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Nutrition and cancer prevention: diet–gene interactions

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Cancer is the major cause of death in the UK, and the Government has set a target to reduce death rate from cancer in individuals < 75 years by ≥ 20 % by 2010. Whilst earlier diagnosis and more effective treatments will contribute to meeting this target, there are considerable opportunities to prevent cancer by improving diet and other aspects of lifestyle. There is now a good understanding of the biological basis of carcinogenesis, which is providing the basis for mechanistic investigation of the chemo-preventive properties of certain foods and food components. It is becoming increasingly clear that there are important interactions between an individual's genotype (characterised by single nucleotide polymorphisms in particular genes) and habitual diet that modulate the risk of developing cancer. The technology to support this post-genomic revolution in nutrition research is now widely available, but brings with it considerable challenges in terms of study design and ethics. However, in the absence of a robust body of evidence on which dietary strategies will benefit which individuals, soundly based genetically-targeted nutrition advice to the public on cancer prevention is a little way in the future.

Cancer prevention: Diet–gene interactions: Nutrition advice

Why focus on cancer prevention?

Throughout the last century there were remarkable improvements in public health in the UK. Substantial falls in infant and childhood mortality and in deaths from infectious diseases in adulthood made major contributions to the increases in life expectancy. At the beginning of the last century 36 % of deaths were in those < 5 years of age compared with only 1 % in this age-group 100 years later. In 1900 only 24 % of deaths were in those aged ≥ 65 years, while this percentage had risen to 84 by 1997 (Department of Health, 1999). This increase in life expectancy has been accompanied by a transformation in the recorded causes of death, so that in the second half of the twentieth century there was an apparent epidemic of deaths from non-communicable diseases, most notably cardiovascular and cerebrovascular diseases. Fortunately, this epidemic of vascular diseases has waned over the past two to three decades, so that cancers are now the leading cause of death in the UK. The UK government's current target is to reduce death rate from cancer in individuals < 75 years by ≥ 20 % by 2010 (Department of Health, 1999).

Whilst there are substantial socio-economic gradients in incidence for cancers at certain sites (e.g. sharp increases in lung cancer incidence from the highest to lowest social class by occupation, with the reverse for melanoma (especially in men)), overall, cancer risk for ages 15–74 years shows little socio-economic patterning (Department of Health, 1998). However, 5-year survival after cancer diagnosis is better for those in affluent communities than for those in deprived communities, and worse in the UK than for the European average or the USA (Department of Health, 1999). For some cancers, e.g. acute lymphoblastic leukaemia and Hodgkin's disease, there have been marked improvements in the efficacy of treatment in the last three to four decades, but for other common cancers, e.g. cancers of the lung and prostate, there has been almost no improvement (Department of Health, 1999), so that achieving the national target requires a focus on prevention.

Opportunities for prevention

Prevention of cancer could be achieved in at least three ways:

1. removal of cause: unlike the case of infectious diseases, for most common cancers, with the exception of tobacco smoking and lung cancer, there is no identifiable causal agent but risk factors may be known or suspected;
2. chemo-prevention: cancer chemo-prevention is the use of specific chemical compounds to prevent, inhibit or

Abbreviation: SNPs, single nucleotide polymorphisms.

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reverse carcinogenesis (Kelloff *et al*. 1996). In contrast with cardiovascular disease, research on the chemo-prevention of cancer is in the early stages. There have been a couple of notable successes (including tamoxifen and retinol for the prevention of breast cancer and skin squamous cell carcinoma respectively), but most trials to date have produced no evidence of benefit (Lippmann *et al*. 1998). The observation that β-carotene supplementation of smokers and those exposed to asbestos (The Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study Group, 1994; Omenn *et al*. 1996) resulted in increased risk of lung cancer has heightened awareness of the need for careful assessment of risk:benefit when giving putative chemo-preventive agents to healthy individuals over long time periods.

3. lifestyle modification: lifestyle modifications may include smoking cessation, greater physical activity (Hardman, 2001) or changes in diet. The public is interested in diet and is motivated by financial inducements and concerns about the effectiveness of the National Health Service to take more responsibility for their health. In addition, the possibility of making health claims is becoming a more important driver in the development of food products. For these reasons, there is an urgent need for reliable evidence of what dietary changes reduce cancer risk.

How could diet influence carcinogenesis? Importance of understanding molecular mechanisms of tumour development

Since the pioneering quantitative epidemiological study by Doll & Peto (1981), it has been accepted that about one-third of the variation in cancer incidence between communities can be accounted for by variation in habitual diet (World Cancer Research Fund/American Institute of Cancer Research, 1997; Department of Health, 1998). The evidence for a marked dietary involvement in their aetiology is stronger for some cancer sites, e.g. large bowel, than for others, e.g. testis (Department of Health, 1998). However, absence of convincing evidence should not be interpreted as lack of effect, given the paucity of research on the role of diet in the aetiology of many cancers.

The complexity of dietary patterns in combination with the biochemical diversity of foods and the not inconsiderable difficulty in quantifying habitual food intake have frustrated efforts to identify protective and cancer risk-enhancing factors in diets using conventional epidemiological tools. This situation is underlined by the rather limited specific recommendations offered by the Committee on Medical Aspects of Food and Nutrition Policy Working Group on Diet and Cancer (Department of Health, 1998). The consistent evidence that diets rich in vegetables and fruits are associated with lower risk of cancer (World Cancer Research Fund/American Institute of Cancer Research, 1997; Department of Health, 1998) has encouraged studies of the protective factors in such foods, and there is growing evidence that optimising intakes of micronutrients could have an important impact on public health by helping prevent cancer and other chronic diseases (Ames & Wakimoto, 2002).

For several decades there has been a sustained investment in research on the biological basis of cancer, which aims to understand, at a molecular level, what goes wrong in cancer cells. The essential hallmarks of cancer are now known (Hanahan & Weinberg, 2000), and these factors have not only indicated potential targets for therapeutic and chemo-preventive agents, but also have illuminated the potential mechanisms by which dietary factors could influence carcinogenesis. For example, the rapid expansion of understanding of the role of apoptosis in tumorigenesis has underpinned research on the mechanisms by which dietary components such as *n*-3 polyunsaturated fatty acids and flavonoids may suppress tumour development (Johnson, 2001).

Genetic and epigenetic phenomena in carcinogenesis

Genetics of cancer

An obvious characteristic of a tumour, especially solid tumours such as carcinomas that arise in epithelial tissues, is that they grow and through sheer mass may damage the organ in which they arise. However, tumours are not just the accumulation of too many cells, but are the result of 'too many aggressive, invasive cells that are in the wrong place at the wrong time' (Sporn & Suh, 2002). The current paradigm states that cancers arise as the result of a multi-step process of clonal selection in which transformed cells acquire selective advantages over normal cells through loss-of-function mutations in tumour suppressor and DNA repair genes and via gain-of-function mutations in proto-oncogenes (Reale & Fearon, 1996). In this sense cancer is a genetic disease, because the Darwinian advantages enjoyed by tumour cells are the result of changes to the genome of those cells. Although the genetic basis of cancer has been acknowledged for a long time, advances in molecular genetics and in molecular and cell biology have revolutionised the field by identifying several of the genes involved, characterising the functions of the proteins encoded by these genes and working out the consequences of mutations in the genes.

The proteins resulting from expression of tumour suppressor genes play key roles in regulating normal cell proliferation, differentiation and apoptosis. Loss-of-function mutations, leading to truncated versions of the protein or the complete absence of the protein, mean that these controls on fundamental cell processes are removed and the transformed cell may grow more rapidly and/or divide indefinitely, fail to differentiate and fail to respond to apoptotic stimuli (Hanahan & Weinberg, 2000). Since DNA is exposed continuously to damage by endogenous and exogenous factors and runs the risk of acquiring errors each time the genome is copied during mitosis, mammalian cells contain sophisticated systems for sensing such damage, correcting it if the damage is not too extensive (Yu *et al*. 1999; Jackson, 2002), or directing the cell along the default pathway of apoptosis. Loss-of-function mutations in the genes encoding these repair systems expose the cell to the accumulation of errors in the DNA, which will include tumour suppressor genes and proto-oncogenes. Oncogenes result from mutations in proto-oncogenes that encode proteins that are

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components of signal transduction pathways regulating cell proliferation and differentiation (Reale & Fearon, 1996). Proto-oncogenes can be activated by several mechanisms (point mutations, chromosomal translocation and DNA amplification) to result in abnormal expression of the onco-protein.

The emerging role of epigenetics

Since the 1960's mammalian developmental biologists have recognised epigenetic phenomena such as the silencing of some genes from one parent during development of the embryo. This phenomenon, known as imprinting, is due to differential methylation of the two alleles of specific genes derived from the mother and father by the enzyme Dnmt1 (Ferguson-Smith & Surani, 2001). The DNA of mature eggs and sperm is highly methylated and there are waves of de-methylation and re-methylation following fertilisation and the development of the blastocyst (Reik *et al*. 2001). Understanding and manipulating genomic methylation patterns are fundamental aspects of mammalian cloning by nuclear transfer (Rideout *et al*. 2001) and are likely to be important if the promise of stem cells for therapeutic purposes is to be realised.

In addition to the effects of mutations, inactivation of tumour suppressor genes can occur by methylation of normally unmethylated CpG islands in the promoter regions of the genes (Fig. 1). Examples of genes silenced by methylation are given in Table 1. This gene silencing is similar to that occurring during development, and its involvement in both the ageing process (Issa *et al*. 2001; Issa, 2002) and in carcinogenesis (Baylin *et al*. 1998) is a current hot topic (Ballestar & Esteller, 2002). The pattern of DNA methylation is heritable from one cell to its daughters but, in contrast to mutations, promoter hypermethylation is potentially reversible, so that expression of the gene can be re-established (Cameron *et al*. 1999). This process offers a major opportunity for investigation of the role of nutrition in the prevention of carcinogenesis, since the methyl groups used for DNA methylation are derived, ultimately, from nutrients such as folate, and there is evidence that manipulation of folate status can alter DNA methylation (Jhaveri *et al*. 2001; Frisco *et al*. 2002). At the same time, gene methylation studies may provide mechanistic explanations for the mode of action of some food- and water-borne carcinogens, e.g. As, which causes liver tumours. Administration of As with a methyl-deficient diet to mice resulted in genome-wide hypomethylation and reduced methylation of the promoter region of the oncogene Ha-*ras* (Okoji *et al*. 2002). This process would be expected to switch on expression of the oncogene and contribute to tumour development.

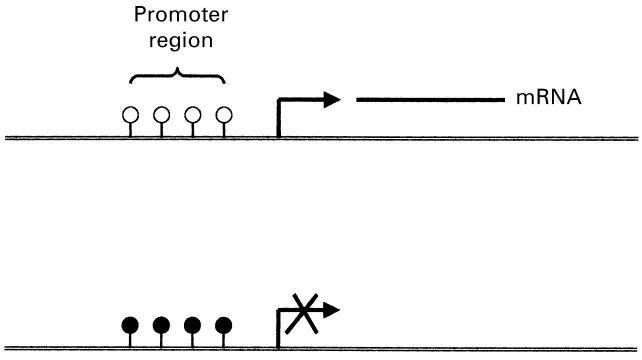

Fig. 1. Methylation of CpG islands in the promoter regions of genes causes gene silencing. (○), Unmethylated CpG; (●), methylated CpG.

Table 1. Examples of tumour suppressor genes in which CpG islands in the promoter regions may be hypermethylated, resulting in gene silencing and contributing to tumorigenesis (adapted from Robertson & Jones, 2000)

Gene	Type of tumour
pRB	Retinoblastoma
BRCA1	Breast cancer
APC	Colo-rectal cancer
hMLH1	Colo-rectal cancer
P16INK4a	Melanoma

Genetic susceptibility or resistance to cancer

Several of the rare forms of familial cancer resulting in disease at a relatively young age, e.g. retinoblastoma, familial adenomatous polyposis and a minority of breast cancers, are the result of the inheritance of a mutated copy of a single tumour suppressor gene, i.e. retinoblastoma (*pRB*), adenomatous polyposis coli (*APC*) and breast cancer (*BRCA*) 1 or 2 respectively. In these cases all cells in the body of affected individuals will carry a non-functional allele of the respective gene, and loss of the second allele via some somatic event leads to the initiation of tumour development. Clearly, in such cases there is a very strong genetic predisposition to cancer, but the time of appearance of the disease varies due to the impact of modifier genes (Dobbie *et al*. 1997), i.e. the consequence of gene–gene interactions and of poorly understood environmental factors. Even in the case of these familial forms of cancer, chemo-prevention is possible, as has been demonstrated with the cyclooxygenase-2 inhibitor celecoxib in individuals with familial adenomatous polyposis (Steinbach *et al*. 2000).

The vast majority of cancer cases are described as sporadic, i.e. they are not the result of an inherited faulty gene but are caused by the accumulation of DNA damage. However, the likelihood of accumulating sufficient damage to result in neoplasia is influenced by our genetic makeup, as a consequence of the carriage of subtly different forms of particular genes known as polymorphic variants. Such variants are responsible for many of the obvious physical characteristics, as well as the detailed biochemistry of each individual, and are the result of changes in individual bases known as single nucleotide polymorphisms (SNPs) within the individual's genome. There are believed to be up to 2×10^6 such SNPs in the human genome, with about 200 000 of these SNPs within exons (Sachidanandam *et al*. 2001). Any two individuals may differ by, on average, about 100 000 SNPs, but there is little information, as yet, on the

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functional importance (if any) of most of these SNPs. The function of the proteins encoded by the gene variants may be modified as a result of, for example, alterations in binding sites for hormones or other signalling molecules, changes in enzyme activity or incorrect positioning of the protein at subcellular sites. From the perspective of cancer development, SNPs in the genes that protect DNA from damage by environmental factors or repair such damage (environmentally-sensitive genetic polymorphisms) are particularly interesting (for reviews of such genes in relation to risk of skin and lung and oral cavity cancers respectively, see Hahn, 2001; Nair & Bartsch, 2001).

Diet–gene interactions

The enzyme manganese superoxide dismutase is synthesised in the cytosol, post-translationally modified and transported to the mitochondrion where it catalyses the dismutation of superoxide radicals, so protecting the organelle from reactive oxygen species. There is accumulating evidence that women who carry a variant of the manganese superoxide dismutase gene in which there is a T→C substitution at position −9 in the mitochondrial targeting sequence, resulting in a valine→alanine change in the signal peptide, are at increased risk of breast cancer (Ambrosone *et al*. 1999; Mitrunen *et al*. 2001), with a greater risk for premenopausal women than for post-menopausal women (Ambrosone *et al*. 1999). In the larger American study (Ambrosone *et al*. 1999) the deleterious effect of being homozygous for the *ala* variant (compared with heterozygotes and those homozygous for the more common *val* variant) was exacerbated by low consumption of fruits and vegetables (Table 2) and was apparent only in those women who did not take supplements of vitamin C (odds ratio 4·8, 95 % CI 2·1, 11·0) and α-tocopherol (odds ratio 3·8; 95 % CI, 1·8, 8·2). It is probable that the secondary structure of the *ala* variant of manganese superoxide dismutase is altered sufficiently to prevent effective targeting of manganese superoxide dismutase to the mitochondrion (Rosenblum *et al*. 1996), so increasing vulnerability to damage by reactive oxygen species. The data from Ambrosone *et al*. (1999) suggest that high intakes of vegetables and fruits or of antioxidant vitamins may overcome the effects of this genetic susceptibility.

There is considerable controversy around whether red meat intake contributes to increased risk of colo-rectal cancer, with the Department of Health (1998) taking the cautious view that those with intakes of ≥140 g/d '… might benefit from, and should consider, a reduction in intake.' It is possible that the individual response to red meat intake will depend on the way in which the meat is cooked (high temperature cooking results in the production of potential carcinogens, e.g. heterocyclic amines) and the genotype of the consumer. Since there are well-known polymorphisms in the genes encoding *N*-acetyltransferases that can activate aromatic amines (Nair & Bartsch, 2001), interactions between *N*-acetyltransferase variants and extent of exposure to well-cooked meats may contribute to the heterogeneity in response to red meat intake. In a case–control study in the north east of England Welfare *et al*. (1997) found that among individuals with the fast-acetylator genotype of *N*-acetyltransferase 2 (*NAT2*), those who reported frequent consumption of fried meat had a 6-fold increase in risk of colon cancer. For those with the slow-acetylator *NAT2* genotype, being a recent smoker or heavy alcohol consumption was associated with markedly increased risk (Welfare *et al*. 1997). Carriage of the fast acetylator genotype of *NAT2 per se* does not seem to have a specific effect on risk of colo-rectal cancer (Ye & Parry, 2002), so that disease outcome will depend on interactions with environmental factors such as components of foods. A limitation of current studies is the lack of robust procedures to be used in epidemiological studies for estimating the intakes of food-derived mutagens or potential carcinogens such as heterocyclic amines. This situation may explain some of the inconsistencies in the literature (Kampman *et al*. 1999).

Table 2. Comparison of effects of low *v.* high intakes of fruits and vegetables on risk of breast cancer among pre- and post-menopausal women; interaction with polymorphism in the manganese superoxide dismutase (*MnSOD*) gene (Ambrosone *et al.* 1999)

Intake of fruits and vegetables…	Low	Low	High	High
MnSOD genotype	A	B	A	B
Premenopausal: Odds ratio	1	6·0	1	3·2
95 % CI		2·0, 18·2		1·2, 8·2
Post-menopausal: Odds ratio	1	1·7	1	1·8
95 % CI		0·8, 3·8		0·9, 3·6

A, $MnSOD^{val/val}$ and $MnSOD^{val/ala}$; B, $MnSOD^{ala/ala}$.

Experimental design issues

Much of the information to date on diet–gene interactions in influencing cancer risk (for example, see Lin *et al*. 1998; Ambrosone *et al*. 1999) has come from observational studies in which researchers obtain information on dietary exposure, on genotype and on disease outcome. In such studies there is likely to be a much greater extent of uncertainty (measurement error) associated with dietary exposure than with ascertainment of genotype. This situation can result in problems of exposure misclassification and bias in the estimation of the interaction effect between diet and genotype. Garcia-Closas *et al*. (1999) have highlighted this problem and suggested means of estimating the impact on sample size calculation. They have also emphasised the importance of improving the accuracy of measurement of the environmental factor (in this case, diet).

Observational epidemiology such as case–control studies can provide useful information on associations, but the strongest evidence for causal relationships comes from intervention trials. This position presents a growing problem for those interested in interactions between genetic polymorphisms and potential anti-neoplastic components in foods. The problem is relatively simple if only one dietary factor and one gene are of interest, but can become unmanageable very rapidly if more than one gene is to be investigated. It would be possible to do genotyping retrospectively; indeed, many of the early studies of diet–gene interactions were conducted in this way. However, this

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approach has the disadvantages that the randomisation process may not allocate treatments to each genotype equally, so that there may be insufficient power to test particular hypotheses and associations may be identified that are chance occurrences rather than causal. The current view is that it is preferable to genotype subjects prospectively and to allocate individuals of each genotype to each treatment at random. This approach may make subject recruitment a major task. Imagine a study in which there are two genes of interest (A and B), each with two polymorphic variants, i.e. A1 and A2 and B1 and B2. If the less-common variant of a given gene occurs in 10 % of the target population, then to accumulate, for example, twenty volunteers with each variant it would be necessary to genotype about 200 individuals, and only forty of these would be recruited into the study. With less common variants and/or with more genes, the recruitment challenge is magnified.

Ethical issues

In terms of physical risk, nutritional studies that include genotyping present no greater ethical issue than many other studies in which biological samples are obtained. The most fundamental issues are around the nature of informed consent and focus on: (1) what is done with the genetic information obtained in the study; (2) what happens to the DNA that has been collected. For the vast majority of gene polymorphisms where there is an association with altered cancer risk, there is insufficient evidence on which to develop any preventive treatment or any advice on lifestyle modification (beyond conventional healthy eating advice). For as long as this situation exists, it is usually argued that volunteers should be invited to give consent to genotyping on the basis that neither they nor any third party, e.g. their general practitioner, will be informed of the outcome of the test. This situation is quite different from one in which genotyping is for mutations in highly-penetrant genes such as *APC* or *BRCA1*, where there is an established enhanced risk, there are large implications for other members of the family and there are well-worked out treatment regimens that can be offered (for further discussion, see Burn *et al.* 2001).

In a particular study researchers may be investigating polymorphisms in one or a small number of genes but, given that new target genes are emerging rapidly, it is very attractive to store DNA for future studies. Indeed, many laboratories have archived samples of blood (or other biological material) that, potentially, could be valuable for further studies of gene-specific responses to dietary interventions. In the wake of public outcries about the storage of body parts without informed consent and in a climate of concern that scientists may be interested primarily in developing profitable products or services using DNA from volunteers, it is essential that all storage of DNA for future investigations is done with the explicit consent of the volunteers. Volunteers should be invited to consent on the clear understanding that their DNA will be stored. If future analyses are envisaged, volunteers could be invited to:

agree to be contacted for further consent once the purpose of those additional genotyping studies is known, or

consent prospectively to studies of a particular type, e.g. genes involved in particular aspects of metabolism or genes believed to be important in a specific disease.

Communication of appropriate messages to the public

The public is increasingly interested in links between food and health and has a heightened awareness of how their genetic make-up influences risk of disease. As the technology for genotyping individuals for selected gene polymorphisms is now relatively inexpensive, companies are beginning to offer genotyping services to the public. In the absence of a robust science base on which to develop lifestyle advice and/or food products that are genotype specific, there is a concern that such services could have serious adverse effects leading to:

the development of inappropriate advice and products;
self-medication in the absence of conventional genotype-specific therapies;
fatalism and high-risk lifestyles;
loss of confidence in science, medicine and the food industry.

This situation presents both a major challenge and a major opportunity for nutrition researchers and communicators. There is a need to discover which dietary strategies will be most beneficial for individuals with particular genotypes, but the answers will take time and a considerable investment of resources. In the meantime, the public should be advised that this area of research is exciting and likely to revolutionise the way links between food and health for the individual are considered, but that soundly-based genetically-targeted nutrition advice to the public on cancer prevention is a little way in the future.

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