bone mass are at increased risk compared to women in the lowest quartile. A relationship between VDR and bone density has been shown in many studies with the bb genotype associated with higher bone density than other genotypes.

### Prostate cancer

Schwartz and Hulka postulated that subclinical prostate cancer progresses to clinical disease in the presence of low levels of vitamin D. This hypothesis was based on the observation of lack of variation in the frequency of prostate cancer reported in autopsy series, contrasting with a north-south gradation in prostate cancer mortality rates in the US, with higher rates in the north. 1,25-dihydroxyvitamin D inhibits proliferation and promotes differentiation in prostate cells in culture. In the four studies of pre-diagnostic serum or plasma levels of 1,25-dihydroxyvitamin D reported so far, no consistent association has been identified.

High calcium intake is associated with decreased 1,25-dihydroxyvitamin D level, because it suppresses the formation of this from 25-hydroxyvitamin D. Ingestion of fructose results in a transient reduction in plasma phosphate, and hypophosphataemia stimulates the production of 1,25-dihydroxyvitamin D. In a cohort study of male health professionals in the US, a high calcium intake was positively associated with advanced and metastatic prostate cancer, while a high fructose intake was inversely associated with the risk of advanced prostate cancer. These findings are compatible with a protective effect of high levels of 1,25-dihydroxyvitamin D on prostate cancer.

Common genetic polymorphisms in the vitamin D receptor gene have been correlated with circulating levels of 1,25-dihydroxyvitamin D and with *in vitro* measures of gene expression. In a study in North Carolina of 108 consecutive prostatectomy cases and 170 urology clinic patients who did not have cancer, most of whom presented with benign prostatic hypertrophy or impotence, the relative risk of prostate cancer for men homozygous for the wild type of the **Taq1** restriction fragment length polymorphism at codon 352 compared to those with a C–T mutation at this site was 0.3 (95% CI 0.16–0.76). In a pilot study in non-Hispanic white men in Los Angeles County of 57 cases of prostate cancer and 169 neighbourhood controls for bladder cancer patients, the relative risk for men homozygous or heterozygous for the long poly-A
length polymorphism compared to those homozygous for the short allele was 4.6 (95% CI 1.34–15.82). This association was stronger for advanced disease than for localised disease. There is linkage disequilibrium between the C→T mutation at codon 352 and the long poly-A allele. Thus, both studies show an increased risk of prostate cancer associated with mutation of the vitamin D receptor gene. Although both mutations are associated with lower serum levels of 1,25-dihydroxyvitamin D, neither is associated with a change in the vitamin D receptor amino acid sequence.

Colorectal cancer
Vitamin D has been considered in the aetiology of colorectal cancer because of its functions in calcium metabolism. An inverse association between colorectal cancer and reported dietary intake of vitamin D has been found in two of three cohort studies, and two case-control studies have shown non-significant inverse associations. An inverse association also has been observed in a case-control study of prediagnostic serum 25-hydroxyvitamin D levels. Treatment of human colon cancer cell lines with 1,25-dihydroxyvitamin D₃, the active form of vitamin D, induces differentiation and inhibits cell proliferation in vitro. The relationship between colorectal cancer and vitamin D receptor polymorphism does not appear to have been investigated.

Other types of cancer
The evidence as to the effects of calcium or vitamin D on the occurrence of other types of cancer is very limited.

Conclusion
Observational studies have shown an inverse association between high levels of intake of vegetables and fruit and the risks of many types of cancer. This evidence has provided leads to a number of micronutrients which may account for these protective effects. In general, these leads have not been confirmed by randomised controlled trials. This apparent conflict does not invalidate the results of observational studies, but raises issues about the timing and duration of a critical period or periods during which micronutrient intake may influence the development of cancer, the dose and possible interaction between high doses of micronutrients and exposures conferring a high risk of cancer. In addition, there may be gene-micronutrient interactions.

Excluding specific genetic syndromes, familial aggregation is apparent for most types of cancer. It is unlikely that this can be accounted for
entirely by the familial clustering of environmental factors\textsuperscript{134}, pointing to the potential importance of genetic susceptibility factors and interaction of these with each other and with environmental factors. Khoury et al\textsuperscript{135} showed that, in the presence of an interaction between genetic and environmental factors, failure to take both of these sets of factors into account leads to bias in the estimation of disease risk. As a result of recent advances in molecular genetics, it is now possible to take measures of genetic susceptibility into account, and the number of genetic polymorphisms for all genes in humans available for research will increase rapidly. It is anticipated that the human genome sequence will be completed in 2005\textsuperscript{136}. Researchers are now beginning to consider how to investigate interactions between the vastly increased range of genetic factors that can be identified and environmental agents.

So far, in most of the studies of genetic polymorphisms thought to influence micronutrient metabolism, no assessment of possible interaction with measures of dietary intake has been made. Large sample sizes are needed to investigate multiplicative interaction, but certain types of gene–environment interaction may be detected with studies of adequate statistical power to detect the main effects of environmental exposures or specific genes\textsuperscript{137}. Therefore, it seems important that in future nutritional epidemiological studies of cancer, the opportunity should be taken to obtain DNA samples to determine genotypes for polymorphisms potentially affecting micronutrient metabolism. The example of the association between the MTHFR C677T polymorphism and colorectal cancer illustrates how such investigation may challenge a suggested mechanistic explanation for a micronutrient—cancer association, and suggest a new hypothesis to be tested. New study designs, such as the case only and case pseudosib designs, are being developed for the investigation of gene-environment interaction\textsuperscript{138,139}. It is important that this line of investigation is pursued in the established framework for making causal inference, that of integrating evidence from diverse sources\textsuperscript{34}. A particularly important criterion of causality has been consistency of association. However, heterogeneity in the strength of associations might well be expected as a result of gene-environment interactions. It will be important to develop hypotheses about this heterogeneity which can be tested in future collaborative studies.

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