Commentary

# Cancer prevention and diet: Help from single nucleotide polymorphisms

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To prevent cancer, an understanding of causality is the prerequisite for effective action. Causality can be established by combining epidemiology, a key tool for identifying major risk factors, with research on mechanism. It is becoming apparent that a better understanding of nutrition and nutrition–genetic interactions will be one important consequence of the genomics revolution. The paper by Skibola *et al.* (1) in this issue of PNAS is one step along the path to understanding. Their work, as well as others we discuss below, shows the power of investigating single nucleotide polymorphisms in epidemiological studies to clarify mechanism and risk factors in the difficult, but extremely important, area of diet and the prevention of disease. Their results provide evidence that folic acid deficiency may be a risk factor for adult acute lymphocytic leukemia (ALL). I discuss their results in the context of adequate dietary fruits and vegetables for the prevention of cancer, why deficiency of folic acid leads to massive uracil incorporation into DNA and chromosome breaks, and why deficiencies of vitamins B6 or B12 might also be risk factors for ALL.

Two polymorphisms, 677 (C>T) and 1298 (A>C), are common in the gene for methylene-tetrahydrofolate (THF) reductase, the enzyme responsible for reducing methylene-THF (the cofactor for methylating dUMP to dTMP in deoxynucleotide synthesis) to methyl-THF (the cofactor in methylating homocysteine to methionine). Each variant homozygote has a decreased enzyme activity (2, 3), which would result in an increased pool of methylene-THF at the expense of a decreased pool of methyl-THF. Thus, as we discuss below, the polymorphism variants may decrease risk of chromosome breaks (because of uracil misincorporation in DNA) and cancer and may increase risk of homocysteine accumulation and associated diseases (see figure 1 in the paper by Skibola *et al.*).

It was previously shown that folate deficiency, a common vitamin deficiency in people who eat few fruits and vegetables, causes chromosome breaks in human genes (4–7). The mechanism of chromosome breaks has been shown to be deficient methylation of dUMP to dTMP and subsequent incorporation of uracil into human DNA (4 million/cell) (4). Uracil in DNA is excised by a repair glycosylase with the formation of a transient single-strand break in the DNA; two opposing single-strand breaks cause a double-strand chromosome break, which is difficult to repair. Both high DNA uracil levels and chromosome breaks in humans are reversed by folate administration (4). Folate supplementation also minimized chromosome breakage in a different study (6). The potential role in human carcinogenesis of uracil misincorporation is supported by two studies that show a 2- to 4-fold lower risk of colon cancer for individuals who are homozygous for the variant 677 (C>T) allele of methylene-THF reductase compared with controls (8, 9). The Skibola paper also supports this idea as either variant polymorphism decreases the risk of ALL, which would be expected if the uracil incorporation, with consequent chromosome breaks, were the culprit. Skibola *et al.* measured both polymorphisms in the gene, which adds to the power of the study and is clearly the way to do future studies. In the Skibola *et al.* study in English subjects, of age- and sex-matched controls, 12% had the variant TT at 677 and 10% had CC at 1298. They looked at all of the combinations of the two variants: the double heterozygote is protective for ALL. Another satisfying aspect of their study is the fact that AML, another type of leukemia that may have other causes (10), derived from myeloid progenitors in contrast to ALL, is not affected by the polymorphism, which suggests folate deficiency is not a major cause of AML. One drawback of the Skibola study is that they could not measure folate intakes. The Skibola study shows that about 35% of the patients are protected by the polymorphism (the homozygotes and the double heterozygote). Although Skibola *et al.* studied genotypes, their findings emphasize the importance of folate and micronutrient nutriture in the prevention of cancer.

A recent paper (11), consistent with an earlier one (12), examined dietary levels of folate, B12, and B6 and the 677 (C>T) polymorphism and found that low intake of each of the three micronutrients in the presence of the TT genotype increased colorectal adenoma risk, particularly in those over age 60. At high intake levels, a slightly decreased risk was observed for the TT genotype. More complexities need to be unraveled. They also found an effect of alcohol, which is known to modify folate metabolism, but this was confined to those with the CC genotype. It will be of great interest when they examine the 1298 (A>C) polymorphism, which may help to clarify matters.

Homozygotes for the 677 (C>T) variant appear to have an increased risk of stroke (13) and neural tube defects (2, 14), although not necessarily of heart disease (13, 15), presumably because of decreases in the methyl-THF pool (which methylates homocysteine to methionine) and increased serum homocysteine. An interesting new paper by James *et al.* (16) on Down's syndrome, which is caused by a chromosomal nondisjunction causing a chromosome 21 trisomy, shows that a maternal genotype of 677 TT or CT is associated with a 2.6-fold increased risk of a child with Down's syndrome compared with 677 CC. This suggests that folate deficiency in the mother, or possibly the grandmother, interacted with the mother's genotype to negatively affect chromosome segregation, a process that may even have been affected during meiosis prophase when the mother was a fetus in her mother.

Germ line damage to the sperm or egg is a likely cause of the cancers of childhood, such as ALL in children (17). Poor diet in the father, or mother, or even the grandmother when she was pregnant with the mother, interacting with genotype may be a contributor to ALL in children, one of the more common types of childhood cancer. We have discussed the evidence

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supporting the idea that childhood cancer, such as ALL, may be caused in part by smoking fathers, with diets low in vitamin C, oxidizing the DNA of their sperm (17).

Unbalanced diets may be as important a cause of cancer as smoking (18). For example, low consumption of fruits and vegetables have been associated with high incidence of cancer in more than 200 epidemiological studies of great consistency (19–21). The quarter of the population with the lowest dietary intake of fruits and vegetables has roughly twice the cancer rate for most types of cancer (lung, larynx, oral cavity, esophagus, stomach, colon and rectum, bladder, pancreas, cervix, and ovary) (19) when compared with the quarter with the highest intake. This might be true of ALL, but there is insufficient epidemiological evidence to draw a conclusion. These observations are consistent with data on the Seventh Day Adventists, who are nonsmokers and mostly vegetarians and have about half the cancer mortality rate and a longer life span than the average American (22). In the U.S., the lowest quartile of adults consumed 2.7 portions or less and the highest quartile 5.6 portions or more (S. M. Krebs-Smith, personal communication). Eighty percent of American children and adolescents (23) and sixty-eight percent of adults (24) did not meet the intake recommended by the National Cancer Institute and the National Research Council: five servings of fruits and vegetables per day. In addition, ≈30% of the vegetables eaten by children and teenagers are potato chips or french fries, richer sources of starch and fat than vitamins (C. Champagne, personal communication).

Publicity about hundreds of minor, implausible, hypothetical risks, such as that from pesticide residues in the diet (25), has contributed to a lack of perspective on disease prevention. Pesticides, for example, are a major public health advance because they lower the price of fruits and vegetables, a major benefit for the poor. Half of Americans do not list fruit and vegetable consumption as a protective factor against cancer (26) and two-thirds think that for good health only two servings per day need to be consumed (27). Fruit and vegetable consumption is lowest among the poor, particularly African Americans (24, 28). Greater consumption of fruits and vegetables is associated with a lower risk of cardiovascular disease, cataracts, and brain dysfunction as well (29).

The understanding of the mechanism for the efficacy of fruits and vegetables is not as clear. Micronutrient deficiency is a plausible explanation for much of the effect of fruits and vegetables (30). For each micronutrient, deficiency distorts metabolism in numerous and complicated ways, many of which may lead to DNA damage. The recommended dietary allowance (RDA) of a micronutrient is mainly based on information on acute deficiency, because the optimum amount for long term health is generally not known. For many micronutrients, a sizable percentage of the population has an inadequate intake (here taken as <50%) relative to the current Recommended Daily Allowance RDA (31). Remedying these deficiencies, which can be done at low cost, is likely to lead to a major improvement in health and an increase in longevity (30). The optimum intake of a micronutrient can vary with age and genetic constitution and be influenced by other aspects of diet. Determining these optima, and remedying deficiencies, and in some cases excesses, will be a major public health project for the coming decades. Long-term health is also influenced by many other aspects of diet (18, 32).

Micronutrient deficiency can mimic radiation in damaging DNA by causing single- and double-strand breaks, oxidative lesions, or both (30). Those micronutrients whose deficiency appears to mimic radiation are folic acid, B12, B6, niacin, C, E, iron, and zinc, with the laboratory evidence ranging from likely to compelling. The percentages of the population that consume less than half the RDA for five of these eight micronutrients are as follows: zinc, 18%; iron, 19% of menstruating women; C, 15%; E, 20+%?; and niacin, 2%. These deficiencies combined with folate, B12, and B6 (discussed below) may comprise *in toto* a considerable percentage of the U.S. population (30).

**Folic Acid.** The level of folate causing chromosome breaks in humans was present in ≈10% of the U.S. population and, based on two small studies done nearly 20 years ago, about half of urban low income (mainly African-American) elderly and adolescents (4). The recent addition of folic acid to U.S. flour, rice, pasta, and cornmeal is changing the picture markedly (33). Folate deficiency has been associated with increased risk of colon cancer (34, 35), and the 15-year use of a multivitamin supplement containing folate lowered colon cancer risk by ≈75% (36). Chromosome breaks could contribute to the increased risk of cancer associated with folate deficiency in humans (4). Folate deficiency causes increased homocysteine accumulation, which has been associated with neural tube defects in the fetus and heart disease, both of which could be eliminated by folate supplements, the new food fortification program, or better diets (37–41). Definitive results on folate deficiency, genotype, and risk of heart disease (42) await studies in which the two methylene THF reductase polymorphisms and homocysteine levels are assayed.

**Vitamin B12.** The main dietary source of B12 is meat. About 4% of the U.S. population consumes below half of the RDA of vitamin B12 (31). About 14% of elderly Americans and ≈24% of elderly Dutch have mild B12 deficiency, possibly in part because of Americans taking more vitamin supplements, although malabsorption plays an important role (43). Vitamin B12 would be expected to cause chromosome breaks by the same mechanism as folate deficiency. Both B12 and methyl-THF are required for the methylation of homocysteine to methionine. If either folate or B12 is deficient, then homocysteine, a risk factor for heart disease (37, 40–42), accumulates. When B12 is deficient, then tetrahydrofolate is trapped as methyl-THF; the methylene-THF pool, which is required for methylation of dUMP to dTMP, is consequently diminished. Therefore, B12 deficiency, like folate deficiency, should cause uracil to accumulate in human DNA. There is accumulating evidence for this (R. T. Ingersoll, S. N. Wickramasinghe, and B.N.A., unpublished work; ref. 44) as well as chromosome breaks (6, 7). The two deficiencies may act synergistically. In a study of healthy elderly men (5), or young adults (6), increased chromosome breakage was associated with either a deficiency in folate, or B12, or with elevated levels of homocysteine. B12 supplementation above the RDA was necessary to minimize chromosome breakage (6).

**Vitamin B6.** About 10% of the U.S. population consumes less than half of the RDA (1.6 mg/day) of vitamin B6 (31). Vitamin B6 deficiency causes a decrease in the enzyme activity of serine hydroxymethyl transferase, which supplies the methylene group for methylene-THF (45). If the methylene-THF pool is decreased in B6-deficiency, then uracil incorporation, with associated chromosome breaks, would be expected, and evidence for this has been found in women at a level of 32 nmol/liter vitamin B6 in blood (0.5 mg/day intake) (R. T. Ingersoll, T. D. Shultz, and B.N.A., unpublished work). In a case-control study of diet and cancer, vitamin B6 intake was inversely associated with prostate cancer (46). In the study on Finnish smokers, "serum folate or pyridoxal-5′-phosphate concentrations showed statistically significant inverse dose-response relationships with pancreatic cancer risk" (ref. 47, p. 535). Vitamin B6 deficiency raises homocysteine levels and appears to contribute to heart disease; supplementation reduces risk (48) and levels above the RDA may be necessary to minimize risk (38). A level of vitamin B6 in blood below 23 nmol/liter is

a risk factor for stroke and atherosclerosis (49). Good sources of vitamin B6 are whole grain bread and cereal, liver, bananas, green beans, and fortified breakfast cereal.

Optimizing micronutrient intake [through better diets, fortification of foods, or multivitamin-mineral pills (41)] can have a major impact on public health at low cost. Other micronutrients are likely to be added to the list of those whose deficiency causes DNA damage in the coming years. Tuning-up human metabolism, which varies with genetic constitution and changes with age, is likely to be a major way to minimize DNA damage, improve health, and prolong healthy lifespan, and a drop of blood and a gene chip could be part of the way to accomplish this.

Missense single nucleotide polymorphisms occur about 1 every 1,000 bases in expressed genes (50, 51), so one expects that there will be many more polymorphisms to be found in micronutrient and dietary studies. It is already apparent that there are many polymorphisms that influence risk in heart disease. Single nucleotide polymorphisms provide a powerful molecular tool for investigating the role of nutrition in human health and disease, and their integration into clinical, metabolic, and epidemiologic studies can contribute enormously to the definition of optimal diets.

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